

**ANAEROBIC BIODEGRADATION
OF SURFACTANTS**

SCIENTIFIC REVIEW

1999

TABLE OF CONTENTS

Foreword and Position Paper

- 1. INTRODUCTION**
- 2. EXECUTIVE SUMMARY**
Environmental relevance of anaerobic biodegradation of surfactants.
- 3. SURFACTANTS IN EUROPE PRODUCTION/CONSUMPTION**
- 4. DEFINITION**
Anaerobic Biodegradation
- 5. ANAEROBIC COMPARTMENTS**
 - 5.1. Overview
 - 5.2. Terrestrial
 - 5.3. Aquatic
 - 5.3.1. Water
 - 5.3.2. Sediment
 - 5.3.3. 'Extreme' habitats
 - 5.3.4. Groundwater
 - 5.4. Waste-Water Treatment
 - 5.5. Landfill
 - 5.6. Animals
- 6. EXISTING METHODS**
 - 6.1. Screening tests
 - 6.1.1. Anaerobic screening tests based on gas volume measurement only
 - 6.1.2. Anaerobic screening tests based on gas production measurement in the gas and the liquid phase
 - 6.1.3. Predictive value of anaerobic biodegradation screening test data
 - 6.2. Simulation tests
 - 6.2.1. Introduction to anaerobic simulation tests
 - 6.2.2. Test systems
 - 6.2.2.1. ¹⁴C-Anaerobic Digester Simulation test
 - 6.2.2.2. Continuous fixed bed simulation test
 - 6.2.2.3. Biological nutrient removal WWTP simulation test
 - 6.2.2.4. Others

7. INTERPRETATION OF AVAILABLE DATA ON ANAEROBIC BIODEGRADATION OF SURFACTANTS

7.1. Anionics

7.1.1. Sulphonates

7.1.2. Sulphates

7.1.3. Fatty acids and soaps

7.2. Nonionics

7.3. Cationics – Amphoteric

8. CRITERIA FOR EVALUATION OF THE IMPORTANCE OF ANAEROBIC BIODEGRADATION

8.1. Flux of surfactants in environmental compartments

8.2. Impact of surfactants on structure and function of anaerobic biodegradation environment compartments

8.2.1. Speciation and bioavailability of surfactants in anaerobic compartments

8.2.2. Water Treatment

8.2.2.1. Anaerobic digester

8.2.2.2. Anaerobic river and lake sediment

8.2.2.3. Soil

8.2.2.4. Septic tanks

8.2.2.5. Landfills for sludge

8.2.2.6. Marine sediments

8.2.2.7. Anaerobic and anoxic zones in sewage treatment plants with biological nutrient removal

9. REFERENCES

APPENDIX : Monitoring Data

Foreword

This document was commissioned by ERASM (Environmental Risk Assessment Steering Committee – a detergent industry group).

ERASM was created in 1991 as a forum for the co-ordination of views and actions in the field of risk assessment and risk assessment issues between the chemical industry (CESIO – Comité Européen des Agents de Surface et leurs Intermédiaires Organiques, a CEFIC Sector Group) and the detergent industry (AISE, Association Internationale de la Savonnerie, de la Détergence et des Produits d'Entretien).

ERASM's mission is to define the industry position in establishing and managing the risk of surfactants to the environment and to propose reasonable and technically sound guidelines for assessing the environmental risk of surfactants. ERASM also ensures that resources are available to carry out necessary research programmes.

The structure of ERASM consists of *ad hoc* task forces established to work on specific topics related to risk assessment methods and data. A task force was set up to address anaerobic biodegradation and the environmental relevance of anaerobic biodegradability of surfactants.

The present dossier gives a compilation and interpretation of the available literature on the fate and biodegradability of commercial surfactants under anaerobic conditions.

The members of this specific task force are :

José Luis Berna (Chairman)	Petresa (Es)
Nigel Battersby	Shell Chemicals (UK)
Luciano Cavalli	Condea Augusta (It)
Richard Fletcher	Unilever (UK)
Andreas Guldner	BASF (De)
Diederik Schowanek	Procter & Gamble (Be)
Josef Steber	Henkel (De)

Position Paper

The anaerobic biodegradation of surfactants is used as an acceptability criterion in some environmental pieces of legislation (eco-label, risk assessment, etc.), without a proper evaluation of the relevance of such a characteristic.

Surfactants form a group of chemicals with considerable environmental importance due to their high volume consumption and widespread use as they are essential ingredients in most laundry and cleaning products. Since the major part of the biosphere is aerobic, priority has been given to the study and assessment of biodegradability under these conditions. Nevertheless there are environmental compartments which can be permanently (e.g. anaerobic digesters) or temporarily anaerobic (e.g. river sediments and soils) and surfactants do reach these.

Available screening test methods to assess anaerobic biodegradation do not simulate the real conditions prevailing in these anaerobic compartments but rather reflect more stringent conditions, due to the high test substance/biomass ratio, possibility of inhibitory effects and limited possibility for adaptation. Therefore positive results are indicative of a similar behaviour under environmental conditions, while a negative result cannot be necessarily interpreted as inherent anaerobic recalcitrance. In addition, low biodegradation results in these tests may be influenced by a limited bioavailability due to the formation of insoluble chemical species.

The relevance of anaerobic biodegradability cannot be separated from other important properties of surfactants such as sorptive behaviour, ecotoxicity profile and above all, aerobic biodegradation rate.

The majority of surfactants entering the environment will be exposed to and degraded under aerobic conditions, and only less than 20 % will potentially reach anaerobic, environmental compartments. In all but a few cases their presence in these will not be permanent.

A systematic evaluation of the risk to the structure and function of these compartments due to the presence of undegraded surfactants led to the conclusion that, in contrast to the adverse effects observed in the absence of aerobic degradation, the lack of anaerobic biodegradation does not seem to be correlated with any apparent environmental problem for most compartments. Particularly for the sediment compartment, data is lacking and it is recommended to fill the missing data gaps to assess structure and function.

In criteria for eco-labelling a conservative set of 'scoring-' or 'weighting'-factors, if any, for anaerobic biodegradability, should follow from a combination of the above characteristics, and it is suggested that these should be of the order of one tenth of the aerobic biodegradability value for readily biodegradable surfactants.

Consequently it is concluded that anaerobic biodegradability does not have the same environmental relevance as the aerobic one. Anaerobic biodegradability should not, therefore, be used as a pass/fail property for the environmental acceptability of surfactants which are readily biodegradable under aerobic conditions.

1. INTRODUCTION

Biodegradation is the most important mechanism for the irreversible removal of chemicals from the aquatic and terrestrial environments. Therefore, the evaluation of biodegradability is an indispensable element of the exposure assessment for the environmental risk assessment process of a chemical substance.

The biosphere is predominantly aerobic and it is understandable that the biodegradation behaviour of chemicals under aerobic conditions has been the focus of attention for a long time. This has led to the development of a number of internationally used and recognised laboratory methods for assessing aerobic biodegradability and a huge amount of test data. The biodegradability of chemicals in the absence of free oxygen, i.e., under anaerobic conditions has been considered to a lesser extent. Nevertheless, there are environmental areas which are permanently or temporarily anaerobic (e.g. sludge digesters of sewage treatment plants, sediments or sub-surface soil layers). It can be argued therefore that a comprehensive evaluation of a chemical's environmental fate should also address its anaerobic biodegradability, provided that there is a possibility for entering anaerobic environments to a significant extent.

Typically, such substances are characterised by a poor water solubility or strong adsorptivity onto solids, resulting in an environmental distribution with a pronounced transportation into anaerobic compartments. Surfactants form a group of chemicals with a high environmental relevance and physical-chemical properties which may result in a significant partitioning between the aqueous and the solid phase in the aquatic environment. Environmental monitoring data for some surfactants support this partitioning assumption, showing their presence in secondary and digester sludges, and sediments. The same phenomenon may also be relevant in the context of the environmental risk assessment of chemicals in the soil compartment as application of digested sludges for agricultural purposes will influence the initial environmental concentration in soils.

In order to establish an overview of the anaerobic biodegradation issue in general (i.e. environmental relevance, test methodology) and of its relevance for surfactants in particular, the Environmental Risk Assessment Steering Committee (ERASM) of the European surfactant producers (CESIO) and the European Detergent Industry (AISE) established a Task Force. This position paper was prepared by experts of the involved industries and aims to form a common basis of knowledge on this topic.

2. EXECUTIVE SUMMARY

Environmental Relevance of Anaerobic Biodegradability of Surfactants

Aim of the review: The objective of this review, prepared by the **AISE/CESIO Task Force ‘Anaerobic Biodegradability’** was to provide a thorough compilation and interpretation of the available literature on the fate and biodegradability of commercial surfactants under anaerobic conditions. This analysis should form the basis for a data-based discussion on the environmental relevance of the property of anaerobic biodegradability (or lack thereof) in a broad range of contexts, such as risk assessment of chemicals and detergent ecolabelling. Regarding the latter, a question currently is whether anaerobic biodegradability is a property to be considered, and if so, what ‘weight’ it should receive.

The earth’s atmosphere contains just over 20 % of oxygen by volume making aerobic compartments the predominant ones in the biosphere. It is therefore not surprising that the prevalent route for biodegradation of chemicals is aerobic and aerobic biodegradation has been extensively studied. In addition, it has long been a legal requirement in the European Union for surfactants used in detergents to be aerobically biodegradable.

Surface active agents are essential ingredients in most household laundry products, domestic and industrial cleaners, as well as in personal care and cosmetic products. Surfactants form a group of chemicals with a high overall environmental relevance, due to a combination of their inherent environmental properties and their very large production volume. They are typically discharged into the environment through the sewage treatment infrastructure (i.e. sewers, sewage treatment plants), or directly in situations where no treatment systems are available.

Anaerobic test methods and their predictive value:

Test methods to determine the ultimate anaerobic biodegradability of organic compounds at screening and simulation level are also available today. First, there are screening tests such as the ECETOC test which determine anaerobic mineralisation by measurement of the methane and carbon dioxide gas production via a pressure reading. Due to the stringent screening-type test conditions (i.e. high test substance/biomass ratio), inhibitory effects of surfactants cannot be excluded and the possibilities for acclimation are limited. These tests are reliable at avoiding false-positive conclusions, but a poor result is not necessarily proof of anaerobic recalcitrance.

Anaerobic biodegradation test systems under more realistic conditions (in terms of sludge concentration, fresh sludge feed, residence times, etc.) include digester simulation tests employing radiolabelled test substances or continuous fixed bed reactors. The latter systems tend to show consistency with the observations in practice. Limitations, however, remain for situations at low substrate concentrations, for toxic surfactants, and for predicting the fate in environments with fluctuating

redox conditions, temporary anaerobiosis and redox gradients (e.g. river sediments, landfills, wetland soils, etc), for which improved testing methodologies and a broader database are warranted. Also, specific analytical methods for following the fate of surfactants and their breakdown products in anaerobic matrices need to be further developed and validated

Anaerobic biodegradability of surfactant groups:

Based on the above laboratory test methods, the available data from published biodegradation studies and available monitoring studies allow the evaluation of the anaerobic biodegradation behaviour of several important surfactant groups:

- Sulphonated anionic surfactants (LAS, SAS, MES): poorly biodegradable
- Sulphated anionic surfactants (alkyl sulphates, alcohol ethoxysulphates): well biodegradable
- Fatty acids and soaps : well biodegradable
- Alcohol ethoxylates: well biodegradable
- Sugar-based surfactants (alkyl polyglucosides, glucamides): well biodegradable
- Alkyldimethyl amine oxides: well biodegradable (based on limited data).
- Alkylphenol ethoxylates: partially degradable leaving alkylphenol residues.
- Mono- or di-alkyl quaternary compounds (TMAC, DTDMAC): poorly biodegradable.
- Esterified mono- or di-alkyl quaternary surfactants (esterquats): well biodegradable.

Factors for evaluating the importance of anaerobic biodegradation:

Mass flux to anaerobic environments: Strictly aerobic and anaerobic environments represent the two extremes of a continuous spectrum of environmental habitats which are populated by a broad variety of micro-organisms with specific biodegradative capabilities. Due to the specific usage patterns, the bulk of the surfactant mass entering the environment will predominantly be exposed to and degraded under aerobic conditions. For readily biodegradable surfactants, less than 20% of the flux will reach anaerobic compartments including for the landfill.

It can be expected that in the coming years the relative importance of sewage treatment will increase with a higher treatment incidence, halting of sludge disposal into the sea and improved treatment systems with biological nutrient removal. Incineration of waste-water sludge will also become a more important disposal route. Consequently, the amount of surfactants which can reach anaerobic compartments via sludge disposal is likely to decrease further over time.

The difference in physico-chemical properties, biodegradability, partitioning and distribution explains the observed differences between the mass fluxes of different surfactants. As such, the relevance of anaerobic biodegradability cannot be separated from other important properties of the surfactant, such as sorptive behaviour, log K_{ow}, solubility, and last but not least, aerobic biodegradation kinetics.

This has already been pointed out by a German Expert Group for Detergents (HAD)¹ who proposed an (arbitrary) relative ranking of anaerobic biodegradability relevance as a function of the above properties.

Bioavailability:

Bioavailability of surfactants changes under anaerobic conditions and may affect the outcome of toxicity/inhibition studies, and to some extent biodegradation or removal rates. The specific chemical structure of some surfactants contributes to a rapid precipitation with water hardness ions (Ca, Mg) into insoluble forms, as well as adsorption to the surrounding solid matrix. This highlights the need to use the real environmental form of a surfactant in inhibition and biodegradation tests, in order to obtain a realistic test result.

Impact of surfactants on the structure and function of anaerobic compartments:

Based on available data, an evaluation was made of the risk to the structure and function of anaerobic compartments being affected by fluxes of undegraded surfactants entering the system. The evaluation led to the conclusion that in contrast to the adverse effects and high risk in the absence of aerobic biodegradation, the lack of anaerobic biodegradation does not seem to be correlated with any apparent environmental or technical problems in most compartments. Nevertheless, it is fair to say that for natural anaerobic environments, not all aspects of structure and function can be adequately assessed today since data is lacking and vigilance is required. For poorly anaerobic biodegradable surfactants, it is recommended to assess the structure and function and fill existing data gaps. It is acknowledged therefore that surfactants biodegradable under both aerobic and anaerobic conditions leave less room open to environmental concerns.

Conclusions :

It is therefore concluded that anaerobic biodegradability as a strict pass/fail criterion is not in line with the environmental interpretation and significance that should be given to the lack of this property for surfactants. For surfactants used today in detergents, rapid aerobic biodegradation as well as their sorptive and ecotoxicological properties, are key to making a realistic assessment of environmental compatibility. If a surfactant is rapidly degradable under aerobic conditions, and its transitory presence in anaerobic environments does not affect the function and structure of that environment (e.g. it is not inhibitory), then its anaerobic degradability is of minor importance. Nevertheless, it is fair to say that for natural anaerobic environments, not all aspects of structure and function can be adequately assessed today since data is lacking and vigilance is required. For poorly anaerobic biodegradable surfactants, it is recommended to assess the structure and function and fill existing data gaps.

¹ Schöberl P. (1994). Die Bedeutung fehlender anaerober biologischer Abbaubarkeit. Tenside Surf. Det. 31, 157-162.

3. SURFACTANTS IN EUROPE PRODUCTION AND CONSUMPTION

Surfactants (anionics, nonionics, cationics and amphoteric) are used in different fields such as cosmetics, metal working, mining, agriculture, paper and leather industries and obviously the detergent industries.

CESIO compiles annual statistics on the production and consumption of surfactants in Europe.

The data for 1996 and 1997 are summarised in the following table:

	<i>Production</i>		<i>Total market</i>	
	<i>in W. Europe</i>		<i>in W. Europe (incl. captive consumption)</i>	
<i>in 1 000 tonnes - as 100 % active</i>	<i>1996</i>	<i>1997</i>	<i>1996</i>	<i>1997</i>
A. ANIONICS				
Alkylbenzene sulphonates (LAS)	400	420	390	390
Alkane sulphonates	77	70	74	60
Alcohol sulphates	111	115	103	108
Alcohol ether sulphates	229	254	210	240
Soap (fatty acids)				
Other anionics	82	80	87	72
Total Anionics	899	939	864	870
Soaps	550	550	550	550
B. NONIONICS				
All ethoxylates	844	879	800	807
Other nonionics	224	236	200	196
Total Nonionics	1068	1115	1000	1003
C. CATIONICS	170	174	145	149
D. AMPHOTERIC	43	60	40	56
GRAND TOTAL	2730	2838	2599	2628

The following conclusions can be drawn from these data:

The four main outlets for surfactants are household, cosmetics and toiletries, food processing and I&I (Industrial and Institutional cleaning) which account already for half of the total surfactant consumption. In those markets a limited number of surfactant types remain dominant : alkylbenzene sulphonates (LAS), ethoxylates, alcohol-sulphates and alcohol ether sulphates.

Other outlets for surfactants are textiles, emulsion polymerization, paint additives, agrochemicals, etc. where besides some commodity surfactants, mainly “specialty” surfactants are used.

The total surfactant consumption in Europe is rather stable for the moment, although some growth mainly in nonionics and to a lesser extent in amphoteric and cationics is expected. Total anionic, as well as soaps, is not expected to grow in the next couple of years.

The consumption of the category of other nonionics is expected to increase mainly because of the growing interest in alkyl polyglucosides.

4. DEFINITION

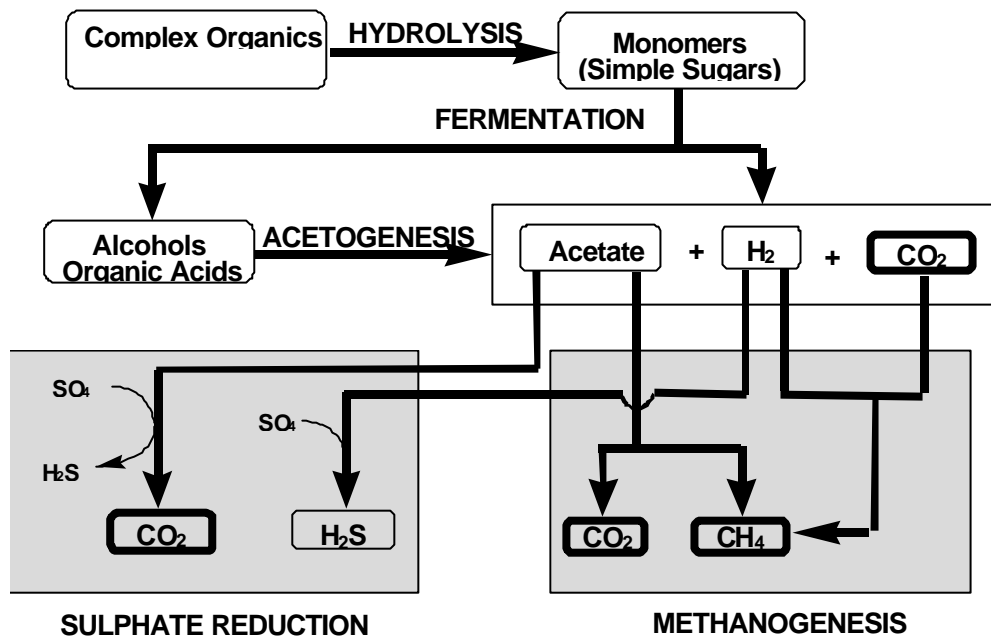
Anaerobic Biodegradation

Anaerobic biodegradation means the microbial degradation of organic substances in the absence of free oxygen (O_2). While O_2 serves as the electron acceptor in aerobic biodegradation processes forming H_2O as the final product, degradation processes in anaerobic systems depend on alternative acceptors such as sulphate, nitrate or carbonate yielding, in the end, hydrogen sulfide (H_2S), molecular nitrogen (N_2) and/or ammonia (NH_3) and methane (CH_4), respectively.

