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ASSESSMENT OF THE BIODEGRADATION OF CHEMICALS IN THE MARINE ENVIRONMENT

CONTENTS

SUMMARY	1
SECTION 1. INTRODUCTION	2
SECTION 2. BACKGROUND	3
SECTION 3. DIFFERENCES BETWEEN MARINE, ESTUARINE AND LIMNIC ENVIRONMENTS IN RELATION TO BIODEGRADATION OF CHEMICALS	4
3.1. INTRODUCTION	4
3.2. FACTORS AFFECTING MICROBIAL NUMBERS AND VARIETY	5
3.3. MICROBIAL POPULATIONS	5
3.3.1. Freshwater and estuarine microorganisms	5
3.3.2. Marine microorganisms	5
3.3.3. Sediment microorganisms	6
3.4. SUBSTRATES USED BY MICROORGANISMS	6
3.4.1. Freshwater substrates	6
3.4.2. Estuarine and coastal water substrates	7
3.4.3. Marine substrates	7
3.5. MICROBIAL ACTIVITY	8
3.5.1. Freshwater microorganisms	8
3.5.2. Marine microorganisms	8
3.5.3. Sediment microorganisms	8
SECTION 4. ASSESSMENT OF EXISTING TEST METHODS	10
4.1. INTRODUCTION	10
4.2. REVIEW OF EXISTING TEST METHODS	10
4.2.1. Screening Methods	10
4.2.2. Simulation Tests	13
4.2.3. Possibilities and Limitations of Present Test Methods	17
4.3. BIODEGRADABILITY DATA FOR MARINE AND ESTUARINE ENVIRONMENT	18
4.4. COMPARISON OF BIODEGRADATION OF CHEMICALS IN THE MARINE, ESTUARINE AND RIVER ENVIRONMENTS	20

SECTION 5.	SUGGESTED APPROACH FOR INVESTIGATING BIODEGRADABILITY OF CHEMICALS IN ESTUARINE AND MARINE ENVIRONMENTS	23
5.1.	INTRODUCTION	23
5.2.	BIODEGRADATION POTENTIAL OF THE MARINE ENVIRONMENT	23
5.3.	TESTING STRATEGY FOR ASSESSING MARINE BIODEGRADABILITY	25
SECTION 6.	PROPOSAL FOR TEST GUIDELINES	28
6.1.	INTRODUCTION	28
6.2.	BASIC REQUIREMENTS FOR TEST PROTOCOLS	28
6.3.	SIMULATION TEST FOR SOLUBLE CHEMICALS	28
6.4.	SIMULATION TEST FOR CHEMICALS ASSOCIATED WITH SEDIMENTS	29
SECTION 7.	CONCLUSIONS AND RECOMMENDATIONS	30
APPENDIX A	GLOSSARY OF TERMS	32
APPENDIX B.	OVERVIEW OF RELEVANT LITERATURE ON BIODEGRADATION IN THE MARINE AND ESTUARINE ENVIRONMENT	35
APPENDIX C	CRITERIA FOR THE INVESTIGATION OF BIODEGRADABILITY IN MARINE OR ESTUARINE ENVIRONMENT	45
C.1.	PREREQUISITES	45
C.2.	DESIGN FEATURES OF A SIMULATION TEST	45
C.2.1.	Size of test vessels	45
C.2.2.	Test media	45
C.2.3.	Collection of test media	46
C.2.4.	Application of test substance and incubation procedures	46
C.2.5.	Sampling and observation	47
C.3.	DATA EVALUATION	47
BIBLIOGRAPHY	48
MEMBERS OF THE TASK FORCE	54
MEMBERS OF THE ECETOC SCIENTIFIC COMMITTEE	55

SUMMARY

Several test methods assessing the biodegradation potential of a chemical has been developed and validated for freshwater situations. Internationally accepted standard methods are now available and the data available covers a wide range of chemicals. In contrast to freshwater situations no generally accepted methods are known to estimate the biodegradation potential of chemicals in marine situations. The literature research on marine biodegradation reveals that apart from a few examples research has focused on degradation of crude oil and petroleum derivatives.

