Biodegradation **7**: 109–120, 1996.

A dynamic river model for biodegradability studies

Investigations with selected aromatic compounds at low concentrations and comparison with aquatic batch tests

Petra Koziollek[1], Hans-Joachim Knackmuss[1], Klaus Taeger[2] & Udo Pagga[2*]
[1] *Institute for Microbiology, University of Stuttgart, Allmandring 31, D-70569 Stuttgart, Germany;* [2] *BASF AG Ecology, D-67056 Ludwigshafen, Germany; (* author for correspondence)*

Accepted 27 May 1995

Key words: batch shake flask tests, biodegradation tests, 2,4-dinitrophenol, low test concentrations, naphthalene-1-sulphonic acid, river model, sulphanilic acid

Abstract

The objective of this publication is to present a new dynamic aerobic biodegradation test method simulating a river. A laboratory cascade test system and standardized batch shake flask tests were used for biodegradation studies with the non-volatile and non-sorbing model compounds 2,4-dinitrophenol, naphthalene-1-sulphonic acid and sulphanilic acid. To be closer to the often very low concentrations of substances in the environment the concentrations of the compounds used were standard test concentrations and lower. ^{14}C labelled compounds were measured at 50 μg/l, capillary electrophoresis at 5000 μg/l and the removal of dissolved organic carbon at 50000 μg/l. The test results obtained confirmed the known ultimate biodegradability of the test compounds and showed that biodegradation degrees, rates and degradation durations depended on the test systems, the concentrations of test compounds and the inocula. The river model is a suitable simulation test for natural dynamic surface waters which can be used to perform biodegradability studies at low test concentrations if adequate analytical tools, preferably radioactive-labelled substances, are available.

Abbreviations: BOD – biochemical oxygen demand, DAWT – DOC-die-away test, DNP – 2,4-dinitrophenol, DOC – dissolved organic carbon, E – effluent of laboratory wastewater treatment plants, MOST – modified OECD screening test, NSA – naphthalene-1-sulphonic acid, P – pond water, SAA – sulphanilic acid (= 4-amino benzene sulphonic acid)

Introduction

The production and use of new and existing chemicals is regulated by national and international laws, directives and standards. Standardized test methods of the International Organization for Standardization (ISO), the Organisation for Economic Co-operation and Development (OECD Guidelines) and directives of the European Union, known and accepted by industry and authorities, are used to characterize the environmental behaviour of chemicals. A knowledge of their biodegradability is important for estimating environmental concentrations in connection with risk assessment studies. A biodegradable substance is expected to cause less ecological problems in the long term than a persistent one.

Biodegradability depends not only on the molecular structure of the test compounds but also on the microorganisms available and the environmental conditions. For the determination of the very important biodegradability in aerobic aquatic environments batch tests are often used with incubation periods of normally 28 days. Such methods are the DAWT (ISO 7827, OECD 301 A), the MOST (OECD 301 E), the respirometric test

(ISO 9408, OECD 301 F) or the CO_2-evolution test (ISO 9439, OECD 301 B). For investigating the ultimate biodegradability, i.e. the complete mineralization of organic test compounds, summary parameters such as DOC removal, BOD or biogenic evolution of carbon dioxide are used as analytical tools. Only a few and simple analytical techniques are therefore in the most important tests required to determine the large number of different organic compounds. Measurements of BOD and CO_2 release in closed batch systems clearly indicate biodegradation processes whereas detection of DOC removal is limited to sufficiently water-soluble compounds and may also include abiotic elimination processes such as adsorption on biomass, sedimentation or stripping. The CO_2/DOC-combination test developed by Strotmann et al. (1995) allows two important parameters to be determined in parallel and to present unequivocally evidence about the biodegradation of the compounds involved.

The disadvantage of summary parameters is that relatively high concentrations of test compounds are required. Tests with lower concentrations can be carried out by using substance-specific analytical procedures such as gas chromatography or capillary electrophoresis. In this case primary biodegradability, i.e. any structural change in the molecules of the test compound, is determined. The lowest test concentrations, which are in the range of real environmental concentrations, can be achieved by the use of radio-labelled compounds which allow the prediction of ultimate biodegradation by carbon balances. However this technique requires suitable laboratory facilities and fairly expensive ^{14}C-radio-labelled substances, which often have to be synthesized especially for this purpose.