Anaerobic biodegradation is a multistep process performed by different bacterial groups. It involves hydrolysis of polymeric substances like proteins or carbohydrates to monomers and the subsequent decomposition to soluble acids, alcohols, molecular hydrogen (H_2) and carbon dioxide (CO_2). Depending on the prevailing environmental conditions, the final steps of ultimate anaerobic biodegradation are performed by denitrifying, sulfate-reducing or methanogenic bacteria (Figure 1).

In contrast to the strictly anaerobic sulphate-reducing and methanogenic bacteria, the nitrate-reducing micro-organisms as well as many other decomposing bacteria are mostly facultatively anaerobic (i.e. they are able to grow and to degrade organic substances under aerobic as well as anaerobic conditions). Thus, strictly aerobic and anaerobic environments represent the two extremes of a continuous spectrum of environmental habitats which are populated by a broad variety of micro-organisms with specific biodegradative abilities. Anaerobic conditions occur where vigorous decomposition of organic matter and restricted aeration result in the depletion of oxygen.

Figure 1 : Anaerobic degradation of organic material by bacterial consortia



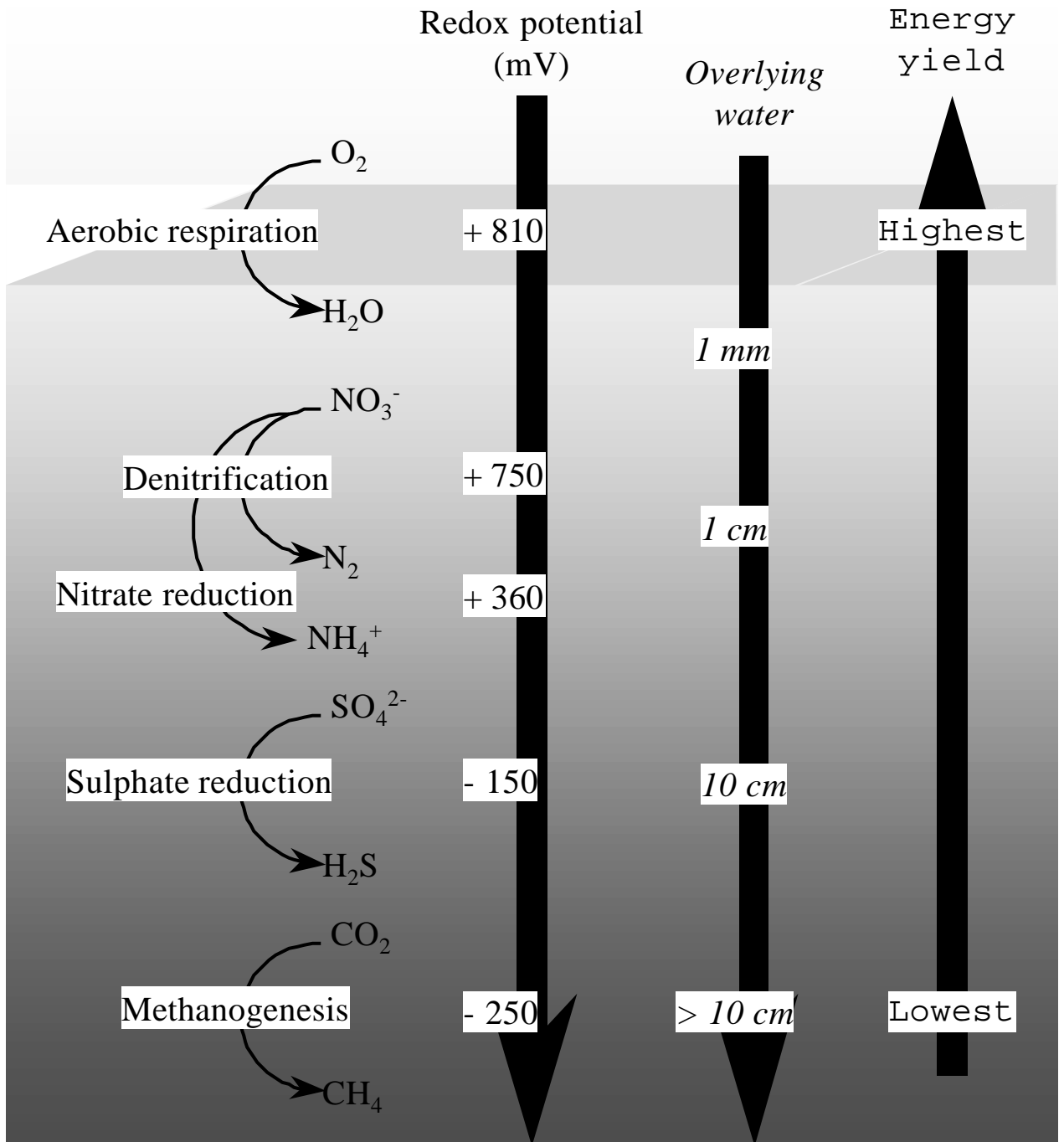
5. ANAEROBIC COMPARTMENTS

5.1. OVERVIEW

The biosphere is predominantly aerobic with an estimated 10^{21} g O₂ being present in the atmosphere and oceans (Press and Siever, 1986). However, anaerobic environments can develop where the consumption of O₂ by the biological oxidation of organic matter exceeds supply. These may be small anaerobic zones in an otherwise oxic system (e.g. Jørgensen, 1977a), or much larger and stable environments such as those found in marine and freshwater sediments. As was mentioned in Chapter 4, in the absence of free O₂ the oxidation of organic matter by micro-organisms continues through the use of a sequence of alternative electron acceptors (e.g. nitrate → sulphate → carbon dioxide). These reactions are dependent on the availability of organic and inorganic substrates, the redox potential of the environment and the types of bacteria present (Zehnder and Stumm, 1988). This is illustrated in Figure 2 and shows a progression down an idealised coastal sediment. It can be seen that the higher the redox potential of the environment, the more energetically favourable is the reaction. Hence, a facultatively anaerobic bacterium existing in an anaerobic zone with a low redox potential, will quickly become more energy efficient once there is an ingress of O₂. This sequence of bacteria preferring reactions which provide the most energy yield can be found in a wide range of aquatic and terrestrial environments.

Anaerobic processes play an important role in nutrient cycling and animal nutrition, and in man-made activities such as wastewater treatment. This chapter summarises the main anaerobic environmental compartments.

Figure 2 : Simplified diagram of the different reactions performed by bacteria depending on the redox potential of the environment. This example is for a coastal marine sediment and is not to scale.



5.2. TERRESTRIAL

Soils are usually aerobic systems (Kaspar and Tiedje, 1982), although anaerobic microsites can occur in poorly drained soils and a flooded soil such as a rice paddy may have an aerobic layer of only 1 cm depth (Richards, 1987). Another important terrestrial anaerobic environment is the peat in water-logged moorland, fens and bogs.

5.3. AQUATIC

5.3.1 Water

An anaerobic body of water can occur if there is high O₂ consumption in the sediment or overlying water, and seasonal or permanent hydrographic conditions such as thermoclines or haloclines prevent the exchange of water. Many freshwater lakes of >10 m depth can become stratified at the end of the summer, leading to the formation of an anaerobic layer above the sediment (Jones, 1982).

The largest anaerobic ecosystems in the biosphere are marine (Schlegel and Jannasch, 1981), with the Black Sea being anaerobic from a depth of ~200 m to the bottom at ~2,000 m. Other permanently anaerobic basins are the Cariaco Trench, off the coast of Venezuela (Richards, 1975) and the Orca Basin in the northern Gulf of Mexico (Shokes *et al.*, 1977). These reports and others have led Sieburth (1987) to conclude that anaerobic basins are fairly common. However, the decomposition of organic material in these regions appears to occur predominantly in the oxygenated water column, with reports of ~75% of organic matter being aerobically oxidised in stratified lakes (Rudd and Hamilton, 1979 ; Harrits and Hanson, 1980) and 85-95% in marine systems such as the Black Sea (Deuser, 1971).

5.3.2. Sediment

Depending on the level of eutrophication, water depth and season, freshwater sediments are usually anaerobic below the surface few mm or cm. In general, aerobic respiration is the predominant decomposition process in these sediments, although anaerobic processes such as dissimilatory nitrate reduction and methane evolution are also important (Jones, 1982).

The aerobic zone of marine sediments can vary from only a few mm in coastal areas to ≥ 1 m in deep sea sediments (Jørgensen, 1982). The depth of this zone is dependent on factors such as the rate of O₂ diffusion into the sediment porewater, bioturbation, microbial respiration rates (which in turn are dependent on organic carbon levels), sediment particle size and tidal flushing. Within the anaerobic layers, sulphate reduction is the predominant terminal step in anaerobic biodegradation and this reflects the abundance of sulphate in sea water (29 mM at a salinity of 35 ‰). In coastal sediments, the mineralization of organic material by sulphate reduction is significant and has been reported to match or even exceed that due to aerobic respiration in intertidal salt marshes and mudflats (Howarth and Teal, 1979; Mountfort *et*

al., 1980) and fjordic sediments (Jørgensen, 1977b). However, these shelf (i.e. shoreline to 200 m depth) sediments cover only around 9% of the world's oceans (Jørgensen, 1982) although they receive a disproportionately large amount of anthropogenic material.

5.3.3. 'Extreme' habitats

These are anaerobic environments which have extreme conditions of temperature

(>100 °C), salinity (saturated NaCl) or pH (<2, >10) and include geothermal vents, hot springs, salt lakes, peat bogs and alkaline hypersaline waters (see Lowe *et al.*, 1993 for a detailed review). The release of surfactants into these habitats is virtually non-existent and as such they are ignored in this document.

5.3.4. Groundwater

Groundwaters can become anaerobic, particularly when a shallow aquifer is contaminated with degradable organic material from a landfill leachate (Smolenski and Sufliya, 1987).

5.4. WASTE-WATER TREATMENT

Mesophilic (i.e. ~35 °C) anaerobic digestion of sewage sludge is widely used in wastewater treatment to reduce volume, stabilise the sludge and produce CH₄-rich biogas which can be burnt to generate energy. Sludge digestion is a strictly anaerobic process, with a redox potential of the order of -250 mV (Mosey, 1985). Digester capacities usually range from 1,000 to 10,000 m³ (Mosey, 1983) and the average residence time is 15 to 20 days. Anaerobic digestion has also been promoted as a means of meeting the fuel demands of developing countries, and large numbers of digesters have been installed in China and India (Compagnion and Nyns, 1986).

Surfactants tend to sorb onto sludge and as such are passed into anaerobic digesters (Field *et al.*, 1995). This is possibly the anaerobic environment with the highest exposure to surfactants (see also the monitoring summary table in the Appendix). In a number of European countries, the digested sludge is eventually disposed to land as a fertiliser, whereby the plant and soil life may be exposed to surfactants or their metabolites.

Other anaerobic environments associated with wastewater treatment are: denitrifying filters and activated sludge systems (Mosey, 1983), anaerobic ponds for high strength industrial waste-waters (Mara and Pearson, 1986), septic tanks, and anaerobic/anoxic zones in waste-water treatment plants with biological removal (see below).

An increasing number of modern waste-water treatment plants are fitted with anaerobic/low dissolved O₂ zones in which enhanced nutrient (nitrogen, phosphorous) removal takes place (currently an average of ~15 % over Europe). These zones are

usually situated prior to the aerobic zone and as such surfactants may be subject to anaerobic/low dissolved O₂ attack prior to their aerobic biodegradation. The residence time of the mixed liquor suspended solids in both the anaerobic and the low dissolved O₂ tank is limited to only a few hours (average of ~ 5). Anaerobic degradation steps can be brought about by the fraction of the biomass active in these zones, which are mainly facultative anaerobic micro-organisms. Fermentation reactions will take place, although the final conversion to methane is not significant due to the limited residence time in the anaerobic zones. Redox conditions vary considerably over time and place in the system. Due to the longer hydraulic and sludge residence times in such systems and the diversity of degradation (redox) conditions, it is expected that surfactant removal will generally be enhanced in comparison to classical activated sludge plants (Rottiers *et al.*, 1998).

5.5. LANDFILL

Decomposition of organic material in landfills, particularly those with compacted deposits, leads to the consumption of O₂ and anaerobic (methanogenic) conditions (Küster and Niese, 1986).

5.6. ANIMALS

Anaerobic environments exist in the alimentary tracts of many animals, in particular the rumen of cattle and the blind sacs (ceca) of termites, wood roaches, rabbits, rats and pigs (Latham, 1979). The fate of surfactants in these environments is beyond the scope of this paper.

6. EXISTING METHODS

As with aerobic biodegradation test systems, a hierarchy of screening, inherent and simulation tests have been proposed for anaerobic conditions. Screening tests are characterised primarily by a high test substance to biomass ratio, while inherent and simulation tests aim to reach realistic concentration ranges of the chemical and the bacterial biomass. In addition, screening and inherent tests usually have a relatively simple test design (e.g. batch tests) making them suitable for routine testing, whereas simulation tests necessitate the use of ^{14}C labelled materials or specific analytical methods.

6.1. SCREENING TESTS

The available anaerobic biodegradation screening tests are based on the determination of the final gaseous products of the anaerobic degradation process, i.e. CO_2 and CH_4 production. Therefore, the tests measure ultimate biodegradability under methanogenic conditions. However, the test conditions differ considerably from the situation in a real digester. The diluted sludge inoculum corresponds to about 10 % or less of the real digester sludge concentration. In addition, for analytical reasons, the test compound concentration is usually in the range of 20-100 mg of organic carbon/l, and is significantly higher than the concentrations usually found in digesters. Therefore, in some cases inhibitory effects are to be expected and also have been observed in these screening tests. This has to be taken into account when negative or poor results are obtained in the test. From these facts it is understandable that anaerobic screening tests are more stringent than test systems simulating realistic environmental conditions. As in aerobic screening tests, it can be concluded that a positive test result indicates good biodegradation under real environmental conditions whereas a negative result is not necessarily proof of recalcitrance.

6.1.1. Anaerobic screening tests based on gas volume measurement only

A number of anaerobic biodegradability screening test procedures are based on the method described by Shelton and Tiedje (1984), e.g. the test methods used in USA (ASTM, 1987), EPA (1988), the UK procedure (HMSO, 1989), Battersby and Wilson (1988) and the method described by Baumann and Schefer (1990).

Test principle

Primary anaerobic digester sludge (with 1 - 3 % w/v dry solids) is diluted to 10 % with a mineral salt medium yielding a sludge concentration in the test of 1 - 3 g of dry solids/l. The test vessels (serum bottles) used have a nominal volume of about 160 ml and contain 100 ml of the sludge mixture as well as the test chemical at a concentration of 50 mg of organic carbon/l. After

sealing, the bottles are incubated at constant temperature (35°C) in the dark for a test period up to 8 weeks. The gas production is measured periodically by determination of the pressure increase in the headspace of the bottles using a calibrated pressure meter. The net amount of gas produced from the degradation of the test substance (test gas production corrected for inoculum blank gas production) is expressed as a percentage of the theoretical gas production (ThGP) calculated from the chemical formula of the substance and taking into account the theoretical ratio of CO₂ and CH₄ formed in the digesting process (Buswell equation) and the empirical solubilities of the gases in the test medium.

Technical aspects

The method is applicable to water soluble and poorly soluble substances provided the concentration of the test material (50 mg of carbon/l) is not inhibitory to the anaerobic organisms. Knowledge of the chemical structure or, at least, of the empirical formula of the test compound is necessary so that the theoretical maximum amount of the gaseous final products (CO₂ and CH₄) can be predicted employing the Buswell equation. Since the test procedure only measures the headspace gas pressure and volume, respectively, it is necessary to make assumptions about the relative solubility of the gases in the test mixture.

The advantage of relatively simple technical requirements for conducting the test faces a few drawbacks:

- i) If the chemical formula of the test material is unknown, the Buswell equation cannot be applied and, thus, the amounts of the individual gases cannot be calculated.
- ii) The ratio of CO₂ and CH₄ evolved in the test may differ more or less considerably from the Buswell equation as was shown in experiments with radiolabelled test substances (ECETOC 1988). If the ratio cannot be predicted reliably, the amounts of the two gases in solution cannot be calculated.
- iii) The true amount of dissolved CO₂ may be variable since the solubility of this gas depends on a number of factors (e.g., temperature, pressure, pH, ratio of headspace/liquid volume, thermodynamic equilibrium established between CO₂ and carbonates/bicarbonates of Ca and Mg) not measured in the test. For that reason, the prescribed test conditions have to be rigidly adhered to (Shelton and Tiedje, 1984 ; Battersby and Wilson, 1988).

Nevertheless, in spite of these problems the method is widely applicable and considered to be a reasonably accurate screening procedure for the evaluation of the anaerobic degradation of test materials.

Test modifications

The method described by Baumann & Schefer (1990) differs somewhat from the previously described procedure by using an extended Buswell equation to

calculate directly the CH₄ production from the chemical oxygen demand of the substance or product tested. In addition, a lower inoculum concentration (0.5 g/l) is used and the problem of the solubility of CO₂ is overcome by adding NaOH to the digesting mixture when gas production has reached a plateau. The test flasks, fitted with stirrers, are larger than in the aforementioned method (250 ml nominal volume containing 200 ml of liquid) and the gas production is measured by means of a mercury manometer fitted to each flask.

6.1.2. Anaerobic screening tests based on gas production measurement in the gas and the liquid phase

The so-called ECETOC test was developed and published by the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC, 1988). The method has been ring-tested and is standardised as ISO 11734 method: “Water quality - Evaluation of the ultimate anaerobic biodegradability of organic compounds in digested sludge - Method by measurement of the biogas production (ISO 1994)”. The adoption of the method by OECD is in progress. The principle of the ECETOC test is shown in Figure 3.

Test principle

A known volume of washed anaerobic sludge (1 - 3 g/l total solids), suspended in an oxygen-free mineral medium, is placed in a suitable vessel (nominal volume 0.1 - 1 l) leaving a headspace (10 - 40 % of the volume of the vessel) into which any gases produced can evolve. Prior to sealing, a small amount of the test compound is added to give a concentration of 20 - 100 mg of organic carbon/l. Controls (without test compound) are prepared in the same manner. Usually the test is carried out using several (e.g. 5) replicates each of controls and test assays. The vessels are incubated at constant temperature ($35 \pm 2^\circ\text{C}$) normally for periods of up to 8 weeks. The headspace pressure resulting from the production of gas is measured with a pressure transducer either once a week or at the end of test. In addition, the DIC (dissolved inorganic carbon) content of the digester liquid is determined at the end of the test. From the cumulative net gas production (net gas = gas in the test vessels minus gas in controls) it is possible, by application of the gas laws, to calculate the amount of test compound-derived organic carbon transformed to gaseous one-carbon products (CH₄, CO₂). In general, the incubation is finished when the cumulative net gas production curve shows a plateau (i. e. when the gas production rate in controls and test vessels is virtually comparable. The net DIC formation is obtained as the difference of DIC concentrations between the test and control vessels. The extent of anaerobic ultimate degradation is calculated by comparison of the amount of carbon equivalent to net gas and DIC production with the initially added organic carbon content of the test chemical.

Technical aspects

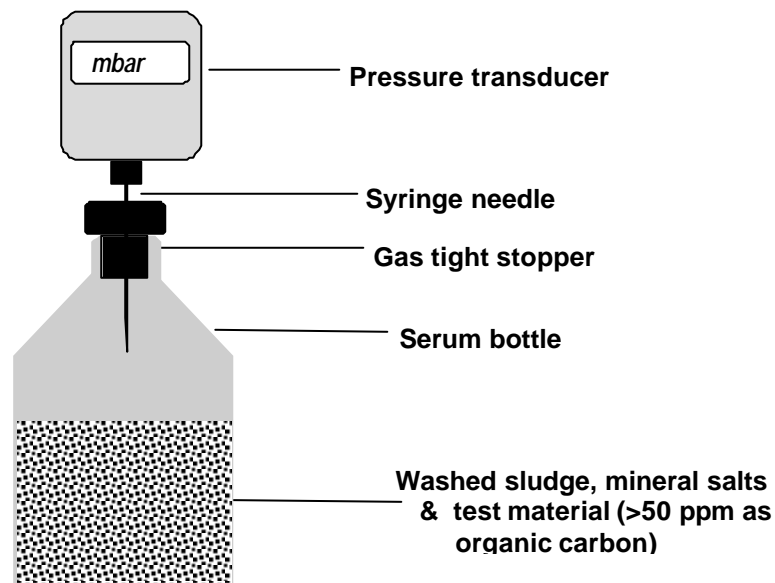
The method is applicable to water soluble and poorly soluble substances provided the test concentration (20 - 100 mg C/l) is not inhibitory to the test inoculum. As with the previous test, inhibition can be easily recognised by the observation of a negative net gas production, i.e. a gas production which is higher in the control vessels than in the vessels containing the test substance.