OECD published recently a set of "Marine Ready Biodegradability Tests" which may, to a certain extent, predict the fate of a chemical in heavily polluted estuaries. It is doubtful whether the result obtained from these test guidelines would be representative for open sea or coastal situations where conditions considerably differ from those in freshwater or estuarine situations.

The marine environment is characterised by a low concentration of nutrients (P,N) and organic substrate. Except for the surface microlayer, where large populations of marine microorganisms have been recorded, the bacterial counts are orders of magnitude lower than those observed in freshwater situations. When developing a biodegradation test for marine situations both physico-chemical and biological parameters which are typical for this environment should be maintained. This implies a low test concentration which precludes the monitoring of the fate of a chemical using non-specific analytical methods.

A strategy is outlined and schematically represented as a tier approach to assess the hazard of a chemical which may reach the marine environment. The test strategy is designed to eliminate as far as possible unjustified testing.

Biological processes for biodegradation in freshwater and the marine environment do not differ. Thus, in theory, those chemicals which are readily biodegradable in freshwater will also degrade under marine situations. The available information only supports this conclusion with regard to ready biodegradability.

Marine biodegradability tests should be carried out using test protocols simulating the situation and the receiving environment as closely as possible. Proposals for the design of possible test protocols are described.

It is recommended that, when more knowledge is obtained about marine biodegradation, the strategy should be reconsidered.

SECTION 1. INTRODUCTION

The current approach to determining the biodegradability of chemicals in aquatic systems is focused on freshwater situations and laboratory simulations of waste water treatment plants.

There has been increasing concern about the pollution of estuaries and the sea, particularly the North Sea, from substances discharged directly or carried into the sea by the rivers. Some synthetic chemicals have been identified in seawater and sometimes even in marine organisms. The OECD has produced a set of guidelines which claim to assess the "ready biodegradability" of chemicals in the marine environment. There is some doubt about the value of these tests which do not simulate estuarine or marine environments. There is nevertheless a need to improve our understanding of the fate of chemicals in the marine environment. Accordingly an ECETOC Task Force was constituted with the following terms of reference:

- review the existing information on the biodegradation of chemicals in the marine environment considering both aerobic and anaerobic conditions,
- critically review the test methods currently used to measure biodegradation in the marine environment,
- develop a strategy for investigating biodegradability of chemicals in estuarine and marine environments.

A glossary of terms is included in Appendix A.

A literature review of the published information on the biodegradability of organic chemicals in the marine environment was made using six data bases¹. Most of the papers retrieved were concerned with either the turnover of naturally-occurring compounds or the biodegradability of two groups of chemicals (hydrocarbons and surfactants). Information on other chemicals appeared to be limited. An overview of the most relevant articles is given in Appendix B.

¹. Chemical Abstracts (from 1967 onwards); Biosis (from 1969 onwards); Oceanic Abstracts (from 1964 onwards); Aquatic Science and Fisheries (from 1978 onwards); Watemet (from 1971 onwards) and Cependex Plus (from 1970 onwards).

SECTION 2. BACKGROUND

The purpose of assessing the biodegradability of substances is to make a judgement on their disappearance or persistence when they reach the environment. The degradation in the environment is brought about by a combination of physico-chemical and biological processes that participate in the natural geo-chemical cycle. The biological transformations are a result of the interactions of the substances with living organisms and theoretically the materials are exposed to the total metabolic capability of the living world. This cannot be simulated in laboratory test systems and consequently in many cases the biodegradation potential of the real environment is frequently underestimated.