Batch test systems are relatively simple but do not always sufficiently simulate the behaviour of substances in natural compartments (Larson 1979). They may for example lead to an underestimation of the biodegradability of chemicals in wastewater treatment plants. Test results need, however, to be unequivocal as they may have important technical and economical consequences for the use of products. This may be in contradiction to the fact that biodegradation is linked to the environmental conditions and the availability of sufficiently adapted micro-organisms. Therefore, it would be best to conduct biodegradability tests under a variety of test conditions but usually only simple batch tests are performed. If in the special case of aerobic biodegradability in dynamic aquatic systems more or detailed information is required, complex dynamic simulation tests may be inevitable. For predicting biodegradation in wastewater treatment plants the activated sludge simulation test (ISO 11733, OECD 303) or the test in a toximeter as described by Pagga (1985) are suitable methods. For the dynamic system of a river no accepted simulation tests for biodegradation studies exist so far. Therefore in the present contribution we describe a continuously operating river test using a cascade model designed to determine the biodegradability of organic compounds at low concentrations. We used the 'riverine model' described by Scholz and Müller (1992), which was developed for the determination of biocenoses in a laboratory-scale microcosm test but which has not yet been used for biodegradability studies. In an earlier diploma thesis the system was adopted in our laboratory for this purpose (Seel 1993). Test results obtained with different analytical techniques at different concentrations were compared with results from the batch tests, and possibilities are suggested for treating and presenting the data obtained in such studies.

The test compounds used in this study should be easily detectable with specific analytical procedures at low concentrations and ^{14}C labelled compounds should be commercially available. Sufficient water-solubility was required to allow DOC measurements and precise metering of stock solutions with the help of a diluter to the trays of the river model. The compounds should be sufficiently and ultimately biodegradable but should not be eliminated from water by abiotic processes such as sedimentation, adsorption or stripping. Biodegradation should only start after a distinct adaptation period to obtain characteristic biodegradation curves but should not be too slow, so that tests can be completed within a period of about one month. Finally the test compounds should not be toxic to the bacteria of the inocula at the chosen test concentrations. These requirements were fulfilled using DNP, NSA, and SAA. It was, however, not the aim of this study to investigate the biodegradability of problematic environmentally important substances but to develop and improve a new test method.

Materials and methods

Test compounds

DNP, NSA and SAA were analytical-grade chemicals obtained from Merck, Darmstadt, Germany, (SAA) and from Merck-Schuchardt, Hohenbrunn, Germany (NSA and DNP). ^{14}C radio-labelled substances were

Table 1. Important parameters of the test methods modified OECD Screening test (MOST) and DOC die away test (DAWT) using the inocula effluent of a laboratory waste water treatment plant (E) and a mixture of this effluent and pond water (EP) and in the dynamic river model (RIVER) using a cascade system.

	MOST/EP	MOST/E	DAWT	RIVER
Test compound	50	50	50	50
concentration (μg/l)	5000	5000		5000
	5000	50000	50000	50000
Analytical techniques	^{14}C substance	^{14}C substance	^{14}C substance	^{14}C substance
capillary	capillary	capillary		capillary
electrophoresis	electrophoresis	electrophoresis		electrophoresis
removal of dissolved	removal of dissolved	removal of dissolved	removal of dissolved	removal of dissolved
organic carbon (DOC)	organic carbon (DOC)	organic carbon (DOC)	organic carbon (DOC)	organic carbon (DOC)
Inoculum				
Source	effluent and	effluent	activated sludge	effluent and
	pond water			pond water
Concentration	0.5 ml/l	0.5 ml/l	30 mg/l dry	biofilm, not
			substance	measured
Test conditions				
Volume (l)	1	1	1	2, each tray
Temperature (°C)	22+2	22+2	22+2	ambient
Normal duration (d)	28	28	28	14–27
Test method	OECD 301 E	OECD 301 E	OECD 301 A	
	ISO 7827	ISO 7827	ISO 7827	ISO proposal

obtained from Sigma Chemie, Deisenhofen, Germany.