The organic carbon content of the test substance must be known; additional substance information (chemical composition, theoretical CO_2/CH_4 -ratio according to the Buswell equation) is not necessary. This is a considerable advantage over other anaerobic screening methods (cf. 6.1.1) since neither correction factors for CO_2 solubility nor assumptions of the theoretical ratios of CO_2 and CH_4 formation are necessary. Thus, the ECETOC method is recommended when more accurate values are needed for chemicals having a known empirical formula, or if a value is required for a test material of unknown composition but its percentage carbon is known. (Painter 1994).

Figure 3 : ECETOC Test

PRINCIPLE:

TEST MATERIAL \longrightarrow $\text{CO}_2 + \text{CH}_4$ \longrightarrow INCREASED PRESSURE + INORGANIC CARBON IN THE LIQUID PHASE



The ECETOC test has been adopted by many laboratories in Europe for several years so that its applicability and practical usefulness can be considered as broadly approved. Information about the reproducibility of the test can be obtained from the degradation data reporting on, respectively, the results of independent tests of the same substance and the mean degradation value including its standard deviation when multiple parallel assays are made (Pagga and Beimborn, 1993). According to these data it can be concluded that the ECETOC test provides reproducible results showing a variation which is typical of biological test systems.

The ECETOC test is, like other biodegradation screening tests, a relatively simple test system not requiring highly specialised technical staff, but nevertheless it does require sound expertise. Thus, it is strongly recommended that inexperienced staff is trained in the use of the test by testing well investigated model substances and comparing the obtained results with published data.

Test modifications

A modified ECETOC test method was used by Madsen *et.al.* (1995) to determine anaerobic biodegradation potential in digested sludge, a freshwater swamp and a marine sediment. In the mineral medium FeCl_2 was replaced by a trace elements mixture. The test medium was inoculated with 10-20% fresh or washed domestic digester sludge (1.5 or 0.15 g of dry solids/l), with 5 % of the freshwater swamp (24 - 88 g of organic carbon/kg), or with 10 % of the marine sediment (9 g of organic carbon/kg), respectively. The gas production in the headspace was measured by a pressure transducer while CH_4 was determined at the termination of the incubation period by gas chromatography. Dissolved inorganic carbon was quantified at the end of the test by acidification of the liquid and subsequent gas pressure measurement.

The authors suggest that the digester sludge inoculum concentration be reduced to 0.15 g of suspended solids/l in order to eliminate the sludge washing step usually required in order to reduce the amount of inorganic carbon when a higher concentrated inoculum is used. This however increases the test concentration to biomass ratio unless the test concentration is lowered accordingly.

6.1.3. Predictive value of anaerobic biodegradation screening test data

As pointed out previously (6.1), it is a common feature of biodegradation screening tests that they are more stringent than the tests simulating the real world. Therefore, a poor degradation result is not necessarily a proof of recalcitrance in the real environment. Examples of anaerobic biodegradation results supporting this argumentation have been published (Birch *et al.* 1989, Steber 1991, Steber and Birch 1995 a.). On the other hand, a positive result

in an anaerobic screening test can be considered as highly predictive for extensive biodegradation in anaerobic environments.

Although the production of CH₄ and CO₂ is the evaluation criterion in the anaerobic screening tests, positive results in such tests may have relevance beyond methanogenic systems. The major part of the anaerobic biodegradation route (hydrolysis, fermentation, acetogenesis) is common to all anaerobic pathways (cf. Fig. 1). These only differ basically in terms of the final stage of the process, i.e. by the different utilisation of the final electron acceptors like carbon dioxide for methane production, sulphate for sulphide formation and nitrate for the production of ammonia and molecular nitrogen, respectively. Thus, it can be concluded that a chemical being degraded in the discussed anaerobic screening tests will also undergo biodegradation in those environmental conditions where the final degradation is brought about by denitrifying or sulphate-reducing bacteria (Steber *et al.* 1995 a.).

An additional aspect of the environmental relevance of anaerobic screening test results also has to be kept in view. The discussed tests determine the ultimate biodegradation of a chemical by measurement of the production of the final gaseous products, i.e. methane and carbon dioxide. Therefore, it has to be acknowledged that the bacterial transformation of the parent chemical (primary biodegradation) by anaerobic bacteria is not reflected by these data. Thus, even a poor degradation result in such a screening test does not necessarily indicate for anaerobic persistence of the parent compound. In other words, if the ultimate degradation of a substance under these test conditions is significant (≥ 20 % gas production) it can be concluded that the extent of primary biodegradation of this chemical is virtually 100 % since already 20 % of the carbon atoms of every molecule have been mineralised. Obviously, this assumption is only valid for pure compounds.

6.2 **SIMULATION TESTS**

6.2.1. **Introduction to anaerobic simulation tests**

The motivation to move beyond the screening level to higher tier testing tiers can be:

- the need for increased environmental realism - including obtaining realistic kinetic information for different anaerobic environments, reactor types or test conditions,
- to avoid inhibition of the anaerobic microorganisms by the test material by working at a lower and more realistic test material to biomass ratio,
- to increase the chances of acclimation by exposing a broader range or organisms
- to improve the signal/noise ratio of the test,
- to study the CO₂ to CH₄ ratio.

The following test systems will be discussed:

- 1) ^{14}C -Anaerobic Digester Simulation Test
- 2) Continuous Fixed-Bed Simulation Test
- 3) Biological Nutrient Removal in Waste Water Treatment Plants (WWTP) Simulation Tests
- 4) Others

None of these higher tier anaerobic or mixed aerobic/anaerobic test systems has as yet been accepted for international standardisation (e.g. OECD, ISO, CEN, MITI). It can be expected, however, that this will occur in the next decade.

6.2.2 Test systems

6.2.2.1 ^{14}C -Anaerobic Digester Simulation Test (see figure 4)

This test system (Steber & Wierich, 1987; Gledhill, 1995; Nuck & Federle, 1996), also known as Anaerobic Mineralization Test, assesses the mineralization of ^{14}C -radiolabelled test chemicals to CO_2 and CH_4 under anaerobic (methanogenic) conditions. The inoculum is usually obtained from an active digester but in principle the system can also be used to simulate other anaerobic compartments, such as septic tanks or sediments. The system follows the formation of CO_2 and CH_4 separately over time. The underlying principle of this method is that the headspace of the anaerobic vessel is continuously purged with N_2 , which is passed through a series of base traps, to capture first the $^{14}\text{CO}_2$ evolved. The effluent gas is mixed subsequently with oxygen and passed through a combustion tube (CuO at $800\text{ }^\circ\text{C}$) to convert the $^{14}\text{CH}_4$ into $^{14}\text{CO}_2$. The latter is trapped in a second series of base traps (see Figure 4). The test can be run in a batch, or fed-batch mode (with addition of fresh sludge/test material at regular intervals). The technical details of this system are provided in Steber and Wierich (1987) and Nuck and Federle (1996).

The anaerobic digester system, if well constructed and operated, provides excellent recovery of the radioactive gases, and therefore good mass balances. Given the simulation of *in-situ* conditions and the realistic ratio of test substance : biomass, the system can generate relevant kinetic data (see Figure 5).

Figure 4 : The anaerobic digester simulation test

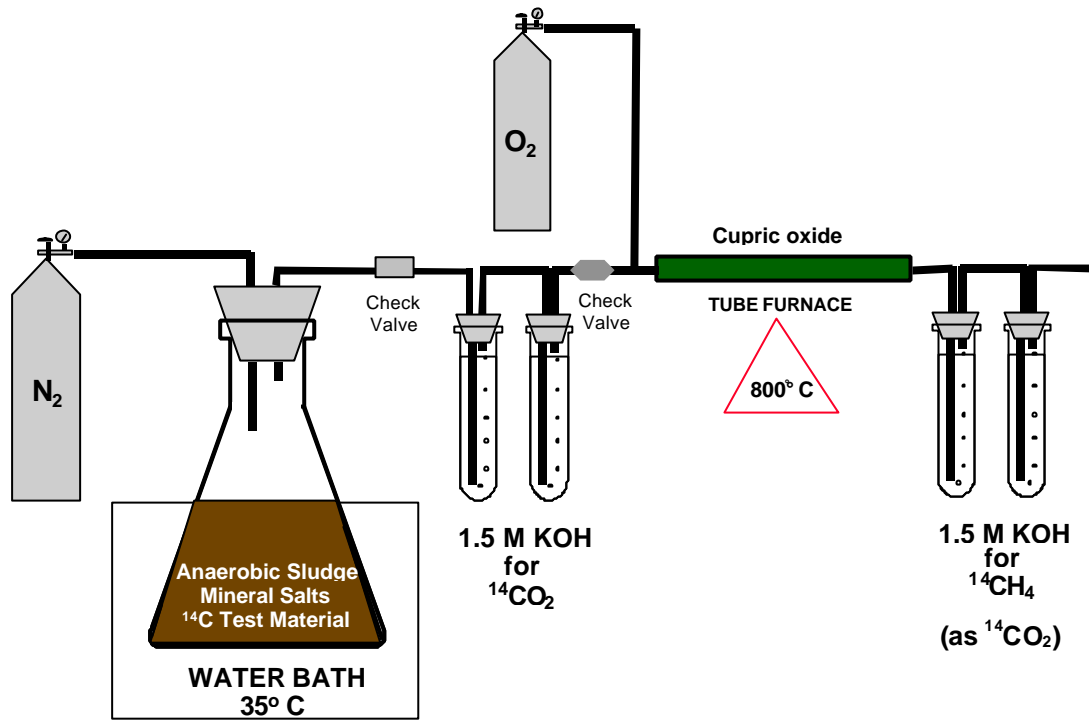
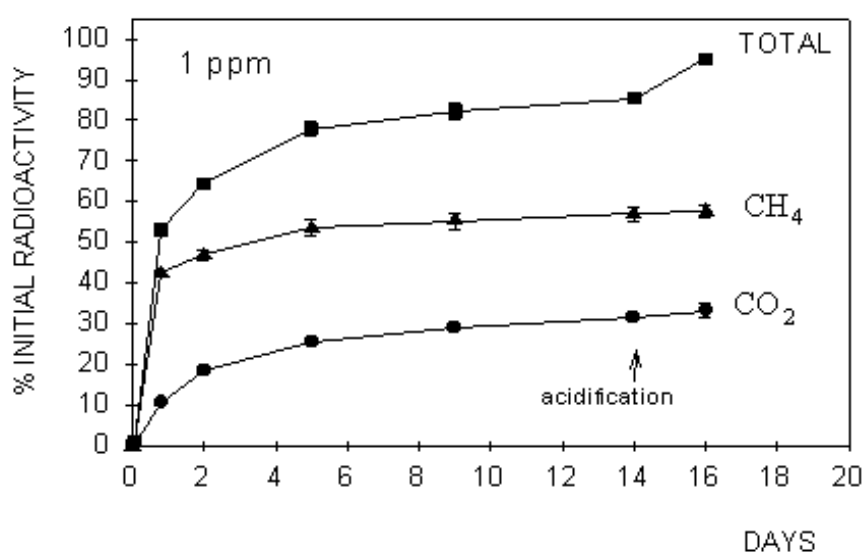


Figure 5 : Example of the anaerobic mineralization of A24E3S (ethoxylate labelled) to CO₂ and CH₄. Results are presented as % of the initial counts added.

**Alkyl ethoxy sulfate (AES) - % mineralized in anaerobic sludge
(Nuck & Federle, 1996)**

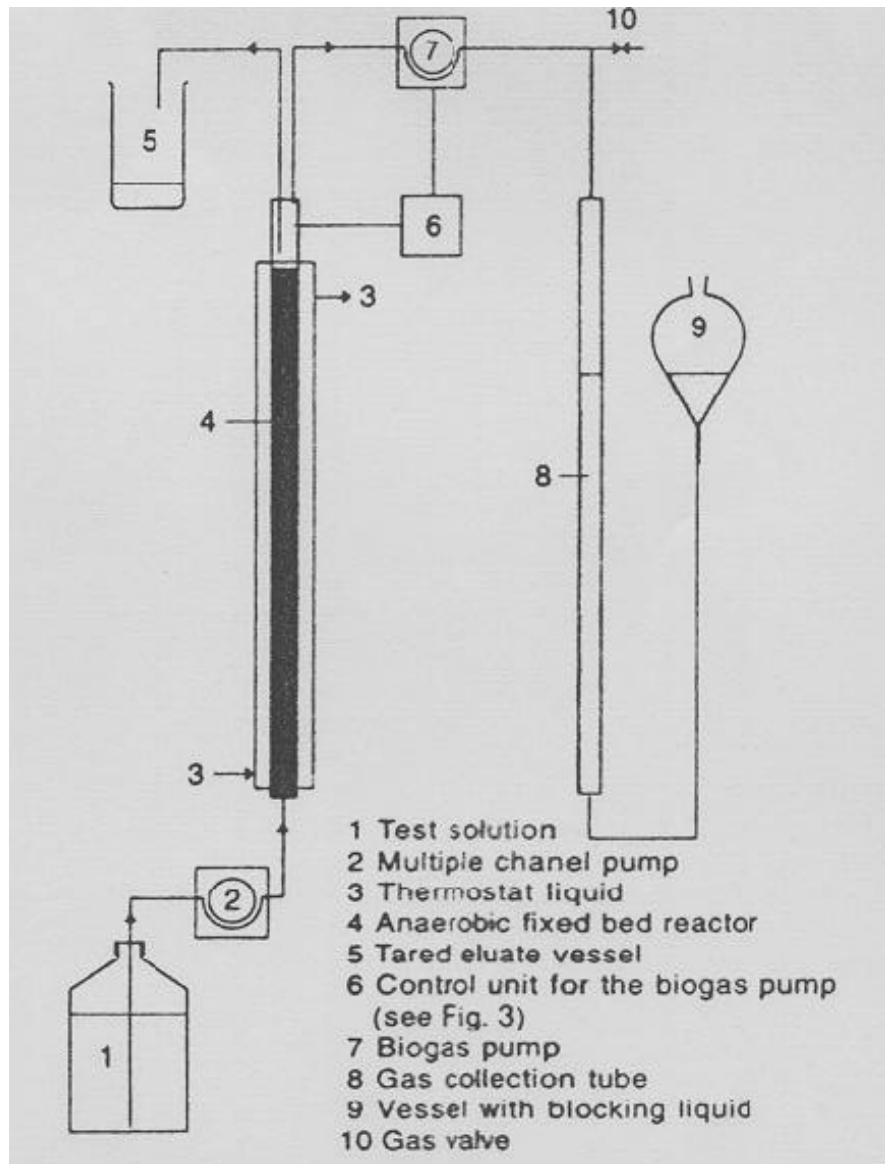


6.2.2.2 Continuous Fixed Bed Simulation Test

Continuous fixed bed systems have been proposed by e.g. Bouwer and McCarthy (1983), Wagener and Schink (1987) and Baumann and Mueller (1997) to study the anaerobic degradation of soluble chemicals or waste waters (Fig. 6). The reactors are filled with sinter-glass rings or any other suitable support material. Inoculation is with digester sludge and the system is maintained on a synthetic medium. In the Wagener and Schink approach, the total biogas volume is monitored, while in the EMPA system (Baumann and Mueller, 1997) the biogas is collected in a glass tube containing sodium hydroxide to absorb the CO₂ fraction. The anaerobic degradability of a test substance can be calculated from the maximum theoretical volume of biogas/CH₄ and the amount of biogas/CH₄ actually produced.

The continuous anaerobic fixed bed system can be used to perform simple mass balance studies, or according to the authors, to gain information on hydrophilic and hydrophobic metabolites (provided adequate analytical methods are available). A typical test concentration for a non-labelled chemical is in the order of 250 - 500 mg/l as COD.

Figure 6: EMPA continuous anaerobic fixed bed reactor system (Baumann & Mueller, 1997).



6.2.2.3. Biological Nutrient Removal WWTP Simulation test: Behr and CAS-UCT units

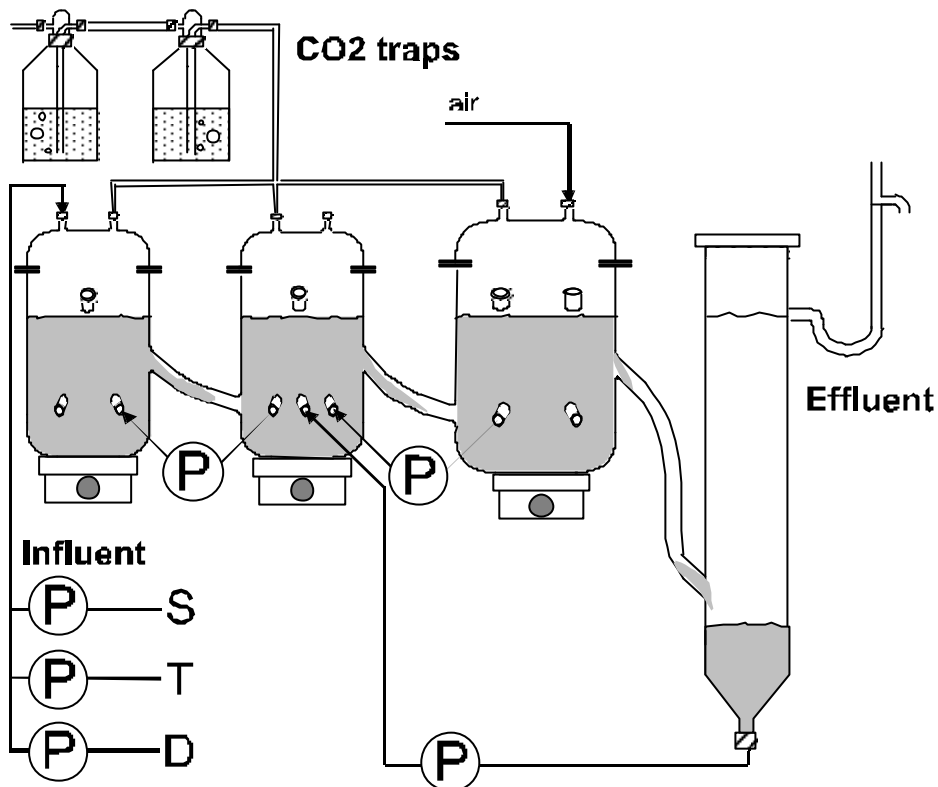
This is not a true anaerobic biodegradation test but it is becoming a common laboratory procedure simulating the state of the art design of WWTP and therefore is included in this chapter for information purposes.

These test systems simulate the behaviour of chemicals in laboratory-scale sewage treatment plants with anoxic (Behr unit, Fig. 7 b), and anaerobic plus low dissolved O₂ zones (CAS-UCT system, Fig 7 a). Behr is a trade name (Behrotest™ KDL4), while UCT stands for University of Cape Town, where this system for biological N/P removal was developed. The systems are recent adaptations of the CAS (OECD 303) test system, and make it possible to perform basically the same type of experiments as with the standard CAS-unit. In addition it allows to follow the removal and metabolism of chemicals in the low dissolved O₂ and/or anaerobic zones.