Over the past thirty years, the development of test methods and strategies for assessing the biodegradability of organic chemicals in the aquatic environment has been predominantly concerned with freshwater systems such as rivers, lakes and wastewater treatment plants. This has resulted in a range of standard test methods which have been incorporated into internationally-accepted guidelines and legislation (e.g. OECD, 1981; EEC 1984). Despite efforts to improve those methods only a limited number of procedures are available which reliably predict the fate of substances in real world situations.

In contrast to freshwater, as yet, there are no generally accepted methods for the determination of biodegradability of substances in brackish water or marine situations (seawater). A first draft with a set of guidelines for assessing "ready biodegradation", derived from the freshwater guidelines was recently published (OECD, 1990).

There are several reasons, which explain the imbalance in the development of methods between fresh and marine water environments. On one hand the potential problems of marine pollution were perceived much later than in freshwater, and on the other many more obstacles arise when trying to develop a test method. There is also a variety of marine situations (estuary, coastal, open sea) which affect the composition of biomass, organic carbon content, salinity and mineral composition. So far no conditions simulating and representative for all marine conditions have been suggested or published.

SECTION 3. DIFFERENCES BETWEEN MARINE, ESTUARINE AND LIMNIC ENVIRONMENTS IN RELATION TO BIODEGRADATION OF CHEMICALS

3.1. INTRODUCTION

The hydrosphere comprises a multitude of extremely varied microbial habitats. Although salinity is the most obvious parameter that differentiates marine, estuarine and freshwaters, it is the low concentration of organic substances and inorganic nutrients (especially N and P) which have the greatest effect on the abundance and activity of the marine microorganisms. Marine ecosystems, particularly away from the coast, are more or less static and here the residence time of a chemical can be extremely long. In contrast freshwaters, particularly river systems, are dynamic in that materials like nutrients, substrates and contaminants, are transported through them and the residence time, during which these materials can be utilised, is limited.

Freshwaters include lakes and rivers whose nutrient status vary from oligotrophic to eutrophic. The nature of the soil, rock and vegetation of the catchment area influences the water composition. The temperature of freshwater, depending on depth is subject to a greater seasonal variation than that of seawater.

Estuaries, at the interface of freshwater and marine environments, exhibit a salinity gradient between the river and sea and may even be stratified. The water composition reflects that of the river above and, in the tidal zone, the coastal water. Sedimentation rates are high and therefore sediments are often an important niche in the estuarine environment. As in many freshwater environments there is a dynamic relationship between the sediment and the water column and this is particularly true in estuarine and coastal waters. Frequent disturbance of the sediment, especially in the tidal zones, mixes the microbial populations and releases inorganic nutrients and metabolites from anaerobic regions in the sediment into the water column. These may be further utilised by aerobes and facultative anaerobes in the water column, many of which are attached to suspended sediment particles.

Coastal marine waters differ substantially from the open ocean in salinity and temperature as well as in concentrations of inorganic nutrients and organic substrates due to terrestrial discharges. This depends on the proximity to an estuary and the depth of the coastal shelf. In the open ocean there are also large variations in water composition with increasing depth and hydrographical conditions.

3.2. FACTORS AFFECTING MICROBIAL NUMBERS AND VARIETY

The principle factors that determine the variety and growth of microbial genera include salinity, pH, temperature, dissolved oxygen content, redox potential, concentration of inorganic nutrients and trace elements and the concentration of organic substrates. Light affects the activity of photosynthetic bacteria and algae. Each of these factors vary not only between the major aquatic environments but also within individual niches. They also change diurnally, seasonally and, particularly in the case of estuaries, with the tidal cycles.

3.3. MICROBIAL POPULATIONS

3.3.1. Freshwater and estuarine microorganisms

The autochthonous populations of river, estuarine and coastal water organisms comprise a wide variety of microbial genera but this is enhanced by an allochthonous population from the continuous inflow of soil and wastewater discharges.

The autochthonous population may be considered as the free swimming microbes in the water column, although it may include organisms attached to suspended particles. In many rivers the majority of the microorganisms are free swimming whereas in estuarine waters the majority of bacteria may be attached to suspended particles (Goulter, 1986).