Biodegradation tests

An overview of important test parameters is given in Table 1. The batch tests DAWT and MOST were performed according to the standard methods using 2-litre Erlenmeyer flasks and standardized inorganic medium which supplied sufficient nutrients and buffer capacity. The vessels were incubated, with shaking. In the case of DOC measurements, for each type of test, blank controls without test compounds were used to determine the organic carbon evolved by the inoculum and subtracted from the DOC of the test assays. Aniline was used as reference substance to check the activity of the bacteria. A mixture of test compound and sodium benzoate was used to identify potential inhibition effects on the inoculum at the highest test concentrations. Special controls on abiotic elimination from water were in this case not necessary as the test compounds were chosen for their poor volatility and sorbability. The batch tests were inoculated with (1) water from a pond near the laboratory (P), (2) effluent (E) and (3) activated sludge from laboratory wastewater treatment plants. These plants were operated according to ISO 11733 with municipal sewage and standardized synthetic medium consisting of peptone, meat extract, urea and inorganic salts and did not receive any test compound to avoid pre-adaptation.

The river model consisted of 4 cascades, installed as an aquatic staircase, storage vessels and metering facilities. Each cascade contained 7 shallow rectangular plastic trays which allowed the downstream flow of water. Photographic washing tanks with side lengths of 45x31 cm were used. The water depth was 1.5 cm and the bottom of each tray was covered with about 1 kg of glass beads, 5 mm diameter, as an artificial sediment to facilitate biofilm growth. In the middle of the lower small side of each tray a hole was located with a small tube for passing test water from one tray to the next. Water, in an amount of 15 l/d, and appropriate aquatic stock solutions of the test compounds were introduced by means of peristaltic Watson-Marlow pumps and Hamilton diluters (Micro Lab M) into the top trays. A mean hydraulic retention time of 24 h in the entire system was obtained which corresponds to about 3.4 h in each tray. One cascade was not supplied with test

compounds and used thus as blank control in the case of DOC determinations. Samples of the water were taken at regular intervals from different parts of the trays for analyses. All cascades were operated under identical conditions and normally illuminated for 8 hours per day with fluorescent tubes to obtain an average light intensity of about 50 $\mu E/m^2 \cdot s$. The batch tests were performed in temperature-controled-rooms at 22+2 °C. The temperature of the river model could not be influenced, and varied, depending on the outdoor temperature, between about 20 and a for a short period up to 35 °C.

The river model was supplied with test water, as well as a 1:1 mixture of E:P. It was metered into the trays for about 8 weeks before the tests were started and during the tests. This permitted sufficient biomass to develop on the beads; biological activity was maintained throughout the tests and a sufficient supply of inorganic salts and sufficient buffering capacity were guaranteed. This constant supply of micro-organisms and nutrients corresponds to real-world conditions, where effluents from wastewater treatment plants are released into rivers.

Analytical procedures

DOC of aquatic samples was determined according to DIN 38409 part 3 after filtration with membrane filters of 0.45 μm pore size with a Shimadzu carbon analyzer TOC 500. Capillary electrophoresis was performed in non-filtered samples with a DIONEX CES I analyzer, and an AI 450 workstation using a buffer of 10 mM borate, 50 mM boric acid, 20 mM sodium dodecylsulphonate, a fused silica capillary column (length 48 cm, internal diameter 75 μm) and UV detection which occurred at the substance-specific absorption maxima. The scintillation measurements of membrane-filtered aquatic samples were performed in a liquid scintillation counter (Packard Instruments 2500 TR) after acidification with phosphoric acid to pH 1–2 and purging for 0.5 h with CO_2. Special intercalibration procedures between the analytical methods and determination of standard errors were not performed because DOC measurements and capillary electrophoresis are part of routine tests.

In the first and last trays of the river model dissolved oxygen concentrations were measured regularly with an oxygen electrode (WTW Oxi 191), ammonium nitrogen (DIN 38 406 part 5) and BOD_5 (DIN 38409 part 51). The concentrations of suspended bacteria in the test water fluctuated considerably. Therefore only approximate values of colony forming units (cfu/ml) are reported. The saprobic index, a numerical value based on indicator species to describe the biota of a water body to indicate its biological quality, was determined by microscopic analyses of important indicator organisms according to DIN 38 410 parts 1 and 2.