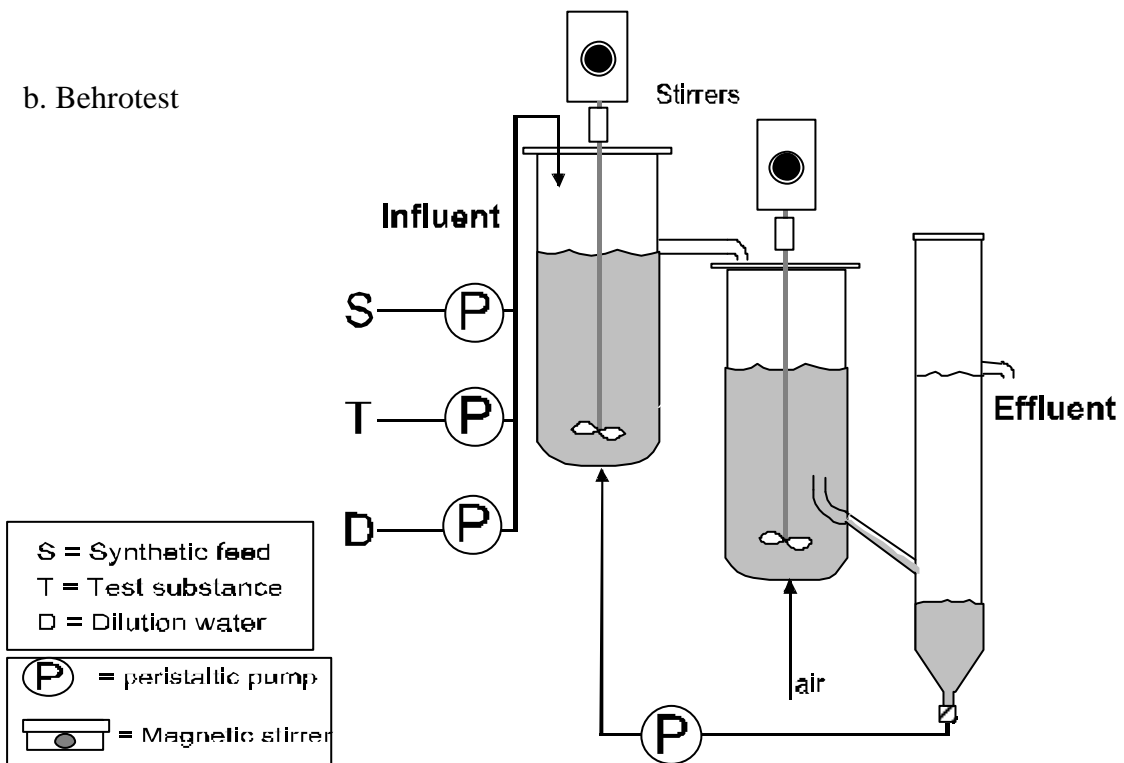
The Behr and CAS-UCT process designs were selected because they are typical configurations for single-sludge wastewater treatment plants with N/P removal. They can also be constructed in such a way that ¹⁴C-mass balance studies are possible. In addition, the availability of active populations of (facultative) anaerobic micro-organisms in the same system (i.e. fermentative, denitrifying and phosphorus accumulating bacteria) makes it possible to perform specific metabolic or inhibition tests with the system's biomass (Schowanek *et al.*, 1996).

Figure 7 : BEHR AND CAS-UCT UNITS

a. CAS - UCT



b. Behrotest



6.2.2.4 Others

In the area of simulation tests, various other anaerobic reactor designs (e.g. septic tank, anaerobic contact process, anaerobic filter, UASB, aquifer column, anaerobic fluidized bed) can also be operated on a laboratory-scale to study the behaviour of chemicals in these specific systems (e.g. Kuhn *et al.*, 1988; Heijnen *et al.*, 1991).

Since these would be considered as non-routine tests, they will not be covered further.

7. INTERPRETATION OF AVAILABLE DATA ON ANAEROBIC BIODEGRADATION OF SURFACTANTS

This chapter gives a compilation of existing literature data on anaerobic biodegradation of all surfactant classes.

Data on laboratory tests are shown in individual tables together with an interpretation of the results.

Monitoring data in the literature do not necessarily reflect the biodegradation behaviour of surfactants under strictly anaerobic conditions. For example, temporary aerobic conditions in an otherwise anaerobic environment may influence the results towards a high degree of degradation. Therefore the data may not be used as a proof of biodegradability/recalcitrance in anaerobic environments, but may form an additional support of the conclusions drawn from the non controlled laboratory test results.

7.1. ANIONICS

7.1.1. Sulfonates

Surfactant type	Characterisation	Test type		Test conc.		Inoculum (dw) conc. in g/l	Test Duration Days	Temp. ° C	Results	Remarks	References
		Screening	Simulation	in mgl active matter	mg/l carbon						
LAS	C10÷C13 commercial	Ecetoc		50		1-5	49	35	0		Steber (1991)
LAS	ring-14C		digester	10		20	27	37	0.3	14C LAS	Steber (1991)
LAS	ring-14C		field system	0.5		WW pond sed.	87	22	0	14C LAS	Federle & Schwab (1992)
LAS	C12 LAS	Ecetoc			50		60			inhibition biogas formation	Battersby & Wilson (1989)
LAS	C8 LAS	Ecetoc			50		60			inhibition biogas formation	Battersby & Wilson (1989)
LAS	C10÷C13 commercial		field system			septic tank			97 (a)	10% 14C LAS	Klein and Mehaughey (1964)
LAS	C10÷C13 commercial		field system	10					99.7 (a)		Larson <i>et al.</i> (1989)
LAS	C12 LAS		research study	5		sediment slurry	6		100 (k < 0.23 d-1) (b)	14C LAS	Heinze & Britton (1994)
LAS	C10÷C13 commercial		research study	5-20		0.02-0.1	29 h	25	100 (k = 0.14 h-1) (c)	specific LAS and S	Denger & Cook (1998)
MES	2-sulpho (14C) palmitic Me ester		digester	10		20	28	35	0.6	14C MES	Steber & Weirich (1989)
SAS	C14÷C17	Ecetoc					17		0		Bruce <i>et al.</i> (1966)

a) Oxygen – limited conditions

b) Mineralization kinetic study in different oxygen-limited conditions

c) Desulfonation kinetic study using an enriched and isolated strain. Primary biodegradation

Conclusions on the anaerobic biodegradability of sulfonates

1. Laboratory data

Sulphonates are not degraded significantly under the anaerobic conditions of the laboratory test methods (Steber, 1991 and Federle, 1992).

In the real environment and also in field system tests, however, oxygen-limited conditions may be more common than rigorously anaerobic conditions. In such conditions sulphonates mineralize even if the rate is not as rapid as that observed under aerobic conditions. Once sulphonate biodegradation has been initiated in aerobic or oxygen-limited conditions, the intermediates can continue to biodegrade anaerobically. This is the reason why in some simulation tests these chemicals can show mineralization results, if some oxygen diffusion had occurred or if limited-oxygen conditions had been created (Larson, 1989; Heinze and Britton, 1994).

Sulphonates can also inhibit biogas formation in laboratory tests if present at relatively high concentrations (Battersby and Wilson, 1989). This inhibition starts at sulphonate concentrations with respect to the total solid matter of about 15 g/kg dw in the laboratory tests, where the added products are used as Na salts. In actual anaerobic environmental compartments (e.g. STP anaerobic digester), however, sulphonates have shown to not inhibit biogas formation even at high concentration (> 30 g/kg dw) because they are present as Ca salts which are poorly soluble and less bioavailable (see 8.2).

It has been recently demonstrated that not all desulphonation reactions require oxygen. Desulphonation of alkylsulphonates as well as of LAS surfactant has been reported to occur by anaerobic bacteria in the laboratory (Denger and Cook, 1998). There is recent evidence that anaerobic desulphonation can take place. Desulphonation with assimilation of the sulphur moiety by strictly anaerobic bacteria (Chien *et al.*, 1995; Denger *et al.*, 1996; Denger and Cook, 1997) was followed by the reduction of the sulphonate as a source of electrons and carbon under anaerobic nitrate-respiring conditions (Lie *et al.*, 1996; Laue *et al.*, 1997; Denger *et al.*, 1997). There is no evidence however that such mechanisms would also occur at significant rates under real-world conditions.

2. Monitoring data (see Appendix)

LAS data for sludges have been obtained in several countries and range from < 500 mg/kg to a maximum value of 30000 mg/kg (Berna, 1989) depending on STP operating conditions and water hardness. Aerobic stabilised sludge always has a LAS content lower than 500 mg/kg, whereas anaerobic stabilised sludge has a LAS concentration typically in the range 5000 - 10000 mg/kg (Waters, 1995 and McAvoy *et al.*, 1993). Results are overall quite consistent between different studies, and between the EU and the US. A few studies have attempted to make a LAS mass balance over full scale anaerobic digesters. A low degree of removal is measured, ranging from 0 - 35% (Berna, 1989 and Giger, 1987). These data fit broadly with the results from laboratory screening or digester tests, and biochemical insight, which

indicate that LAS, as well as any other sulphonate surfactants, does not degrade under strictly anaerobic conditions. It is not entirely clear to which process(es) the small degree of removal observed in field systems can be ascribed (binding, humification, co-metabolism, anaerobic desulphonation). While LAS concentrations on a wet sludge basis actually increase in an anaerobic digester due to the dewatering and digestion of solids, there is no evidence that LAS causes any inhibition of the essential processes (biodegradation, biogas formation, dewatering, digestion) ongoing in the reactor.

LAS has been found in river sediments. The levels range from zero to a maximum value of 174 mg/kg, believed to be the most reliable one and found just below a STP outfall (Rapaport, 1990). Downstream the same STP outfall the LAS concentrations in sediments drop to 5-11 mg/kg (Rapaport, 1990). No mass balancing studies are known to us. Several interesting studies have looked at the levels of LAS and other surfactants in cores or river/lake sediments as accumulated over time. Residual LAS levels can be measured, and in the trends over time (i.e. depth) clear parallels can be seen with changes from branched alkyl sulphonates to LAS (Schöberl, 1996 ; Reiser, 1995).

We are aware of only another study on anaerobic degradation of sulfonates in full scale systems, not related to LAS but instead to SAS (Field, 1992). The SAS monitoring in anaerobic sludge ranges from 270 to 800 mg/kg. Like LAS, there is no evidence for a significant anaerobic degradation of SAS.

7.1.2. Sulphates

Surfactant type	Characterisation	Test type		Test conc.		Inoculum conc.	Test duration	Temp.	Results	Remarks	References
		Screening	Simulation	in mg/l active matter	carbon	in g/l	in days	oC	%		
Alcohol Sulphates	C12		Digester	10	5	20	28	35	90	14C-labelled (31% CO ₂ + 59% CH ₄)	Steber <i>et al.</i> (1988)
	C12-14	Mod. Shelton & Tiedje		96	50	1	40-50	35	77-84	CH ₄	Salanitro & Diaz (1995)
									98-99	MBAS	
	C14		Digester	1	0.5	26	15	35	80	14C-labelled (CO ₂ + CH ₄)	Nuck & Federle (1996)
	C14-15	Mod. Shelton & Tiedje		93	50	1	40-50	35	65-78	CH ₄	Salanitro & Diaz (1995)
									97	MBAS	
	C18	ECETOC		50	29	3	56	35	88	Biogas + IC	Ecetoc (1988)
	C18		Digester	10	6	20	28	35	94	14C-labelled (43% CO ₂ + 51% CH ₄)	Steber <i>et al.</i> (1988)
Laurylether Sulphates	C12	Mod. ECETOC		40-100	20-50	0.06-0.12	55-56	35	14-41	Biogas (includes IC after acidification)	Madsen & Rasmussen (1994)
Alcohol Ethoxy Sulphate	C12-14, 2 EO	ECETOC			50	1-5	41	35	75	Biogas + IC	Steber (1991)
	C14-15, 2 EO		Digester	1-10	0.5-5	26	17	35	88	14C-labelled (CO ₂ + CH ₄)	Nuck & Federle (1996)
	C14-18, 3 EO		Septic tank	26-52	14-29	No data	240	No data	72-81	MBAS	Painter (1992)

Conclusions on the anaerobic biodegradability of sulphates

Alcohol Sulphates

Primary alcohol sulphates are anaerobically biodegradable, with a conversion to CH₄ and CO₂ (biogas) of 80-94% in tests simulating anaerobic digesters. They are also extensively mineralised (65-88%) in screening tests, although as with other anionic surfactants, the unnaturally high surfactant to biomass ratio means that low biodegradation or inhibition of biogas production are sometimes observed.

Alcohol Ethoxysulphates

Fewer data are available on the anaerobic biodegradability of ethoxysulphates but the available information indicates that they too are extensively biodegraded (>75%) during anaerobic digestion. Low biodegradation has been reported for laurylether sulphate but this can be attributed to the very high test substance to biomass ratio used in the study quoted.

7.1.3. Fatty Acids and Soaps

Surfactant type	Characterisation	Test type		Test concentr.		Inoculum concentration in g/l	Test duration Days	Temp. °C	Results %	Remarks	References
		Screening	Simulation	in mg/l active matter	carbon						
Fatty acid	dodecyl (C 12)	ECETOC		20		0.15 sludge	56	35	> 75	gas after acidific.	Madsen <i>et al.</i> (1995)
		ECETOC		20		4.4 organic C/L	56	35	> 75	gas after acidific.	Madsen <i>et al.</i> (1995)
						(freshwater swamp sediment)					
		ECETOC		20		0.9 organic C/L	96	35	> 75	gas after acidific.	Madsen <i>et al.</i> (1995)
						(marine sediment)					
	coconut (C12-18)	ECETOC		18		0.06/0.12 sludge	55	35	40 - 57	gas after acidific.	Madsen & Rasmussen (1994)
	(u-14C) palmitic (C 16)		static	10		20 sludge	28	35	96.5	56.6% CH ₄ +39.9 % CO ₂	Steber & Weinrich (1987)
Soap	Na-laurate (C 12)		semicont.	200		30 sludge	20 (retention time)	35	95	CH ₄ measured, CO ₂ calc.	Mix-Spagl (1990)
	Ca-laurate (C 12)		static	1000		30 sludge	5 - 6	35	90	CH ₄ measured, CO ₂ calc.	Petzi (1989)
	Na-palmitate (C 16)	ECETOC		70		1 - 5 sludge	28	35	94	gas + DIC	Birch <i>et al.</i> (1989)
	Na- palm kernel (C 8-18)		semicont.	200		30 sludge	20 (retention time)	35	67	CH ₄ measured, CO ₂ calc.	Petzi (1989)
	Na-tallow (C 16/18)		semicont.	200		30 sludge	20 (retention time)	35	60	CH ₄ measured, CO ₂ calc.	Petzi (1989)
	Na-stearate (C 18)		semicont.	200		30 sludge	20 (retention time)	35	51	CH ₄ measured, CO ₂ calc.	Mix-Spagl (1990)
	Ca-stearate (C 18)		static	1000		30 sludge	10	35	85	CH ₄ measured, CO ₂ calc.	Mix-Spagl (1990)
	Na-oleate (C 18 unsat.)		semicont.	200		30 sludge	20 (retention time)	35	69	CH ₄ measured, CO ₂ calc.	Mix-Spagl (1990)
	Na-behenate (C 22)		semicont.	200		30 sludge	20 (retention time)	35	14	CH ₄ measured, CO ₂ calc.	Mix-Spagl (1990)
	Ca-behenate (C 22)		static	1000		30 sludge	10	35	90	CH ₄ measured, CO ₂ calc.	Mix-Spagl (1990)

Conclusions on the Anaerobic Biodegradability of Fatty Acids and Soaps

Fatty acids and their sodium/calcium salts (soaps) are well biodegradable under anaerobic conditions. This was shown in several laboratory studies using different test methods (see Table). The data for dodecanoate and palmitate obtained in the stringent ECETOC screening test showed very high mineralization rates ($> 75\%$ $\text{CO}_2 + \text{CH}_4$ formation) within the test period of 4-8 weeks. The bacterial inocula used in these investigations were digester sludges as well as anaerobic sediments from fresh water and marine environments. The data for C_{12-18} fatty acids reported by Madsen and Rasmussen (1994) are lower (40-57%) but this may indicate slower biodegradation kinetics due to the unusually high test substance/inoculum ratio used in the test. The positive evaluation of the anaerobic biodegradability of fatty acids and soaps was confirmed in a digester simulation study using radiolabelled palmitate and showing an almost quantitative ultimate degradation to carbon dioxide and methane (Steber and Wierich 1987). Additional static and semicontinuous digester simulation tests proved the extensive ultimate biodegradation of Na- and Ca-salts of fatty acids with an alkyl chain length of 8-22 carbons (Petzi 1989, Mix-Spagl 1990). While the Ca-soaps (C_{12} , C_{18} , C_{22}) exhibited high gas formation rates ($\geq 85\%$) in the static system within a 10-day test duration, the semicontinuously run investigations with Na-soaps showed that the time needed for mineralization was increasing with the chain length and concomitantly with the water solubility. The relatively poor water solubility of Ca/Mg-soaps may also account for the high soap concentrations found in the raw sludges of sewage treatment plants (up to 5% of sludge dry matter); nevertheless, the mass balances of soap based on a monitoring study of the concentrations in sludges of a digester influent and effluent, respectively, showed unequivocally that the removal of soap was about 70% (Moreno *et al.* 1993).