The numbers of viable bacteria in river waters are normally about 10^6 /ml in upland rivers and up to 10^7 /ml in the lower reaches (Bent and Goulter, 1981; Goulter, 1986; Rimes and Goulter, 1986). These are higher than the number found in agricultural land drainage water of between 10^2 and 10^5 /ml (Evans and Owens, 1973) which suggests that the autochthonous population accounts for 90%, or more, of the total population. This does not detract, however, from the metabolic importance of allochthonous genera. Thurston (1991) found that the majority of nitrifying bacteria in the Tees estuary originated from runoff rather than growth of an autochthonous population in the estuarine waters. In addition a significant number of heterotrophic viable bacteria (10^7 /ml, Kuenemann and Battersby, 1992) are released from sewage treatment plants.

3.3.2. Marine microorganisms

The numbers of bacteria in marine waters are considered to be lower than those in freshwaters (Bartholomew and Pfaender, 1983; Guerin, 1989; Gonzalez *et al*, 1990). In a literature review Austin (1988) concludes that the bacterial population in seawater is generally between 10^3 to 10^6 /ml. There are large populations of marine microorganisms at the surface microlayer where up to 10^8 /ml

have been recorded. There is a decline in the numbers down the water column so that the average numbers are very low.

Many marine microorganisms are halophilic but others are only halotolerant. In the open ocean most are adjusted to life in oligotrophic conditions in contrast to the eutrophic conditions of many freshwater environments. Microorganisms in marine habitats exist either in a free-swimming state or in association with suspended particles.

3.3.3. Sediment microorganisms

The upper layer of sediment is colonised by many aerobic and facultative anaerobic microorganisms attached to particles that frequently become suspended in the water column whenever the sediment is disturbed.

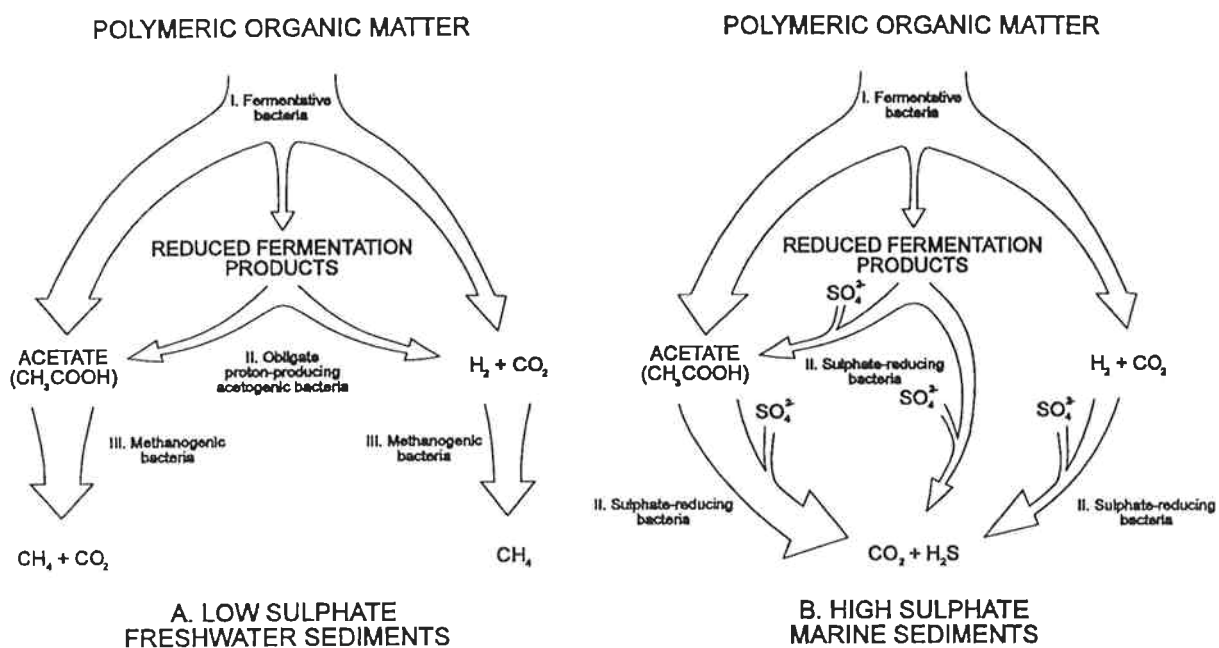
Much of the sediment is anoxic and colonised by four distinct groups of bacteria. Fermentative bacteria convert primary polymeric organic matter into a variety of metabolites, including alcohols, fatty acids, acetate, molecular hydrogen and carbon dioxide. In sediments, low in sulphate (e.g. freshwater), obligate proton reducing acetogenic bacteria convert the reduced fermentation products to acetate, molecular hydrogen and CO₂. Methanogenic bacteria then convert these substrates to methane and CO₂. In sulphate rich marine sediments, sulphate reducing bacteria have a competitive advantage and oxidise the fermentation products via low molecular weight organic acids to CO₂ with the concomitant reduction of sulphate to sulphide (Ward and Winfrey, 1985). These reactions are illustrated in Figure 1 and indicate that the critical stages in breaking down an organic chemical to simple, easily metabolisable compounds are common in both freshwater and marine sediments.