Evaluation and interpretation of test data

The data measured in fairly complex test systems such as the river model can be handled in different ways in order to obtain the required information. The following parameters were used to describe the biodegradability of the test compounds and to compare the results of the the river model with batch tests. This does not exclude the use of more or other suitable data, for example the half-life time of a test substance or information on metabolites, or the reduction of data, depending on the purpose of the test. The initial *test concentration* (μg/l) is calculated from the concentrations and the amounts of the stock solutions added once or continuously to the test system. The *actual concentrations* of the test compound are determined in regularly taken samples of the test water, in the case of DOC measurements after subtracting the corresponding blank values. The *end concentration* is the mean value of the measured actual concentrations at the end of the test, in the case of biodegradable substances when the plateau phase is reached. The amount of the *biodegraded test compound* is determined from the difference between the test and the end concentration and is used to calculate the *degree of biodegradation* in per cent relative to the initial test concentration. The exact calculation of the *rate of biodegradation* (μg/h) depends on information such as the increase in biomass or the order of reaction which was not available. Therefore for the purpose of this investigation only a rough estimation was done by calculating the amount of test compound removed during the phase of linear disappearance in the biodegradation phase. An alternative could be in the case of the river model the calculation of the biodegradation per unit of surface area of the biofilm.

The measured actual concentrations versus the test duration are used to plot *biodegradation curves*. The *lag phase* or adaptation phase starts in the batch tests with the addition of the test compound to the test system and ends when about 10% of the compound has been degraded. The lag phase is followed by the *biodegradation phase,* which is the period of maximal disappearance of the test compound. This phase ends with the beginning of the *plateau phase*, when the test

compound has been degraded. The end of the plateau phase corresponds to the end of the test. In the case of the river model a three-dimensional diagram was used giving the actual concentrations of the test compounds (y-axis), the day of sampling (z-axis) and the sampling points of the trays (x-axis). Each sampling point at the end of a tray corresponds to a specific mean hydraulic retention time of about 3.4 h under the chosen test conditions. The lag phase starts as well with the addition of the test compound. The degradation phase is determined by the number of trays where significant degradation starts up to the tray where the end concentration is reached and the plateau phase begins. To express the biodegradation phase in hours the number of trays was multiplied by the factor 3.4.

As it is often difficult or impossible to differentiate the phases of the biodegradation curves clearly, especially in the river model, a *period of substance existence* was defined and expressed in days. It is the time from the first addition of the test compound to the test system until the end concentration has been reached. In the case of biodegradable substances it is lag-phase and biodegradation phase together, in the case of non-biodegradable substances it is equal to the total test duration. In the river model the period of substance existence is obtained by counting the number of days from the start of the test until a stable end concentration has been reached in one tray, usually in the last tray of the cascade.

The criterion *readily biodegradable*, which is strictly defined by the OECD methods, was fulfilled if in the MOST or DAWT with 50000 μg/l test concentration and DOC measurement a biodegradation degree of >70% was measured within a biodegradation phase of 10 days.

Results and discussion

The main aim of this publication is the presentation of the river model, a demonstration how the measured data can be handled and the comparison of the test results with batch tests. The most important test results are summarized in Table 2. In Fig. 1–6 examples of biodegradation curves are presented for each test system, test compound and test concentration. These biodegradation curves are helpful for demonstrating and explaining how the test results were obtained.

An example of batch tests is Fig. 3. The test concentration of SAA was 50000 μg/l, calculated from the stock solution and confirmed by the measured value.

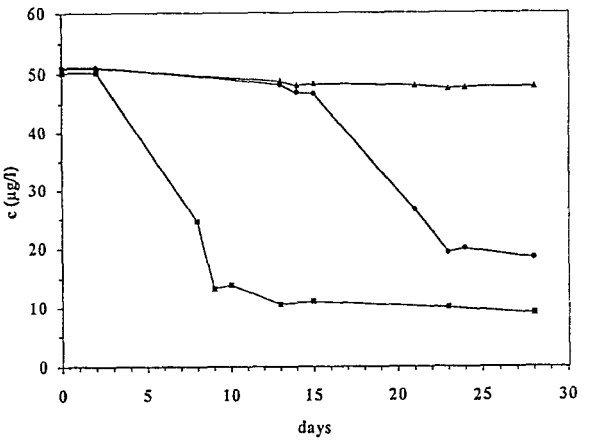

Fig. 1. Biodegradation of 2,4-dinitrophenol in the batch shake flask tests DOC-die-away test with activated sludge as inoculum (DAWT - ■ -), modified OECD screening test with effluent (MOST/E - ● -), and modified OECD screening test with effluent and pond water (MOST/EP - ▲ -) at a test concentration (c) of 50 μg/l using ^{14}C determination as analytical parameter.