7.2. NONIONICS

Surfactant type	Characterisation	Test type		Test Conc.		Inoculum Conc.	Test duration days	Temp °C	Results	Remarks	References
		Screening	Simulation	in mg/l active matter	carbon						
Alcohol Ethoxylates	C9-11, 8EO	ECETOC			20-50	0,15 g dry matter/l	56	35	> 75%	inoculum:digested sewage sludge, freshwater swamp	Madsen <i>et al.</i> (1995)
	C9-11, 8EO	ECETOC			20-50	0,15 g dry matter/l	56	35	30-75	inoculum:marine sediment	Madsen <i>et al.</i> (1995)
	C9-11, 8EO	Mod.ISO 11734 (1995)			20-50	0,6 g dry matter /l	55	35	65-82% biogas	Inoculum: digester sludge	Madsen & Rasmussen (1994)
	C10-12, 7,5 EO	screening		10-1000			37		70 % biogas	Inoculum, anoxic freshwater sediment	Wagener & Schink (1987)
	C12, 23 EO	screening		10-1000			37		80 % biogas	Inoculum, anoxic freshwater sediment	Wagener & Schink (1987)
	C12, 23 EO		simulation	1000			90		>90 % CH ₄ , acetate, propionate	Laboratory-scale anaerobic fixed-bed reactor fed with synthetic wastewater	Wagener & Schink (1987)
	C12, 9 EO	14C-screen		1,7 ug/g sediment			87	22	13 - 40 % 14C; glucose: 40%	WW pond sediment	Federle & Schwab (1992)
	Isotridecanol, (5,10,20) EO	ECETOC, modified			20	2-3 g/l as solids	110	35	0-30 % ThGP		Siegfried <i>et al.</i> (1996)
	linear primary C12-C15 7EO	ECETOC, modified			20	0,15 g dry matter/l			35 % ThGP	inhibition during the first 3 weeks	Madsen <i>et al.</i> (1996)
	Linear C12-14,(5,10,20)EO	ECETOC, modified			20	2-3 g/l as solids	110	35	29 - 94 % ThGP		Siegfried <i>et al.</i> (1996)
mono br. C14-15,(10,20)EO	ECETOC, modified			20	2-3 g/l as solids	89	35	0-23 % ThGP		Siegfried <i>et al.</i> (1996)	
C18, 7EO		14C-sim.	10		12-25g dry matter/l	28	35	83-87% 14C as biogas		Steber & Weirich (1987)	
Alkylphenol Ethoxylates	C10-12 alkylphenol, 9 EO	screening		10-1000			37		45-50 % CH ₄	Inoculum: anoxic freshwater sediment	Wagener & Schink (1987)
	Nonylphenol, 10 EO	ECETOC		50		1-5 mg dry matter/l	84	35	20,5 +- 12,6 % CO ₂ +CH ₄		Steber (1991)
	Nonylphenol, 9 EO	ECETOC		50		1 g dry matter/l	40-50	35	32-43 % CH ₄		Salanito & Diaz (1995)

	Nonylphenol, 0/1/2 EO		simulation							NP is metabolite of APEO, accumulates	Tschui & Brunner (1985)
Surfactant type	Characterisation	Test type		Test Conc.		Inoculum Conc.	Test duration days	Temp °C	Results	Remarks	References
		Screening	Simulation	in mg/l active matter	carbon						
Glucose Derivatives											
Glucoside	Ethyl 6-O-decanoyl glucoside	Mod. ISO 11734 (1995)			20		56	35	59-65% biogas	Inoculum: digester sludge	Madsen & Rasmussen (1994)
	APG (branched) C8, DP=1.6	ECETOC, modified		30-40	20	0,15 g dry matter/l			22 % ThGP	primary domestic sludge	Madsen <i>et al.</i> (1996)
	APG (linear) C12-14, DP=1.4	ECETOC, modified		30-40	20	0,15 g dry matter/l			72 % ThGP	primary domestic sludge	Madsen <i>et al.</i> (1996)
	C12 Ethylglucoside monoester EGE	ECETOC, modified		30-40	20	0,15 g dry matter/l			82 % ThGP	primary domestic sludge	Madsen <i>et al.</i> (1996)
	C12 6-O-Ethylglucoside monoester	ECETOC			20-50	0,15 g dry matter/l	56	35	> 75 %	inoculum: digested sewage sludge, freshwater swamp	Madsen <i>et al.</i> (1995)
	C12-C14 APG	ECETOC			20-50	0,15 g dry matter/l	56	35	> 75 %	inoculum: digested sewage sludge, freshwater swamp	Madsen <i>et al.</i> (1995)
	C10 6-O-Ethylglucoside monoester	ECETOC			20 - 50	0,15 g dry matter/l	55	35	> 75%	inoculum: digested sewage sludge, marine sediment, freshwater swamp	Madsen <i>et al.</i> (1995)
	C12-14-APG	ECETOC		100		3g dry matter/l	56	35	84 +/- 15 % (CO ₂)+(CH ₄)		Steber <i>et al.</i> c.(1995)
	C8-10-APG	ECETOC		100		3g dry matter/l	56	35	95 +/- 22 % (CO ₂)+(CH ₄)		Steber <i>et al.</i> c.(1995)
Glucose Amide	C12 Glucose Amide		14C-sim.	1			35		86 +/- 0,3 % (CO ₂ + CH ₄)		Federle and Nuck (1997)
Amine Oxides	DimethylDodecyl Amine Oxide		14C Digester	1		~20	7	35	> 80		Vandepitte and Debaere (1992)

DP = degree of polymerization;
APG = alkylpolyglucoside)
THGP = Theoretical gas production

Conclusions on the anaerobic biodegradation of nonionic surfactants

Alcohol Alkoxylates:

Alcohol ethoxylates (linear C9-C18-alcohols, 5-23 EO) are well biodegradable in anaerobic screening tests. Degradation of usually > 70% (biogas) has been reported in digested sewage sludge and freshwater sediment.

Degradation > 80% (biogas formation and ¹⁴C) in digester simulation tests have been reported in the case of lauryl alcoholethoxylates and stearyl alcoholethoxylates.

Actual data on the AE concentration in digester sludges support the conclusion that AE's are well biodegradable in full scale digestors (Klotz, 1998).

Alkylphenol ethoxylates showed poor to moderate mineralisation rates (20-50 % biogas formation) in screening and simulation tests.

Simulation tests indicated that degradation proceeds via de-ethoxylation to alkylphenol which is poorly degradable. Consequently, high concentrations of nonylphenol (up to 2530 mg/kg DM) were measured in digestors (Tschui and Brunner, 1985).

Lower concentrations (35-95 mg/kg DM) of nonylphenol in digester sludges were reported recently (Küchler, 1995).

Sugar derivatives

Alkylpolyglucosides (C8-C12-linear alkyl) and glucoside fatty acid esters are well degradable in anaerobic biodegradation screening tests (> 60 % biogas formation).

Glucose Amides showed high mineralisation rates (> 80%) in a ¹⁴C-simulation test.

Amine oxides are likely to be anaerobically degradable (1 positive data point - DDAO)

7.3. CATIONICS – AMPHOTERICS

Surfactant Type	Characterisation	Test Type		Test Conc.		Inoculum Conc.	Test Duration	Temp.	Results	Remarks	References
		Screening	Simulation	mg/l Active Matter	mg/l Carbon						
CATIONICS											
DTDMAC	Dimethyl-di(14C) Stearyl-ammonium chloride (C18)		14C-digester	10	~ 8	~ 20 (as dry matter)	28	35	8.2 (CH ₄) + 6.7 (CO ₂) = 14.9	degradation due to impurity ?	Steber (1991)
STAC	14C1-StearylTriMethyl Ammonium chloride (C 18)		14C-digester	0.98 mg/kg sediment		ww-pond sediment 500 ml/l	87	22	0		Federle and Schwab (1992)
MTEA Esterquat	Esterquat	ECETOC		50		1 to 5 (as dry matter)	42	35	101.1 +/- 12.8		Puchta <i>et al.</i> (1993)
DEEDMAC	Esterquat (C16-18)	ECETOC		~ 38		1 to 5	60	35	90		Giolando <i>et al.</i> (1995)
CTMAB	Cetyltrimethyl ammonium bromide (C 16)	Mod. Shelton & Tiedje		50	50	2-3 gl	60	35	inhibition		Battersby and Wilson (1989)
Amphoterics											
	no data found.										

Conclusions regarding anaerobic degradability of cationic surfactants

Cationics

Anaerobic degradability results for cationic surfactants need to be evaluated with caution, since they may be inhibitory at low levels (i.e. low mg/l range) to anaerobic metabolism. Nevertheless, some apparent trends can be found :

- mono-alkyl or di-alkyl quaternary nitrogen compounds with straight (C-C) alkyl chains are not anaerobically degradable (e.g. TMAC, DTDMAC, etc).
- esterified mono-alkyl or di-alkyl quaternary nitrogen compounds are biodegradable (e.g. MTEA esterquat, DEEDMAC, etc). After ester hydrolysis, both the fatty acid as well as the alcohol cleavage products can be further completely mineralized

Since only a few compounds have been tested, it is difficult to draw general conclusions for cationics with a high degree of confidence. The effect on the degradability of factors such as the position of the ester bond, the type of substitution on the nitrogen, branching, etc., have not been systematically investigated.

Amphoterics

No data were found.

8. CRITERIA FOR EVALUATION OF THE IMPORTANCE OF ANAEROBIC BIODEGRADATION

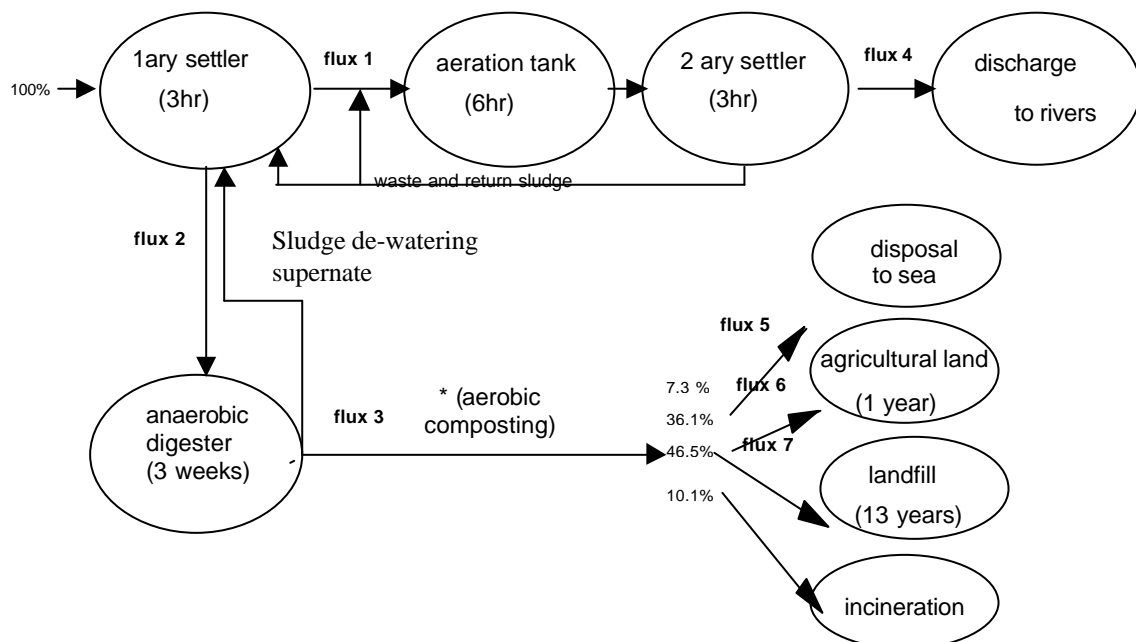
8.1. FLUX OF SURFACTANTS IN ENVIRONMENTAL COMPARTMENTS

To produce a simplified approach to the flux of surfactants in the environment the following parameters have been used.

- The removal of surfactant on primary solids which will determine what percentage goes to aerobic and anaerobic zones of a sewage treatment plant.
- The degradation of the surfactant in these two zones.
- The degradation in the receiving environmental compartment.
- Direct discharge of raw sewage is not included since other factors such as ammonium toxicity, lack of dissolved oxygen will have a major environmental impact.

The flux of surfactants through a Waste Water Treatment Plant (WWTP) and into the environment is depicted in Figure 8. Data from an ISWA study conducted in 1990 and reported in 1995 (ISWA, 1995) on the percentage sludge disposed to sea, agricultural land, landfill and incineration within the EU is used to quantify the percentage of surfactant going to each of these compartments.

Figure 8: Diagrammatic representation of surfactant flux through a WWTP.



*Sludge used for agricultural use may undergo aerobic composting prior to disposal

The figures given in brackets are average residence times. In the case of agricultural land and landfill they are the figures used to calculate removal in these environments

A study of the relative amounts of surfactant types in different environmental compartments relies on the availability of environmental monitoring data. This is relatively extensive for LAS but not so great for other detergent types. Therefore as a first step in a simplified approach the environmental flux of three hypothetical surfactants may be considered. In Case 1 the surfactant is both aerobically and anaerobically degradable whereas in Case 2 the surfactant is aerobically but not anaerobically degradable. Case 3 is aerobically nor anaerobically degradable, that is, it differs from Case 2 in the fact it is not aerobically degradable. Such a surfactant would not be allowed in the market place through legislation because of its inability to degrade aerobically.

The figures given for the individual fluxes are a percentage of the amount of surfactant entering a WWTP and do not take into account the degradation that occurs in the sewer.

To arrive at values for the various fluxes and to reach conclusions as to the amount of surfactant present in the various terrestrial anaerobic compartments in the above table, the following suppositions have been made. It should be noted that these figures are only approximations.

- 25% of surfactant is removed on primary solids with the remaining 75% going to aerobic treatment.

For surfactants that are aerobically degradable

- 98% are removed in a WWTP
- 95% are removed in landfill
- 99% are removed in agriculture land

For surfactants that are anaerobically degradable in addition

- 80% are removed by anaerobic digestion

As noted in Figure 8 some WWTPs have the capacity for aerobic composting of the sludge after anaerobic digestion. This would remove ~70% of aerobically degradable surfactant and so the fluxes given in the table would have to be modified accordingly should composting occur.

Table 1. Flux of hypothetical surfactants through a WWTP and final concentrations present in the anaerobic compartments landfill and agricultural land.

	Case 1	Case 2	Case 3
Flux 1	75%	75%	75%
Flux 2 (digester)	25%	25%	25%
Flux 3	5%	25%	25%
Flux 4 (rivers)	2.0%	2.0%	75.0%
Flux 5 (sea)	0.37%	1.8%	1.8%
Flux 6 (agriculture)	1.8%	9.0%	9.0%
% present in agricultural land after 1 year	0.02	0.09	>9 *
Flux 7 (landfill)	2.3%	11.6%	11.6%
% present in landfill after 13 years	0.02	0.6	> 12 *

* The amounts cannot be accurately estimated and hence the given value is considered as a minimum since it is fair to assume a continuous increase in this compartment.

From Table 1 it becomes apparent that provided a surfactant is aerobically biodegradable, even if it is anaerobically recalcitrant, then less than 1% of the surfactant released to WWTPs will be present in the permanently/temporarily anaerobic compartments of agricultural lands and landfill sites (case 2). In comparison, the amount of a surfactant that is both aerobically and anaerobically biodegradable present in these compartments will be lower by a factor of 5 (case 1). However, it should be remembered that the impact of a substance to an environmental compartment depends essentially on the resulting concentration and the ecotoxicological effect levels. In compartments which may be temporarily anaerobic like terrestrial land, the environmental relevance of anaerobic recalcitrance is strongly dependent on aerobic biodegradation behaviour, i.e. the aerobic biodegradation rate and the resulting environmental concentrations. In contrast, landfill sites are intendedly designed as artificial sinks for waste and do not represent natural ecosystems. Therefore the presence of any substances at elevated concentrations in that waste is inconsequential.

In the case of an aerobically recalcitrant surfactant (case 3) the percentage present in agricultural land may be estimated to be at least 9%, which not only is twenty times higher than in case 2 but additionally it must be expected that the concentrations will increase continuously and may cause, in the end, ecotoxicological problems. At the same time, the flux which may finish up on river sediments is fifty times higher, again with the danger of increasing environmental concentrations. It is for this reason that such surfactants are not allowed to be used by the detergent industry.

A similar table may be drawn up for various surfactants where monitoring data is available. Not surprisingly the environmental fate of LAS has been extensively studied since it is the most widely used surfactant. Although monitoring exercises on

the fate of other surfactants have been performed and are discussed later, the data is not sufficient to complete all fluxes.

Giger *et al.* (1989) studying fluxes in Zurich found the removal of LAS onto primary solids to be 27%, Berna *et al.* (1989) doing similar work on a WWTP in Madrid found 16% removal, whereas Prats *et al.* (1997) working in Alicante found this to be 37%. The discrepancy between the latter case and the other two is explained by the unusually high water hardness, and consequently the sewage, in the Alicante region.

Such regions are not unique, but there again not commonplace, and so it seems that a removal of 25% on primary solids would be a fair estimate. This would mean that 75% of the LAS entering a WWTP will go to aerobic treatment, with that on primary solids going to the anaerobic digester. Prats *et al.* (1997) found a removal of 18% of LAS by the anaerobic digester, Giger *et al.* (1987) found 20 - 30% removal and Osburn (1986) 0-35%.

Although degradation tests under strictly anaerobic circumstances indicate that sulphonates are not anaerobically degradable, studies on these materials, when there has been a short exposure to aerobic conditions, show extensive degradation. The removals found in the monitoring studies may therefore be due to that fraction of LAS entering the digester which had been pre-exposed to aerobic conditions. In addition to this, in some WWTPs some anaerobic sludge is returned to the primary settler prior to aerobic composting or discharge to the chosen sludge disposal route. To simplify matters it seems reasonable to take 15% removal of LAS by anaerobic treatment as a fair estimate. Prats *et al.* (1997) found that the composting stage removed a further 70% of the LAS remaining. The sludge may be disposed of to agricultural land, landfill, the marine environment (due to end 1998) or incineration.

Of the LAS going to the aerobic process of a sewage treatment plant Prats *et al.* (1997) found the amount leaving in effluent to be 0.6% of that entering the WWTP, equivalent to 99.4% removal by the aerobic treatment. Rappaport and Eckhoff (1990) found 98% removal by activated sludge plants although this was slightly lower for trickling filter plants. Feijtel *et al.* (1995) found an average of 99.2% removal when monitoring WWTPs in the Netherlands. A figure of 99% seems to be not unreasonable and this amount will enter a water course and could go onto river sediment.

Holt *et al.* (1989) found over 98% removal of LAS in sludge amended soils over a year and calculated a half life for this surfactant of 7-22 days. Ferrer *et al.* (1996) found 89.2% removal within 62 days giving a half life of 19.3 days which is in agreement.

Marcomini *et al.* (1988) monitoring a landfill site accepting sewage sludge, found that at the surface of the site where sludge application was most recent, the level of LAS was 9160 mg/kg whereas near the bottom this had dropped to 245 mg/kg. This equates to a removal of 97% over a 13-year period.

As noted before there have been other monitoring studies on other surfactant types but not as extensive as that done for LAS. In a parallel study to that on LAS, Prats *et al.* (1997) followed the progress of nonionic surfactants through a WWTP. However,

whereas in their study of LAS where the material was followed specifically by HPLC, nonionics were followed using Wickbold activity, a technique which is non specific and will measure natural nonionic materials as well as anthropegenic ones.

Field *et al.* (1995) studied the flux of secondary alkane sulphonates (SAS) through a WWTP, although in their study there were no data for the effect of composting the sludge after anaerobic digestion. Ahel *et al.* (1994) studied the flux of nonyl phenol ethoxylate (NPE) and its metabolic products, nonyl phenol, nonylphenol 1EO and nonyl phenol 2EO through WWTPs. In this study of a number of WWTPs in Switzerland they found on average 8% of parent nonyl phenol ethoxylate leaving the plants in the effluent but during the sewage treatment process short ethoxylate chain nonyl phenols and nonyl phenols were formed.

In all these studies rough conclusions can be made as to the amount of surfactant leaving WWTPs to be disposed of to various environmental compartments but there are insufficient data available to assess their removal in these compartments. However it is possible to fill these gaps using circumstantial evidence. Since SAS, like LAS is aerobically but not anaerobically degradable, we can assume similar removals in landfill and agricultural land of 98 and 97% respectively. Alcohol ethoxylates (AE) and NPE are both aerobically and anaerobically degradable and so removals of 99% could be expected.

Alcohol ethoxy sulphates (AES), alcohol sulphates (AS), AE and soap were also monitored in the Dutch study (Feijtel *et al.*(1995)) with highly specific analysis using high performance liquid chromatography (HPLC) and mass spectrometry. In addition the Biochemical Oxygen Demand (BOD) flux to rivers was measured.

Table 2: Disposal of surfactants and BOD to rivers, ie Flux 4, found during monitoring for a Dutch risk assessment.

Parameter	Flux 4
B.O.D.	1.9%
L.A.S.	0.8%
A.E.	0.2%
A.E.S.	0.4%
A.S.	0.8%
soap	0.9%

Therefore the flux to rivers of the individual surfactants was less than that of the BOD, or conversely the surfactants were removed to a greater extent than the sewage and at least as efficiently as soap.

From the above monitoring data on these surfactants, a table of fluxes as drawn up for the hypothetical surfactants can be produced.

Table 3: Estimated flux of surfactants from above monitoring data.

Data in italics and with a shaded background are calculated from assumptions given earlier in the text.

	LAS	SAS [a]	AE [b]	NPE [c]
Flux 1	75% [b,e,f]	84.4%	44%	80%
Flux 2 (digester)	25% [b,e,f]	14.6%	41%	20%
Flux 3	21.3% [b,e,g]	16.3%	14%	20%
Flux 4 (rivers)	1.0% [b,d,h]	0.3%	0.2% [d]	8.0%
Flux 5 (sea)	1.6%	1.2%	0.3%	1.5%
Flux 6 (agriculture)	7.7%	5.9%	1.6%	7.2%
% present in agricultural land after 1 yr	0.15% [i]	<i>0.12%</i>	<i>0.02%</i>	<i>0.07%</i>
Flux 7 (landfill)	9.9%	7.6%	2.1%	9.3%
% present in landfill after 13 years	0.3% [j]	<i>0.23%</i>	<i>0.02%</i>	<i>0.09%</i>

[a] Field *et al.* (1995), [b] Prats *et al.* (1997), [c] Ahel *et al.* (1994)

[d] Feijtel *et al.* (1995) [e] Giger *et al.* (1989) [f] Berna *et al.* (1989)

[g] Osburn (1986) [h] Rappaport and Eckoff (1990) [i] Holt *et al.* (1989)

[j] Marcomini *et al.* (1988)

The references given above are where the available data has been extracted from.