3.4. SUBSTRATES USED BY MICROORGANISMS

3.4.1. Freshwater substrates

Rivers upstream of urban and industrial areas normally contain little organic material, although following heavy rain they become rich in soil and debris from vegetation. Downstream of these areas, effluent discharges greatly increase the substrate load available to the microbial population. As a result of this increase the biochemical oxygen demand (BOD) of this population often exceeds the oxygen transfer rate. As a result the dissolved oxygen levels may decrease from near saturation to a lower level, or exceptionally even to zero, throughout the water column except for a very shallow surface layer (Bernier, 1971; Patrick and De Lanne, 1972; Howarth and Teal, 1979).

Figure 1. Carbon and electron flow models for the decomposition of organic matter in anaerobic sediments (from Ward and Winfrey, 1985)



The thickness of the arrows is proportional to the amount of carbon and electron flow

3.4.2. Estuarine and coastal water substrates

Compounds which are not degraded during the residence time of the material in the river may ultimately be discharged into the sea. The concentrations of substrates in the estuaries and the sea will vary with the input, the freshwater flow into the estuary and coastal water, tidal cycle, season and decay rate. Compounds which are not removed or degraded during the residence time of the compound in wastewater treatment works are ultimately discharged into a river estuary or into the sea. Lung *et al* (1990) estimated that 20 to 30% of all United States publicly owned treatment works (POTW) discharges enter estuaries and coastal waters directly. In the Netherlands, less than 5% of the POTWs discharges directly enter estuaries and coastal waters (de Greef and de Nijs, 1990).

3.4.3. Marine substrates

Primary production of biomass in the marine environment is estimated at between $22-28 \times 10^{15}$ g C/annum (Zehnder, 1982). The marine environment away from the coastal zones is low in organic substrates and there is also a strong competition between phytoplankton and bacteria for available

nutrients (N and P). Material released by decaying phytoplankton are the major source of organic carbon for marine bacteria. Weisse *et al* (1990) reported that in phytoplankton more than 50% of the primary production is channelled through the microbial loop.

Those chemicals which are not removed under freshwater conditions are normally flushed out of rivers into the sea. The sea and underlying sediments are therefore a major sink of most non-volatile chemicals. Even poorly soluble materials such as PCBs may be found back in the sea. Ernst (1988) demonstrated that several known persistent xenobiotic compounds are found in the North Atlantic, albeit at very low levels (10-150ng/l).