From the monitoring data we can conclude that less than 20% of surfactant entering sewage plants has the potential to reach an anaerobic environmental compartment. This figure does not include that percentage entering anaerobic digesters in WWTPs since there will be no environmental impact so long as the surfactant is not inhibitory to the digester.

Landfill sites are man-made environments which do not require the same environment potential, which means that the quantity of surfactant entering the important ones of the seas, rivers and agricultural land is less than 10%.

This monitoring data is in general agreement with that obtained for the hypothetical surfactants mentioned earlier. AE are both aerobically and anaerobically degradable whereas LAS is not practically anaerobically degradable, ie. these two surfactants are in fact real examples of hypothetical surfactants cases 1 and 2. Comparing the values obtained for the amounts present in agricultural land for cases 1 and 2 versus AE and LAS, both are in the same order of magnitude. Whereas the amount of LAS present in agricultural land was calculated from actual monitoring data, that for AE made the assumption that there would be a 99% removal in this environment. However it is fair to say that using the figures obtained, the presence of an anaerobically degradable surfactant will be almost 10 times lower than that of one which is only aerobically degradable. This factor is lower than that calculated earlier for the hypothetical cases, but nevertheless it is appreciated. Because of the very low levels involved however, the practical implications of this can only be assessed by determining how such levels affect the various anaerobic compartments.

8.2 IMPACT OF SURFACTANT ON STRUCTURE AND FUNCTION OF ANAEROBIC ENVIRONMENTAL COMPARTMENTS.

8.2.1. Speciation and bioavailability of surfactants in anaerobic compartments.

In the aquatic (aerobic) environment the risk assessment of surfactants is always based on measurements and determinations of the soluble species (usually Na salt) which are the most bioavailable and the biodegradable forms.

The speciation of surfactants present in anaerobic compartments has never been established. Assessments have always been based on the same soluble species, as present in aquatic media. Extraction and detection procedures of surfactants from anaerobic matrixes (soils, sediments, sludges) end up in a soluble form of surfactant.

Some anionic surfactants have a strong tendency to precipitate as insoluble salts with Ca, Mg ions (water hardness) as they carry a negative charge in the molecule. This phenomenon might also occur with other surfactants (cationics) as a consequence of the interaction with various anionic species. LAS is probably the surfactant most widely assessed in environmental compartments and there are several indirect indications of its presence in solid environmental samples under the form of Ca-Mg derivatives. A clear chemical speciation differentiation of surfactants in anaerobic compartments however poses a truly scientific challenge because of the inherent complexity of such compartments. There are several clear, although indirect, indications showing the presence of such insoluble (not bioavailable) derivatives.

- **Very low solubility of Ca derivatives of LAS (Verge 1997)**

Solubility of Ca – LAS homologues in water is very low. For C₁₂ LAS the solubility at 250 mg/l water hardness is in the order of 9 mg/l.

- **Relationship between LAS concentrations in sludges and water hardness.**

Several studies have indicated (Berna 1989) the influence of water hardness on the LAS concentration in primary solids of W.W.T.P. as well as on anaerobically digested sludges of sewage treatment plants.

- **Concentration of Ca & Mg ions in the sludges of sewage treatment plants**

A comparison of the mass balance of Ca and Mg ions in the raw sewage, treated water and digested sludges (Berna 1992) indicates a remarkable accumulation of those ions in the sludge. While the amount of Ca + Mg is nearly half of the amount of Na in Raw Sewage as well as in treated water, in the final sludge [Ca + Mg] is almost 30 times higher than [Na].

	Na	Ca	Mg
Raw Sewage (mg/l)	63	28	9
Treated Water (mg/l)	60	26	8
Sludge (mg/kg)	1,200	21,500	9,000

- Normal operation of anaerobic digesters in WWTP with LAS concentrations above the known inhibitory level (as Na salt) (Painter and Mosey, 1992). The bioavailability of insoluble forms of surfactants in environmental compartments needs to be evaluated, in order to assess their ecotoxicological relevance.

(See Table in appendix with data of surfactant monitoring in sludges)

8.2.2. Water treatment

8.2.2.1. Anaerobic digester

There is little published information available on the inhibitory effects of surfactants on anaerobic digestion. Bruce *et al.* (1966) reported results obtained from laboratory digesters fed on a daily basis with surfactant-free raw sludge which had been amended with different concentrations of surfactant. LAS (C10-C13, sodium salt) at a concentration of 15 g/kg dry solids was not inhibitory; although biogas production was reduced over the first 24 h after feeding, total gas evolution after 48 h was similar to that in controls fed with surfactant-free sludge only. At LAS levels between 15 and 20 g/kg dry solids the reliability of digestion was impaired and more serious inhibition occurred at higher concentrations. Similar results were obtained for sodium alkyl sulphonate. Tallow (C16 - C18) alcohol sulphate and a 'synthetic' primary alcohol sulphate both had only a minor short-term inhibitory effect on gas production at concentrations up to 40 g/kg dry solids. At the end of the 18 day incubation period, gas production in the test digesters was higher than in the controls and 90% of the surfactants had been degraded (MBAS removal). Nonyl phenol ethoxylate had only a slight inhibitory effect on gas production at levels up to 200 g/kg dry solids.

Published concentrations of surfactants in digester sludges show that the majority of the data are for LAS, with average levels of around 5-10 g/kg dry solids. This is consistent with monitoring data for the period 1981-1986 for sewage treatment plants in the USA and Germany, which showed that the average concentration of LAS in the anaerobic sludges analysed was 5 g/kg dry solids (Rapaport and Eckhoff, 1990). In their review on the behaviour of LAS in sewage treatment, Painter and Zabel (1989) also reported LAS levels of around 5-10 g/kg dry solids in both primary (raw) and anaerobically digested sewage sludges. Hence, LAS levels in sewage sludges at most sewage treatment works should not be high enough to inhibit digestion. Although 'anionic surfactants' have been blamed for a small number of anaerobic digester failures in the past in the UK, inadequate design or operation, or trade wastes have been identified as more likely causes (Swanwick *et al.*, 1969).

The concentration of linear alcohol ethoxylate in digester sludges is an order of magnitude lower than LAS at < 0.5 g/kg dry solids (Klotz, 1998). Although there are few measured concentrations for the other surfactant groups, it is reasonable to conclude that inhibition of full-scale digestion is unlikely to occur unless they are much more toxic than LAS. This is deemed to be unlikely based on the findings of Bruce *et al.* (1966), and the high biodegradabilities of alcohol ethoxylates, alcohol sulphates, alcohol ethoxysulphates and other surfactants at the unnaturally high surfactant to sludge dry solids ratios (~ 50 g/kg drysolids) used in the ECETOC and ISO anaerobic biodegradability screening tests (see Chapter 6).

8.2.2.2. Anaerobic river and lake sediments

Freshwater sediments are usually anaerobic below the surface few mm or cm, and stratified in terms of redox potential and the metabolic processes that occur within these zones. In rivers, the structure of the sediment will be affected by the velocity and volume of the river flow, which in turn causes the transport of sediment particles either by suspension or by sliding and rolling (Press and Siever, 1986). Anaerobic, freshwater sediments have important functions in the decomposition of organic matter, and in the cycling of nitrogen, sulphur and phosphorus. These processes will be dependent on the redox potential of the sediment and include nitrate reduction to either ammonia or nitrogen, sulphate reduction to sulphide and methanogenesis (Jones, 1982). Sediments can also act as sinks for chemicals which sorb to sediment particles or organic matter. The sediment is home for a wide range of fauna, flora and micro-organisms, and perturbation of these organisms can have a detrimental effect of the ecosystem (e.g. Burton and MacPherson, 1995).

In a review of the literature, Painter and Zabel (1988) reported that concentrations of LAS in river sediments were generally in the range 1-10 mg/kg dry solids, although levels of 100-300 mg/kg dry solids were reported for river sediments close to where untreated sewage was discharged. Rapaport *et al.* (1995) reported average sediment LAS levels of 1-2 mg/kg dry solids below the outfall of USA activated sludge plants, with higher levels of 60-180 mg/kg dry solids below trickling filter plants. In all of these studies, no indication was given of the redox potential of the sediments sampled. However, it is likely that they were grab samples of the surface few cm of river sediment and may therefore had contained some anaerobic sediment.

No information of the toxicity of LAS (or other surfactants) to anaerobic, sedimentary bacteria could be found. However, data on the toxicity of LAS to aerobic bacteria (see Painter and Zabel, 1988) and anaerobic digestion (see above) indicate that the levels of LAS in river sediments should not inhibit microbial processes. As regards higher organisms, Pittinger *et al.* (1991) reported that the larvae (benthic organism) of *Chironomus riparius* (midge) was not affected by LAS at a concentration of 319mg/kg dry solids, which again is higher than the levels measured *in situ*.

8.2.2.3. Soil

According to the European Technical Guidance documents (EU-TGD, 1996) for sludge application to agricultural soil, an application rate of 0.5 kg dry weight/m² per year is assumed. In Denmark the sludge distribution on farm land is mainly regulated by phosphorus rules. This means that according to these rules an average maximum rate of 0.4 kg dry weight/m² in one year over a three years period is allowed at present (Krogh, 1997). Assuming typical European LAS concentration in anaerobic sludge of 5000-10000

mg/kg, considering a bulk soil density of 1.8 and a mixing soil depth of 0.2 m, one expects an initial soil LAS concentration of 7-14 mg/kg after a fresh sludge application of 0.5 kg/m². Sludge, however, is usually stored for an average period of several months (ca. 6) before use in agriculture where aerobic biodegradation can lower the effective surfactant concentration at the moment of the sludge application. In addition, for exposure of the endpoints in soil the concentration of the chemical needs to be averaged over a certain period, 30 or 180 days depending on endpoints (EU-TGD, 1996). Aerobic conditions prevail in sludge-amended soil, as shown by several works which demonstrated that LAS disappears with kinetics having half lives of 15-30 days (De Wolf, 1998; Cavalli, 1998). All this means that after the mandatory averaging time the LAS concentration in sludge-amended soil should be lower than the expected initial value of 7-14 mg/kg (Cavalli, 1998).

No experimental field monitoring has been performed to monitor LAS, and of any other surfactant, in soil amended by sludge following strictly the European or Danish rules, even at the initial moment of sludge application. Monitorings are available which refer to LAS laboratory and pilot field studies where the initial concentration in soil is often quite high (16-250 mg/kg) and corresponds to unrealistic sludge application rates (1-12 kg/m² in single shots even for several consecutive years), used mainly for research purposes (De Wolf, 1998; Cavalli, 1998). In the studies the disappearance of LAS in soil is in any case rather quick and levels to a typical concentrations of 0-5 mg/kg after the test period. That should be true for any other surfactant having the same aerobic biodegradation characteristics as LAS.

As to species living in the terrestrial compartment there are only some chronic data points available for LAS. The PNEC for LAS in soil should reasonably be between 5 and 15 mg/kg (Kloepper-Sams, 1996; Cavalli, 1998). Research is in progress in Denmark to generate additional data for LAS in order to carry out a definitive terrestrial risk assessment of this surfactant.

No LAS was detected in the groundwater near a leaching field (De Wolf, 1998). Biodegradation and adsorption play a role in removing LAS. Some laboratory studies carried out on sludge stored on compacted soil floors demonstrate that LAS penetrated, even after an extended time period of 1 year, only to a minimum degree into the soil. Most of the LAS migration was found in the 3 cm thick soil layer. No LAS was present in the water percolating through the soil. Leaching of LAS from the sludge amended soil to ground water should not be expected to happen (Figge, 1991)

Surfactants, applied with the use of sewage sludge in agriculture, can increase the mobility of organic micropollutants. These effects, however, were only detected at very high surfactant concentrations which are not relevant in the agricultural practice (Küchler, 1995).

8.2.2.4. Septic tanks

Septic tanks are onsite (pre-)treatment systems for black water or unsettled domestic sewage. Their effluent can be sent to a sewer system, or can be allowed to percolate into the soil (i.e. into a tile field). In Europe and the US a large fraction of the population in rural areas (up to 30 %) has septic tanks

installed. The septic tank itself and the tile field in the immediate vicinity of the tank, are usually strictly anaerobic environments. Surfactants released via a septic tank system are therefore exposed first to an anaerobic compartment without dilution.

The main function of a septic tank is to act as a settling tank for the solids in domestic sewage, and needs to be periodically emptied (e.g. once per year). This sludge is treated elsewhere, usually in a nearby treatment plant, or is disposed to land. The water phase in the tank has a residence time between 1-2 days.

The sludge at the bottom of the tank undergoes facultative anaerobic and anaerobic decomposition, with the formation of biogas and H₂S. This digestion is the second function of a septic tank. Since the temperature in the tank is close to that in the surrounding soil (< 20° C), digestion typically occurs at a slow rate.

Most surfactants and detergent formulations have been tested for septic tank safety. The protocol for such tests includes a parameter related to anaerobic digestion and settling rate under realistic circumstances. This is mostly done in the laboratory, but some validation on real septic tanks has been performed. When used as directed, the surfactants and other ingredients in detergents and cleaners are safe for septic tank systems. This includes also those surfactants which are not anaerobically degradable. Data to support this are available within the Industry member companies. Some studies are also available in the literature on the fate and behaviour of non-degraded surfactants leaving the tank into a tile field (McAvoy *et al.*, 1994 ; Shimp *et al.*, 1994).

8.2.2.5. Landfills for sludge

Landfilling is widely used to get rid of waste, including digested and dewatered sludge. So landfilling is seen as a sink of any material. Conditions in the bulk can be both aerobic and anaerobic.

There is a lack of monitoring data for concentrations of surfactants. One study (Marcomini, 1989) gives data on TPS (Tetrapropylenebenzene Sulphonate) and LAS for four German landfills, which were used as deposits for digested sludge only for up to 30 years.

The switch from TPS to LAS can be used as a time marker to help to set the age of the landfill. Considering that LAS consumption, and consequently its input to landfills, has only slightly increased along the years taken into account, it appears that, with a residence time of 13 years, the LAS concentration decreased from about 9 g/kg to 0.2 g/kg in one landfill.

Lack of anaerobic biodegradation, however, is not relevant in this compartment, provided of course there is no leakage to ground water.

8.2.2.6. Marine sediments

The structure and function of marine sediments are essentially similar to freshwater sediments, although there are some key differences. In estuarine and coastal areas, sediments are usually anaerobic below the surface few mm but pelagic sediments can be oxic at sediment depths > 1 m (Jørgensen, 1982). The stratification of anaerobic decomposition and nutrient cycling processes with decreasing sediment redox potential are similar to freshwater sediments. However a big difference is the abundance of sulphate in seawater which means that sulphate reduction replaces methanogenesis as the terminal stage in the anaerobic oxidation of organic matter. In coastal waters this process can oxidise as much organic matter to CO₂ as do aerobic organisms.

There is a paucity of monitoring data for concentrations of surfactants in estuarine or marine sediments and what values are available are mostly confined to LAS. In their review on the environmental safety of LAS, Painter and Zabel (1988) reported levels of 5-17 mg/kg dry solids in estuarine sediments close to the outfalls of probably untreated sewage; LAS was undetectable further out to sea. No information could be found on the toxicity of surfactants to marine sediment dwelling organisms.

8.2.2.7. Anaerobic and anoxic zones in sewage treatment plants with biological nutrient removal

The recent EU Urban Waste Water Treatment Directive 91/271/EEC imposes WWTP effluent criteria for nitrogen and phosphorus in nutrient sensitive areas. Full-scale Biological Nutrient Removal (BNR) treatment plant designs such as the UCT, Bardenpho, Bardenpho, SBR, A/O, A₂/O process, etc., are being built with increasing frequency (US – EPA, 1987). Alternatively, existing plants are retrofitted for BNR (e.g. Matsché, 1987; Randall *et al.*, 1992; Kayser, 1994). All of the above systems include low dissolved O₂ and/or anaerobic treatment zones in addition to an aerated zone, through which the activated sludge is circulated. In Sequencing Batch Reactors (SBR) the redox zone is substituted by a time period. In BNR systems there can be a direct discharge of surfactants in an (engineered) anaerobic environmental compartment, although the residence time is only a few hours before surfactants are exposed again to aerobic conditions.

Two aspects are of particular interest in the context of anaerobic degradability of surfactants :

- 1) the presence of alternative e-acceptors (e.g. NO₃⁻, SO₄²⁻, C) in addition to oxygen may lead to other degradation pathways and/or kinetics under anoxic/anaerobic conditions.
- 2) two additional essential biological processes take place, i.e. excess phosphorus uptake and biological nitrogen removal via nitrification/denitrification. This may imply an increased sensitivity of the plant to inhibitory substances (Kroiss *et al.*, 1992; Strotmann & Eglsäer,

1995). It is important that the above functions of the STP are adequately protected.

To date, few data on the influence of surfactants on these specific processes are available. Lab studies (Rottiers *et al.*, 1998) and circumstantial evidence from the field have shown, however, that there is no particular reason for concern, since the biological nutrient removal systems can function adequately even in the presence of considerable amounts of surfactants in the influent (e.g. 10 - 20 mg/l in domestic sewage). It is expected that more information will come available over the next years, following the expansion of BNR systems across Europe.

9. REFERENCES

- Ahel, M., Giger, W., Koch, M. (1994). Behaviour of Alkyl Polyethoxylate surfactants in the aquatic environment - 1. Occurrence and transformation in sewage treatment. *Wat. Res.* 28, No 5, pp.1131 – 1142.
- ASTM E 1196-87. (1987). Standard Method for Determining the Anaerobic Biodegradation Potential of Organic Chemicals. pp. 738-742.
- Battersby, N.S., Wilson, V. (1988). Evaluation of a serum bottle technique for assessing the anaerobic biodegradability of organic chemicals under methanogenic conditions. *Chemosphere* 17, 2441-2460.
- Battersby, N., Wilson, V. (1989). Survey of the anaerobic biodegradation potential of organic chemicals in digesting sludge. *Appl. Environ. Microbiol.* 55, pp. 433-439.
- Baumann, H., Schefer, W. (1990). Simple test system to judge the anaerobic biodegradability of organic substances. *Textilveredlung* 25, p. 248.
- Baumann, U., Mueller, M.T. (1997). Determination of anaerobic biodegradability with a simple continuous fixed bed reactor. *Wat. Res.* 31, pp. 1513 -1517.
- Berna, J. L., Ferrer, J., Moreno, A., Prats, D., Ruiz, F. (1989). The fate of LAS in the environment. *Tenside Surf. Det.* 26, pp. 101 –107.
- Berna, J.L., Moreno, A., Ferrer, J. (1992). An assessment of the ultimate biodegradation of LAS. *Proceedings of CESIO World Surfactants Congress, London (UK)*, 3, pp. 59-72.
- Birch, R.R., Biver, C., Campagna, R., Gledhill, W.E., Pagga, U., Steber, H., Reust, H., Bontinck, W.J. (1989). Screening chemicals for anaerobic biodegradability. *Chemosphere* 19, pp. 1527 - 1550.
- Bouwer, E.J., McCarty, P.L. (1983). Transformation of halogenated organic compounds under denitrification conditions. *Appl. Environ. Microbiol.* 45, pp. 1295 - 1299.
- Bruce, A.M., Swanwick, J.D., Ownsworth, R.A. (1966). Synthetic detergents and sludge digestion: Some recent observations. *The Journal and Proceedings of the Institute of Sewage Purification* 5, pp. 427-447.
- Burton Jr., G.A., MacPherson, C. (1995). Sediment toxicity testing issues and methods. In *Handbook of Ecotoxicology*, eds. D.J. Hoffman, B.A. Rattner, G.A. Burton Jr. and J. Cairns Jr., 70-103. CRC Press Inc.
- Cavalli, L. and Valtorta, L. (1998). Surfactants in sludge-amended soil, 1998, XXVIII CED, Barcelona, May 6-8.

- Chien Chih-Ching, Leadbetter, E. R., Godchaux III, W. (1995). Sulfonate-sulfur can be assimilated for fermentative growth, *FEMS Microbiology Letters*, 129, pp. 189-194.
- Compagnion, D., Nyns, E.-J. (1986). Biomethanation in developing countries. In *Biotechnology Vol. 8. Microbial Degradations*, ed. W. Schönborn VCH, pp. 239-267.
- Denger, K., Kertez, M. A., Vock, E. H., Schon, R., Cook, A. M. (1996). Anaerobic desulfonation of 4-tolylsulfonate and 2-(4-sulfophenyl) butyrate by *Clostridium* spp, *Appl. Environ. Microbiology* 62, pp. 1526-1530.
- Denger, K., Cook, A. M. (1997). Assimilation of sulfur from alkyl- and arylsulfonates by *Clostridium* spp, *Arch. Microbiol.* 167, pp. 177-181.
- Denger, K., Lane, H., Cook, A. M. (1997). Anaerobic taurine oxidation: a novel reaction by nitrate-reducing *Alcaligenes* spp, *Microbiology* 143, p.1919.
- Denger, K., Cook, A. (1998). LAS bioavailable to anaerobic bacteria as a source of sulfur, *Appl. Environ. Microbiol.* in press.
- Deuser, W.G. (1971). Organic carbon budget of the Black Sea. *Deep Sea Research* 18, pp. 995-1004.
- De Wolf, W. and Feijtel, T. (1998). TRA for LAS in sludge-amended soil, *Chemosphere*, 36, pp. 1319-1343.
- ECETOC. (1988). Evaluation of anaerobic biodegradation. Technical Report 28.
- EPA (1988). EPA 40 CFR Ch. 1 (7-1-88 Edition) §796.3140: Anaerobic biodegradability of organic chemicals. pp. 568-572.
- EU-TGD (1996). Technical Guidance Documents (TGD) in support of EU Directive 93/67 EEC and EU Regulation 1488/94 published by the EU Chemical Bureau, 1996.
- Federle, T.W., Schwab, B.S. (1992). Mineralization of surfactants in anaerobic sediments of a laundromat wastewater pond. *Wat. Res.* 26, pp.123-127.
- Ferrer, J. de, Moreno, A., Vaquero, M^a.T., Comellas, L. (1996). Monitoring of LAS (Linear Alkylbenzene Sulfonate) in direct discharge situations : untreated sewage and on sludges amended soils, CESIO 4th World Surfactant Congress, Barcelona (Spain).
- Feijtel, T.C., van den Plassche, E. (1995). Environmental Risk Characterisation of four Major Surfactants used in the Netherlands, Report No 679101025 by NVZ – RIVM.
- Field, J., Miller D. J., Field, T. M., Hawthorne, S. B., Giger W. (1992). Quantitative determination of sulfonated aliphatic and aromatic surfactants in sewage sludge by ion-pair / supercritical fluid extraction and derivatization GC/MS, *Anal. Chem.* 64, pp. 3161-3167.

Field, A., Field, T., Poiger, T., Siegrist, H., Giger, W. (1995). Fate of Secondary Alkane Sulfonate surfactants during municipal Wastewater Treatment. *Wat. Res.* 29, No 5, pp. 1301 – 1307.

Figge, K. (1991). NATEC report, Fate of LAS in digested sewage sludge deposits, February 1991.

Giger, W., Brunner, P.H., Schaffner, C. (1984). 4-Nonylphenol in Sewage sludge: Accumulation of Toxic Metabolites from Non-ionic Surfactants. *Science*, August. pp. 623-625.

Giger, W., Brunner, P. H., Ahel, M., Mc Evoy, J., Marcomini, A., Schaffner, C. (1987). Organische Waschmittelinhaltsstoffe und deren Abbauprodukte in Abwasser und Klärschlamm, *Gas Wasser Abwasser* 67, pp. 111-122.

Giger, W., Brunner, P.H., Marcomini, A., Siegrist, H. (1989). Behaviour of LAS in sewage and sludge treatment plant. *Tenside Surf. Det.* 26, pp. 95 –100.

Giolando, S., Rapaport, R.A., Larson, R.J., Federle, T.W. (1995). Environmental fate and effects of DEEDMAC: a new rapidly biodegradable cationic surfactant for use in fabric softeners. *Chemosphere* 30, pp. 1076-1083.

Gledhill, W.E. (1995). Review of the anaerobic biodegradation test methodology: relevance to the environmental anaerobic conditions and detergent environmental data - A literature review for the US Soap & Detergent Association.

Harrits, S.M., Hanson, R.S. (1980). Stratification of aerobic methane-oxidizing organisms in Lake Mendota, Madison, Wisconsin. *Limnology and Oceanography* 25, pp. 412-421.

Heijnen, J.J., Mulder, A., Weltevrede R, Hols, J., Van Leeuwen, H.L.J.M. (1991). Large-scale anaerobic-aerobic treatment of complex industrial wastewater using biofilm reactors. *Water Science Technol.* 23 (7-9), pp. 1427-1436.

Heinze, J., Britton, L (1994). Anaerobic biodegradation: environmental relevance, *Proceedings 3rd World Conference on detergents: global perspectives* (ed. A. Cahn), p. 235, AOCS Press, Champaign, IL.

HMSO. (1989). The Assessment of Biodegradability in Anaerobic Digesting Sludge 1988. *Methods for the Examination of Waters and Associated Materials*. Her Majesty's Stationery Office : London.

Holt, M., Matthijs, E., Waters, J. (1989). The concentrations and fate of LAS in sludge amended soils, *Water Research*, 23, No 6, pp. 749 – 759.

Howarth, R.W., Teal, J.M. (1979). Sulfate reduction in a New England salt marsh. *Limnology and Oceanography* 24, pp. 999-1013.

- ISWA (International Solid Waste Association). (1995). Handling, treatment and disposal of of sludge in Europe. Situation Report 1 ISWA Working Group on Sewage sludge and and Water Works, 94 pages, Copenhagen.
- Jones, J.G. (1982). Activities of aerobic and anaerobic bacteria in lake sediments and their effect on the water column. In *Sediment Microbiology*, eds. D.B. Nedwell and C.M. Brown, pp. 107-145. Academic Press: London.
- Jørgensen, B.B. (1977a). Bacterial sulfate reduction within reduced microniches of oxidised marine sediments. *Marine Biology* 41, pp. 7-17.
- Jørgensen, B.B. (1977b). The sulfur cycle of a coastal marine sediment (Limfjorden, Denmark). *Limnology and Oceanography* 22, pp. 814-832.
- Jørgensen, B.B. (1982). Mineralization of organic matter in the sea bed - the role of sulphate reduction. *Nature* 296, pp. 643-645.
- Kaspar, H.F., Tiedje, J.M. (1982). Anaerobic bacteria and processes. In *Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties*, pp. 989-1009. ASA-SSSA: Madison.
- Kayser, R. (1994). Upgrading the wastewater treatment plant of the city of Oldenburg. *Wat. Sci. Tech.* 29, pp. 89-95.
- Klein, Megauhey. (1964). The fate of detergents in septic tank systems and oxidation ponds, Univ. of California, Report No. 64-1, Jan.
- Klopper-Sams, P., Torfs, F., Feijtel, T., Gooch, J. (1996). Effects assessments for surfactants in sludge-amended soils: a literature review and perspectives for TRA, *The Science of the Tot. Environment*, 185, pp. 171-185.
- Klotz, H. (1998). Alcohol ethoxylates (AE) in sewage treatment sludges. Interim results of a German ring test. *Analytica Conference* 98, April 21-24, München.
- Krogh, P. H., Holmstrup and Jensen J. (1997). NERI report N. 69, p. 16.
- Kroiss, H., Schweighofer, P., Frey, W., Matsché, N. (1992). Nitrification inhibition - a source identification method for combined municipal and/or industrial wastewater treatment plants. *Wat. Sci. Tech.* 26, pp. 1135-1146.
- Küchler, T. (1995). Behaviour of surfactants and their influence on the mobility of organic micropollutants in sandy soils with weak sorption capacities, Fraunhofer Institute, Thesis, Potsdam 1995.
- Kuhn, E.P., Zeyer, J., Eicher, P., Schwarzenbach, R.P. (1988). Anaerobic degradation of alkylated benzenes in denitrifying aquifer columns. *Appl. Environ. Microbiol.* 54, pp. 490 - 496.
- Küster, E., Niese, G. (1986). Dumping of refuse and sludges. In *Biotechnology Vol. 8. Microbial Degradations*, ed. W. Schönborn, VCH, pp. 349-362.

- Larson, R. J., Federle, T. W., Shimp, R. J., Ventullo, R. M. (1989). Behaviour of LAS in soil infiltration and ground water, *Tenside Surf. Det.* 26, p. 116.
- Latham, M.J. (1979). The animal as an environment. In *Microbial Ecology: A Conceptual Approach*, eds. J.M. Lynch and N.J. Poole, pp. 115-137. Blackwell Scientific Publications: Oxford.
- Laue, H., Denger, K., Cook, A. M. (1997). Taurine reduction in anaerobic respiration of *Bilophila wadsworthia* RZATAU, *Appl. Environ. Microbiol.*, in press.
- Lie, T. J., Pitta, T., Leadbetter, E. R., Godchaux III, W., Leadbetter, J. R. (1996). Sulfonates: novel electron acceptors in anaerobic respiration, *Arch Microbiol.*, pp. 166, 204-210.
- Lowe, S.E., Jain, M.K., Zeikus, J.G. (1993). Biology, ecology, and biotechnological applications of anaerobic bacteria adapted to environmental stresses in temperature, pH, salinity, or substrates. *Microbiological Reviews* 57, pp 451-509.
- Madsen, T., Rasmussen, H.B. (1994). Evaluation of a method for determining the anaerobic biodegradability of surfactants. Report No. 38 VKI Water Quality Institute, Horsholm, Denmark.
- Madsen, T., Rasmussen, H.B., Nilsson, L. (1995). Anaerobic Biodegradation Potentials in Digested Sludge, *Chemosphere* 31, pp. 4243-4258.
- Madsen, T., Petersen, G., Seiero, C., Torslov J. (1996). Biodegradability and Aquatic Toxicity of Glycoside Surfactants and a Nonionic Alcohol Ethoxylate; *JAOCS*, 73, pp. 929-933.
- Mara, D.D., Pearson, H. (1986). Artificial freshwater environment: Waste stabilisation ponds. In *Biotechnology Vol. 8. Microbial Degradations*, ed. W. Schönborn, pp. 177-206.
- Marcomini *et al.* (1998). Reference 26 from monitoring overview.
- Marcomini, A., Cape, P. D., Lichtensteiger, Th., Brunner, P. H., Giger, W. (1989). Behaviour of aromatic surfactants and PCBs in sludge- treated soil and landfills, *J. Environ. Qual.* 18, pp. 523-528.
- Matsché, N. (1987). Kombiniertes Einsatz von biologischer und chemischer P-Elimination. *Gewässerschutz, Wasser, Abwasser* 98, pp. 13-33 (1987).
- McAvoy, D.C., White, C.E. Moore, B.L., Rapaport, R.A. (1994). Chemical fate and transport in a domestic septic system: sorption and transport of anionic and cationic surfactants. *Env. Tox. Chem.* 13, pp. 213-221.
- Mix-Spagl, K. (1990). Untersuchungen zum Umweltverhalten von Seifen. In : *Umweltverträglichkeit von Wasch- und Reinigungsmitteln (Münchener Beiträge zur Abwasser-, Fischerei- und Flußbiologie, Vol. 44)* R. Oldenbourg Verlag, München.

Moreno, A., Bravo, J., Ferrer, J., Bengoechea, C.(1993). Soap determination in sewage sludge by high-performance liquid chromatography. *JAOCS* 70, pp.667-671.

Mosey, F.E. (1983). Anaerobic processes. In *Ecological Aspects of Used-water Treatment*. Vol. 2. Biological Activities and Treatment Processes, eds. C.R. Curds and H.A. Hawkes, 219-260. Academic Press, London.

Mosey, F.E. (1985). Redox potentials in wastewater treatment. *The Chemical Engineer*, May 1985, pp. 21-24.

Mountfort, D.O., Asher, R.A., Mays, E.L., Tiedje, J.M. (1980). Carbon and electron flow in mud and sandflat intertidal sediments at Delaware Inlet, Nelson, New Zealand. *Applied and Environmental Microbiology*, 39, pp. 686-694.

Nuck, B.A., Federle, T.W. (1996). A batch test for assessing the mineralisation of ¹⁴C-radiolabeled compounds under realistic anaerobic conditions. *Environmental Science and Technology* 30, pp. 3597-3603.

Osburn, Q.W. (1986). Analytical methodology for linear alkylbenzene sulfonate (LAS) in waters and wastes. *J. Am. Oil.Chem. Soc.* 63, pp 257-263.

Pagga, U., Beimborn, D.B. (1993). Anaerobic Biodegradation Test for Organic Coumpounds. *Chemosphere* 27 ; p. 1499.

Painter, H.A., Zabel, T.F. (1988). Review of the Environmental Safety of LAS. Report for ECOSOL and SDIA, Water Research Centre, Medmenham.

Painter, H.A., Zabel, T. (1989). The behaviour of LAS in sewage treatment. *Tenside Surfactants Detergents* 26, pp. 108-115.

Painter, H., Mosey, F., (1992). The question of the anaerobic biodegradability of LAS. *Proceedings of CESIO World Surfactant Congress* , London (UK), 3, pp. 34-43.

Painter, H.A. (1992). Anionic surfactants. In *The Handbook of Environmental Chemistry Vol. 3 Part F*, ed. O. Hutzinger, Springer-Verlag: Berlin.

Painter, H.A. (1994). Detailed Review paper on Biodegradability Testing. OECD Paris.

Petzi, S. (1989). Untersuchungen zum anaeroben Abbau von Seifen. *Seifen-Öle-Fette-Wachse* 115 , pp. 229-232.

Pittinger, C.A., Kimerle, R.A. (1991). Abstract #455, p. 112. SETAC 11th Annual Meeting, Alexandria, VA.

Prats, D., Ruiz, F., Vasquez, B., Rodriguez-Pastor, M. (1997). Removal of anionic and nonionic surfactants in a wastewater Treatment plant with anaerobic digestion. A comparative study. *Wat. Res.* 31, No 8, pp. 1925-1930.

- Press, F., Siever, R. (1986). Rivers: Currents, channels, and networks. In *Earth*, pp. 78-209.
- Puchta, R., Krings, P., Sandkühler, P. (1993). A New Generation of Softeners. *Tenside Surf. Det.* 30, p. 186.
- Randall, C.W., Barnard, J.M., Stensel, H.D. (Eds). (1992). *Water Quality Management Library Vol 5: Design and retrofit of wastewater treatment plants for biological nutrient removal*. Technomic Publishing Company, Lancaster, Pennsylvania.
- Rapaport, R.A., Eckhoff, W.S. (1990). Monitoring linear alkyl benzene sulfonate in the environment: 1973-1986. *Environmental Toxicology and Chemistry* 9, pp. 1245-1257.
- Rapaport, R.A., Larson, R.J., McAvoy, D.C., Nielson, A.M., Trehy, M. (1995). The fate of commercial LAS in the environment. *The CLER Review* 1, pp. 20-31.
- Reiser, R., Toljander, H., Giger, W. (1995). Historic record of alkylbenzenesulfonates in recent lake sediments : input changes and postburial fate of detergent derived chemicals, Organic Geochemistry meeting, S. Sebastian.
- Richards, F.R. (1975). The Cariaco basin (Trench). *Oceanography and Marine Biology Annual Review* 13, pp. 11-67.
- Richards, B.N. (1987). *The Microbiology of Terrestrial Ecosystems*. Longman Scientific and Technical: Harlow.
- Rottiers, A., Boeije, G., Corstanje, R., Decraene, K., Feijtel, T., Matthijs, E., Schowanek, D. (1998). Adaptation of the CAS test system and synthetic sewage for biological nutrient removal. Accepted for publication in *Chemosphere*.
- Rudd, J.W.M., Hamilton, R.D. (1979). Methane cycling in Lake 227 in perspective with some components of the carbon and oxygen cycles. *Archiv für Hydrobiologie ergebnisse der Limnologie* 12, pp. 115-122.
- Salanitro, J.P., Diaz, L.A. (1995). Anaerobic biodegradability testing of surfactants. *Chemosphere* 30, pp. 813-830.
- Schlegel, H.G., Jannasch, H.W. (1981). Prokaryotes and their habitats. In *The Prokaryotes: A Handbook on Habitats, Isolation and Identification of Bacteria*, Vol. 1, eds. M.D. Starr *et al.*, pp. 43-82. Springer-Verlag: New York.
- Schöberl, P., Spilker, R. (1996). Alkylbenzenesulfonat (LAS)-Konzentrationen in Lippe-Sediment eines Rhein-Altermes, *Tenside Surf. Det.*, 33, p 400.

- Schowaneck, D., Feijtel, T., Rottiers, A., Matthijs, E., Decraene, K., Boeije, G., Verstraete, W. (1996). Development and validation of new continuous activated sludge test systems with biological nutrient removal. Proc. IAWQ-NVA Conference Advanced Wastewater Treatment, Amsterdam, September 23-25, 1996, pp. 431 - 433.
- Shelton, D.R., Tiedje, J.M. (1984). General Method for Determining Anaerobic Biodegradation Potential. *Appl. Env. Microbiol.* 47, pp. 850-857.
- Shimp, R.J., Lapsins, E.V., Ventullo, R.M. (1994). Chemical fate and transport in domestic septic system : biodegradation of Linear Alkylbenzene Sulfonate (LAS) and Nitritotriacetic Acid (NTA). *Environmental Toxicology and Chemistry*, 13 (2), pp. 205-212.
- Shokes, R.F., Trabant, P.K., Presley, B.J., Reid, D.F. (1977). Anoxic, hypersaline basin in the northern Gulf of Mexico. *Science* 196, pp. 1443-1446.
- Sieburth, J.M. (1987). Contrary habitats for redox-specific processes: Methanogenesis in oxic waters and oxidation in anoxic waters. In *Microbes in the Sea*, ed. M.A. Sleight, 11-38. Ellis Horwood: Chichester.
- Siegfried, M., Müller, M.T., Baumann, U. (1996). Anaerobic Degradation and Toxicity of Alcohol Ethoxylates in Anaerobic Screening Test Systems; CESIO Congress Barcelona, 3, pp. 61-275.
- Smolenski, W.J., Suflita, J.M. (1987). Biodegradation of cresol isomers in anoxic aquifers. *Applied and Environmental Microbiology* 53, pp. 710-716.
- Steber, J., Wierich, P. (1987). The Anaerobic Degradation of Detergent Range Fatty Alcohol Ethoxylates. Studies with ¹⁴C-Labelled Model Surfactants, *Water Res.* 21, pp. 661-667.
- Steber, J., Gode, P, Guhl, W. (1988). The ecological evaluation of a group of important detergent surfactants - fatty alcohol sulfates. *Soap/Cosmetics/Chemical Specialities* 64, pp. 44-50.
- Steber, J., Wierich, P. (1989). The environmental fate of fatty acid α -sulfomethyl esters: biodegradation studies with a carbon - 14 labelled model surfactant *Tenside*, 26, pp. 406-411.
- Steber, J. (1991). Wie vollständig sind Tenside abbaubar ?, *Textilveredelung* 26, pp. 348-354.
- Steber, J., Birch, R.R. (1995 a). Chemical structures and their biodegradability in the anaerobic environment. Final Report of EU Project Contract No. STEP-CT 91-0154.
- Steber, J., Herold, C.-P., Limia, J.M. (1995 b). Comparative evaluation of anaerobic biodegradability of hydrocarbons and fatty derivatives currently used as drilling fluids. *Chemosphere* 31, pp. 3105 – 3118.