3.5. MICROBIAL ACTIVITY

3.5.1. Freshwater microorganisms

The activity of freshwater microorganisms varies considerably with the quality of the freshwater system. In upland waters the major substrates are from soil and vegetation and activity is normally substrate limited. In contaminated waters activity can be high but is often limited by the oxygen transfer rate and/or the toxicity of influent organic material from domestic as well as industrial sources (Austin, 1983).

3.5.2. Marine microorganisms

In the open ocean Meyer and Morita (1989) claimed that survival of marine heterotrophic bacteria depends on an ability to withstand long periods of nutrient deprivation. Most of the organic matter entering the sea is extensively degraded in surface waters. Consequently levels of carbon below the photic zone are low at 0.4 to 0.7mg/l for dissolved organic carbon (DOC) and 3 to 10µg/l for particulate organic carbon (POC). As this material consists mainly of refractory compounds, metabolic activity is usually low in the marine environment. Zehnder (1982) quotes average ages of deep water DOC between 3,400 and 6,000 years. This DOC originates from natural sources.

3.5.3. Sediment microorganisms

Microbes attached to sediment particles that are regularly suspended in the water column, often classified as part of the water column population, are important in the degradation of organic debris. Present evidence suggests that the particle-associated bacteria may be more metabolically active than their planktonic counterparts in marine environments (Wardell *et al*, 1983).

In anoxic sediment, as described above, the levels of sulphate determine the relative activity of acetogenic, methanogenic and sulphate reducing bacteria. The importance of sulphate reducing

bacteria in sediments has been reviewed by Gibson (1990). The sediments of estuarine and marine waters are the primary habitats of sulphate reducing bacteria. Jørgenson (1977, 1982) found that over 50% of the organic carbon from detritus input to coastal marine sediments rich in sulphate was finally released as carbon dioxide through the activity of sulphate reducing bacteria.

SECTION 4. ASSESSMENT OF EXISTING TEST METHODS

4.1. INTRODUCTION

Experimental systems for studying the fate of chemicals in the marine environment have tended to concentrate on the fate and effects of oil spills or the biogeochemical cycling of organic matter. In recent years attempts have been made to modify existing screening or "ready biodegradability" tests for use with marine inocula and to develop test systems where the biodegradation of a chemical can be studied under conditions which simulate a particular marine environment. These various test methods are reviewed below.

4.2. REVIEW OF EXISTING TEST METHODS

4.2.1. Screening Methods

Screening tests for assessing biodegradability must be applicable to a wide range of chemicals. Hence, non-specific analysis is used and biodegradation is measured as the difference in oxygen consumption, DOC removal or carbon dioxide evolution between vessels containing a known amount of test substance and blanks. The precision of the test is dependent on the size of this difference. A reliable measure of biodegradation requires high concentrations of test substance (e.g. 2 to 100mg/l) as compared to environmentally relevant levels (e.g. <1µg/l). This may affect the biodegradability of the test chemical *in situ*.

Street (1984) proposed that a screening method for assessing the biodegradability of chemicals in seawater was needed, as predictions of environmental fate based on the results of standard "ready biodegradability" tests failed to take into account the lower microbial concentration and reduced species diversity in the marine environment compared with freshwaters. Biodegradation may also be limited by the low concentration of essential nutrients (P and N) in the open sea. Further nutrient limitation becomes an even greater problem when unrealistically high concentrations of test substance are used in screening tests.

Street (1984) compared unamended seawater with standard BOD dilution water (inoculated with secondary effluent) as a medium to assess the biodegradability of organic compounds using the five day BOD test (BOD₅). The rates of biodegradation (oxygen uptake) for sodium acetate, sodium benzoate, sodium dodecyl sulphate and glucose/glutamic acid mixture were similar in both media but the BOD₅ values in seawater were generally 10-20% lower than those in BOD dilution water. These differences were not thought to be due to nutrient limitation as the addition of N and P only