Steber, J., Guhl, W., Stelter, N., Schröder, F.R. (1995 c). Alkyl polyglycosides – ecological evaluation of a new generation of nonionic surfactants, *Tenside Surf. Det.* 32, pp. 515-521.

Strotmann, U.J., Eglsäer, H. (1995). The toxicity of substituted phenols in the nitrification inhibition test and luminescent bacteria test. *Ecotoxicol. Environ. Saf.*, 30 (3), pp. 269-273.

Swanwick, J.D., Shurben, D.G., Jackson, S. (1969). A survey of the performance of sewage sludge digesters in Great Britain. *Journal of the Institute of Water Pollution Control* 6, pp. 639-661.

Tschui, M., Brunner, P.H. 1985). Die Bildung von 4-Nonylphenol aus 4-Nonylphenolmono- und diethoxylat bei der Schlammfäulung, *Vom Wasser*, 65, pp. 9-19.

US-EPA (1987). Design manual Phosphorus Removal. EPA Technology Transfer Report EPA 625/1-87/001, Center for Environmental Research Information, Cincinnati, OH 45268.

Vandepitte, V., Debaere, G. (1997). Biodegradation (aerobic/anaerobic) of dimethyl dodecyl amine oxide. Procter & Gamble ETC, Poster SETAC Congress San Francisco.

Verge, C., Moreno, A., Berna, J.L. (1997). Influence of water hardness on the bioavailability and toxicity of LAS. SETAC, San Francisco (USA).

Wagener, S., Schink, B. (1987). Anaerobic degradation of nonionic and anionic surfactants in enrichment cultures and fixed-bed reactors, *Wat. Res.* 21, pp. 615-622.

Waters, J., Feijtel, T. (1995). AISE-CESIO environmental surfactant monitoring program: outcome of five national pilot studies on LAS, *Chemosphere* 10, p. 1939.

Zehnder, A.J.B. and Stumm, W. (1988). Geochemistry and biogeochemistry of anaerobic habitats. In 'Biology of Anaerobic Microorganisms', ed. A.J.B. Zehnder, 1-35, Wiley-Liss.

APPENDIX

**OVERVIEW OF SURFACTANT MONITORING AND MASS BALANCING
IN ANAEROBIC ENVIRONMENTAL COMPARTMENTS**

Overview of surfactant monitoring and mass balancing in anaerobic environmental compartments									
Name	Surfactant	Type	Monitoring site	Concentration levels (mg/ kg DM)	Environmental conditions	Removal results (%)	Residence Time	Remarks	References
Sulfonates									
	LAS	Commercial	Digester Sludges	2900 - 11900	Anaerobic	M			McEvoy & Giger (1985)
	LAS	Commercial	Digester Sludges	4200 (1200)	Anaerobic	20-30 %		29 digestors/ grab samples	Giger <i>et al.</i> , (1987)
	LAS	Commercial	Digester Sludges	9300 - 18800	Anaerobic	M		Grab samples; one digester	Holt & Bernstein (1992)
	LAS	Commercial	Digester Sludges	6660	Anaerobic	0%			Sedlak <i>et al.</i> , (1986)
	LAS	Commercial	Digester Sludges	4660 (1540)	Anaerobic	M		US five plants - 49 grabs	Rapaport & Eckhoff (1990)
	LAS	Commercial	Digester Sludges	6000 - 9400	Anaerobic	M		Monitoring in Spain & Italy	Waters & Feijtel (1995)
	LAS	Commercial	River sediment	100 - 322	undefined	M			Osburn (1986)
	LAS	Commercial	river SS	2 - 209	not applicable	M			Schoeberl <i>et al.</i> , (1996)

	LAS	Commercial	river sediments	0 -3.3	Undefined	M		Monitoring cores of Lippe sediment from 1939 -1991	Schoeberl & Spilker (1996)
	LAS	Commercial	Anaer. pond water	5.2 - 6.3 mg/l	Undefined Eh	ca. 20 %	20 to 60 days	Over a period of one year	Moreno <i>et al.</i> , (1994).
	LAS		Anaer. pond sedim.	43600					Moreno <i>et al.</i> , (1994).
	LAS	Tridecylbenzene	Fresh water pond	0,48 (spiked)	Anaerobic	0%		Lab study with pond sediment inoculum	Federle & Schwab (1992)
	LAS	Commercial	river sediments	0.49-5.3	undefined	M		Monitoring in Spain & Italy	Waters & Feijtel (1995)
	LAS	Commercial	river sediments	0.01 - 20	undefined	M		Monitoring in Missisipi	Tabor & Barber (1996)
	LAS	Commercial	river sediments	0.6 - 567	undefined	M		Monitoring in Tokyo area	Takada & Ishiwatari (1987)
	LAS	Commercial	river sediments	5.6	undefined	M			Marcomini & Giger (1987)
	LAS	Commercial	Marine sediments	10 to 30	Undefined Eh	M			Matthijs <i>et al.</i> (1986)
	LAS	Commercial	Marine sediments	11 to 30	Undefined Eh	M			Hon-Nami & Hanya (1980)
	LAS	Commercial	Landfill	245 (bottom) - 9160 (top)	Undefined Eh	M		Landfill receiving digested sludge	Marcomini <i>et al.</i> , (1989)
	SAS	Commercial	Digester Sludges	648 - 738	Anaerobic	0%	40 days	10 day sampling	Field <i>et al.</i> , (1995)
Sulfates	AES		Septic Tanks		Anaerobic	72-81 %		No environment characterisation	Birch <i>et al.</i> (1992)
	AS		Digester			66%			to be completed
Soaps									
	Soap		Digester Sludge	18000 - 51900	Anaerobic	70-75 %	26 days	Sampling over several treatment plants	Moreno <i>et al.</i> , (1993)

Alcohol Ethoxylates	AE	Commercial	Digester	2600 (400)	Anaerobic	65%	26 days	One day sampling	Prats <i>et al.</i> , (1997)
	AE	Commercial	Digester	< 500 typically	Anaerobic	M		Monitoring programme in Germany (Tegewa)	Klotz, 1998
Alkylphenol Ethoxylates	NP		Digester Sludges	900 (600)	Anaerobic	M		29 digestors	Giger <i>et al.</i> , (1987)
	NP		Digester Sludges	450 - 2530	Anaerobic	M		Several digestors	Giger <i>et al.</i> , (1984)
	NP		Digester Sludges	1600	Anaerobic	M			Ahel & Giger (1985)
	NP + NPEO		Digester Sludges	3 - 540	Anaerobic	M			Madsen <i>et al.</i> (1997)
	NP		Digester Sludges	35 - 95	Anaerobic	M			Kujawa <i>et al.</i> (1996)
	NP		Digester Sludges	545 - 1000	Anaerobic	M		Non Indicated	Ahel <i>et al.</i> , (1994)
	NP		Digester Sludges	130 - 400	Anaerobic	M			Kunkel (1987)
	NP		Digester sludges	640 - 2200	Anaerobic	M			Brunner <i>et al.</i> , (1988)
	NP		Digester sludges	137 - 470	Anaerobic	M			Lee & Peart (1995)
	NP		Digester sludges	1200	Anaerobic	M			Marcomini & Giger (1987)
	NP		Digester sludges	400 - 1200	Anaerobic	M			Wahlberg <i>et al.</i> , (1990)
	NP		Digester sludges	20-350	Anaerobic	M			Chaloux <i>et al.</i> , (1994)
	NP		Digester sludges	21-1193	Anaerobic	M			Jobst (1995)
	NP		River sediment	0.29 - 6.73	undefined	M			Lee & Peart (1995)
	NP		River sediment						
	NP		River sediment	0.9	undefined	M			Marcomini & Giger (1987)

	NP		River sediment	0.02-0.04	undefined	M			Chaloux <i>et al.</i> , (1994)
	NP		Marine sediment	0.006-0.07	undefined	M			Chaloux <i>et al.</i> , (1994)
	NP		Subsurface (aquifer)	0.2 - 0.96 ug/l (range 0.0 - 33)	Mixed Eh/Aerobic	90%	2-4 h	Soil biodegradation of NP	Ahel <i>et al.</i> , (1996)
	NP1EO		Digester Sludges	5 - 40	Anaerobic	M			Kunkel (1987)
	NP2EO		Digester Sludges	<3	Anaerobic	M			Kunkel (1987)
	NP1EO		Digester sludges	90 - 680	Anaerobic	M			Brunner <i>et al.</i> , (1988)
	NP2EO		Digester sludges	20 -220	Anaerobic	M			Brunner <i>et al.</i> , (1988)
	NP1EO		Digester sludges	220	Anaerobic	M			Marcomini & Giger (1987)
	NP2EO		Digester sludges	30	Anaerobic	M			Marcomini & Giger (1987)
	NP1EO		Digester sludges	20 -190	Anaerobic	M			Wahlberg <i>et al.</i> , (1990)
	NP2EO		Digester sludges	1.0-50.0	Anaerobic	M			Wahlberg <i>et al.</i> , (1990)
	NP1EO		river sediment	0.8	undefined	M			Marcomini & Giger (1987)
	NP2EO		river sediment	0.7	undefined	M			Marcomini & Giger (1987)
	NP1EO		Subsurface (aquifer)	0.04 - 0.91 ug/l (range 0.0 - 4.9)	Mixed Eh/Aerobic	99%	2-4 h	Soil biodegradation of NP	Ahel <i>et al.</i> , (1996)
	NP2EO		Subsurface (aquifer)	0.01 - 0.33 ug/l (range 0.00 - 23)	Mixed Eh/Aerobic	99%	2-4 h	Soil biodegradation of NP	Ahel <i>et al.</i> , (1996)
	NP1EC		Subsurface (aquifer)	2.9 - 10.9 ug/l (range 0.00 - 13.1)	Mixed Eh/Aerobic	80%	2-4 h	Soil biodegradation of NP	Ahel <i>et al.</i> , (1996)

REFERENCES FIELD MONITORING

Ahel, M., Giger, W. (1985). Determination of alkylphenols and alkylphenol mono- and diethoxylates in environmental samples by high performance liquid chromatography. *Anal. Chem.* 57, pp. 1577-1583.

Ahel, M. Giger, W, Koch, M. (1994). Behaviour of alkylphenol polyethoxylate surfactants in the aquatic environment - I. Occurrence and transformation in sewage treatment. *Wat. Res* 28, pp. 1131-1142.

Ahel, M. Schaffner, C., Giger, W. (1996). Behavior of Alkylphenol ethoxylate surfactants in the Aquatic environment - III. Occurrence and elimination of their persistent metabolites during infiltration of river water to groundwater. *Wat. Res.* 30, pp. 37-46.

AISE/CESIO monitoring on European sludges.

Amano, K., Fukushima, T. and Nakasugi, O. (1992). Diffusive exchange of linear alkylbenzenesulfonates (LAS) between overlying water and bottom sediment. *Hydrobiologia* 235/236, pp. 491-499.

Berna, J.L., Moreno, A. Prats, D., Bevia. F.R., (1989). The fate of LAS in the environment. *Tenside* 26, pp. 101-107.

Bitrch, R.R., Geldhill, W.E., Larson R.J. and Nielsen, A.M. Proc. 3rd CESIO International Surfactants Congress and Exhibition, London, 1992. pp. 26-33.

Brunner, P.H., Capri, S., Marcomini, A. and Giger, W. (1988). Occurrence and behaviour of linear alkylbenzene sulphonates, nonylphenol, nonylphenol mono- and nonylphenol diethoxylates in sewage and sewage sludge treatment. *Wat. Res.* 22, pp. 1465-1472.

Cavalli, L., Gellera, A., Landone (1993). LAS removal and biodegradation in a waste water treatment plant. *Env. Tox. Chem* 12, pp. 1777-1788.

Chaloux, N., Bayona, J.M. and Albaiges, J. (1994). Determination of nonylphenols as pentafluorobenzyl derivatives by capillary gas chromatography with electron capture and mass spectrometric detection in environmental matrices. *J. Chromatogr.* 686, pp. 275-281.

DiCorcia, A., Samperi, R., Bellioni, A., Marcomini, A., Zanette, M., Lenr, K., Cavalli, L. (1994). LAS Pilot study at the "Roma-Nord" sewage treatment plant and in the Tiber river. *Riv. Ital. Sost. Grasse* 71, pp. 467-475.

Federle, T.W., Schwab, B.S. (1992). Mineralization of surfactants in anaerobic sediments of a laundromat wastewater pond. *Wat. Res.* 26, pp.123-127.

- Fernandez, P., Alder, A.C., Suter, M.J.F., Giger, W. (1996). Determination of the quaternary ammonium surfactant ditallowdimethylammonium in digested sludges and marine sediments by supercritical fluid extraction and liquid chromatography with post-columns ion-pair formation. *Anal. Chem.* 68, pp. 921-929.
- Field, J.A., Field, T.M., Poiger, T., Siegrist, H., Giger, W. (1995). Fate of secondary alkane sulfonate surfactants during municipal wastewater treatment. *Wat. Res.* 29, pp. 1301-1307.
- Giger, W., Brunner, P.H., Schaffner, C. (1984). 4-Nonylphenol in Sewage sludge: Accumulation of Toxic Metabolites from Non-ionic Surfactants. *Science* 225, vol. 10, August. pp. 623-625.
- Giger, W., Brunner, P.H., Ahel, M., McEvoy, J. Marcomini, A., Schaffner, C. (1987). Organic detergent components and their degradation products in waste water and sludge. *Gas, Wasser, Abwasser* 67, pp. 111-122.
- Holt, M.S., Bernstein, S.L. (1992). Linear alkylbenzenes in sewage sludges and sludge amended soils. *Wat. Res.*; 26: pp. 613-624.
- Holt, M.S., Mitchell, G.C. and Watkinson, R.J. (1992). The environmental chemistry, fate and effects of non-ionic surfactants. In: *the Handbook of Environmental Chemistry*, Vol. 3, Part F. Anthropogenic Compounds, Detergents. (ed. N.T. de Oude), pp. 89-144, Springer Verlag, Berlin.
- Hon-Nami, H., Hanya, T. (1980). Linear alkylbenzene sulfonates in river, estuary and bay water. *J. Jap. Limnol* 41, pp. 1-5.
- Jobst, H. (1995). Chlorphenole und Nonylphenole in Klarschlammen Teil 1: Vorkommen in Klarschlammen westdeutscher Klaranlagen aus den Jahren 1987 bis 1989. *Acta hydrochim hydrobiol.* 23, pp. 20 - 25.
- Kujawa, M., Schnaak, W. and Kuechler, T. (1996). Occurrence of organic pollutants in sewage sludge and influence of surfactants on their mobility in amended soils. Fraunhofer Internal Report
- Kunkel, E. (1987). Umwelanalytik von Tensiden. *Tenside Surf. Det.* 24, pp. 280 -285.
- Lee, H.B., Peart, T.E. (1995). Determination of 4-nonylphenol in effluents and sludge from sewage treatment plants, 67, pp. 1976 - 1980.
- Madsen, T., Kristensen, P., Samsoe-Petersen, L., Torslov, J., Rasmussen, J.O. (1997). Application of sludge on farmland - quality objectives, level of contamination and environmental risk assessment. Proc. Specialty conference on management and fate of toxic organics in sludge applied to land. Copenhagen, Denmark, 30 April - 2 May 1997.
- Marcomini, A., Giger, W. (1987). Simultaneous determination of linear alkylbenzene sulfonates, alkylphenol polyethoxylates, and nonylphenol by high performance liquid chromatography. *Anal. Chem.* 59, pp. 1709 - 1715.

- Marcomini, A., Capel, P.D., Lichtensteiger, T., Brunner, P.H. and Giger, W. (1989). Behaviour of aromatic surfactants and PCBs in sludge treated soil and landfill. *J. Environ. Qual.* 18, pp. 523-528.
- Matthijs, E., De Henau, H. (1987). Determination of LAS in aqueous samples, sediments sludges and soils using HPLC. *Tenside* 24, pp. 193-199.
- McAvoy, D.C., Eckhoff, W.S., Rapaport, R.A. (1993). Fate of Linear Alkylbenzene Sulfonate in the environment. *Environ. Tox. and Chem.* 12; pp. 977-987.
- McEvoy, J., Giger, W. (1985). Accumulation of linear alkyl benzene sulphonates in sewage sludges. *Naturwissenschaften* 72, pp. 429-431.
- Moreno, A. Bravo, J., Ferrer, J., Bengoechea, C. (1993). Soap determination in sewage sludge by high-performance liquid chromatography. *JAOCS* 70, pp. 667-671.
- Moreno, A., Ferrer, J., Ruiz, B.F., Prats, D., Vazquez, B., Zarzo, D. (1992). Monitoring of Linear alkylbenzene Sulfonate removal in a lagoon treatment system CESIO international surfactants congress, London .
- Osburn, Q.W. (1986). Analytical methodology for linear alkylbenzene sulfonate (LAS) in waters and wastes. *J. Am. Oil Chem. Soc.* 63, pp. 257 - 263.
- Painter, H.A., Zabel, TF (1989). The behaviour of LAS in sewage treatment. *Tenside Surfactants Detergents* 26, pp.108-115.
- Prats, D., Vazquez, B., Rodriguez-Pastor, M. (1997). Removal of anionic and nonionic surfactants in a wastewater treatment plant with anaerobic digestion. A comparative study. *Wat. Res.:* 31:8., pp. 1925-1930.
- Rapaport, R.A. and Eckhoff, W.S. (1990). Monitoring Linear alkyl benzene sulfonate in the environment: 1973-1986. *Environ. Tox.* 9; pp. 1245-1257.
- Schoeberl, P., Klotz, H., Spilker, R. (1996a). Alkylbenzolsulfonat (LAS)-Monitoring. Teil 3: LAS -Gehalte der Schwebstoffe verschiedener deutscher Fließgewässer. *Tenside Surf. Det.* 33, pp. 329-335.
- Schoeberl, P., Spilker, R. (1996b). Alkylbenzolsulfonat (LAS)-Konzentrationen im Lippe-Sediment eines Rhein-Altarmes. *Tenside Surf. Det.* 33, pp. 400-403.
- Sedlak, R.I., Booman, K.A. (1986). A Study of LAS and alcohol ethoxylate removal at a municipal wastewater treatment plant. US SDA annual Convention, Boca Raton, Fla.
- Sweetman, A.J. (1994). Development and application of a multi-residue analytical method for the determination of n-alkanes, linear alkylbenzenes, polynuclear aromatic hydrocarbons and 4-nonylphenol in digested sewage sludges. *Wat. Res.* 2!, pp. 343-353.

Tabor, C.F., Barber, L.B. (1996). Fate of linear alkylbenzene sulfonate in the Mississippi river. *Environ.Sci. Technol.* 30, pp. 161 - 171.

Takada, H., Ishiwatari, R. (1987). Linear alkylbenzenes in urban riverine environments in Tokyo: distribution, source, and behaviour. *Environ. Sci Technol.* 21, pp. 875 - 883.

Takada, H., Ishiwatari, R. and Ogura, N. (1992). Distribution of linear alkylbenzenes (LABs) and linear alkylbenzene sulfonates (LAS) in Tokyo Bay sediments. *Estuarine, Coastal and Shelf Science* 35, pp. 141-156.

Tschui, M. and Brunner, P.H. (1985). Die Bildung von 4-Nonylphenol aus 4-Nonylphenolmono- und diethoxylat bei der Schlammfäulung. *Vom Wasser* 65, pp. 9-19.

Wahlberg, C., Renberg, L and Wideqvist, U. (1990). Determination of nonylphenol and nonylphenol ethoxylates as their pentafluorobenzoates in water, sewage sludge and biota. *Chemosphere* 20, pp. 179-195.

Waters, J. & Feijtel, T.C.J. (1995). AISE/CESIO Environmental surfactant monitoring programme; outcome of five national pilot studies on linear alkylbenzene sulphonate (LAS). *Chemosphere* 30, pp. 1939-1956.