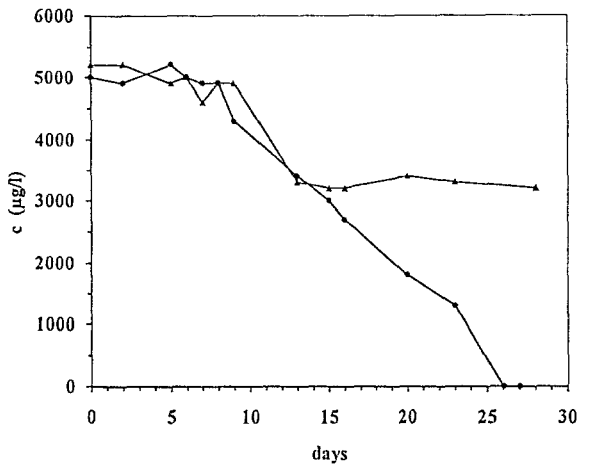

Fig. 2. Biodegradation of naphthalene-1-sulphonic acid in the batch shake flask tests modified OECD screening test with effluent (MOST/E - ● -), and modified OECD screening test with effluent and pond water (MOST/EP - ▲ -) at a test concentration (c) of 5000 μg/l using capillary electrophoresis as analytical parameter.

The end concentrations after taking of the blank values into account were 1400, 2100 and about 50000 μg/l. The calculated biodegradation degrees were 97%, 96% and <10%. The lag phases were 12, 15 and >35 days, the biodegradation phases 3, 19 and 0 d and the periods of substance existence therefore 15, 34 and >35 d. The amounts of biodegraded test compounds are 48600, 47900 and 0 μg/l. Divided by the biodegradation phases expressed in hours (72, 456, 0), the

Table 2. Biodegradation results in batch tests and in the river model. Test concentrations (TEST), end concentrations (END), biodegradation degrees (DEG), biodegradation rates (RATE) and periods of substance existence (EXIST) of the the test compounds 2,4-dinitrophenol (DNP), naphthalene-1-sulphonic acid (NSA) and sulphanilic acid (SAA) in the batch shake flask tests modified OECD Screening test (MOST) and DOC die away test (DAWT) using the inocula effluent of a laboratory waste water treatment plant (E) and a mixture of this effluent and pond water (EP) and in the dynamic river model (RIVER) using a cascade system.

Parameter	TEST (μg/l)	END (μg/l)	DEG (%)	RATE (μg/h)	EXIST (d)	END (μg/l)	DEG (%)	RATE (μg/h)	EXIST (d)	END (μg/l)	DEG (%)	RATE (μg/h)	EXIST (d)
Compound		DNP	DNP	DNP	DNP	NSA	NSA	NSA	NSA	SAA	SAA	SAA	SAA
MOST/EP	50	50	<10	<0.1	>28	41	10–20	0.1–1	>28	34	30–40	0.1–1	>28
MOST/E	50	19	60–70	0.1–1	23	13	70–80	0.1–1	13	12	70–80	0.1–1	22
DAWT	50	11	70–80	0.1–1	9	5	>90	0.1–1	6	7	80–90	0.1–1	16
RIVER	50	2	>90	1–10	9	6	80–90	1–10	14	11	70–80	1–10	24
MOST/EP	5000	5000	<10	<0.1	>28	3300	30–40	10–50	>28	5000	<10	<0.1	>28
MOST/E	5000	5000	<10	<0.1	>28	<500	>90	10–50	26	<500	>90	10–50	18
RIVER	5000	<500	>90	100-500	15	<500	>90	100-500	14	<500	>90	100-500	17
MOST/EP	50000	4100	>90	500-1000	13	50000	<10	<0.1	>28	50000	<10	<0.1	>28
MOST/E	50000	4600	>90	500-1000	13	39000	20–30	100-500	>28	2100	>90	100-500	30
DAWT	50000	400	>90	100-500	12	1600	>90	100-500	19	1400	>90	500-1000	15
RIVER	50000	6600	80–90	>1000	12	5700	80–90	>1000	21	11800	70–80	>1000	21

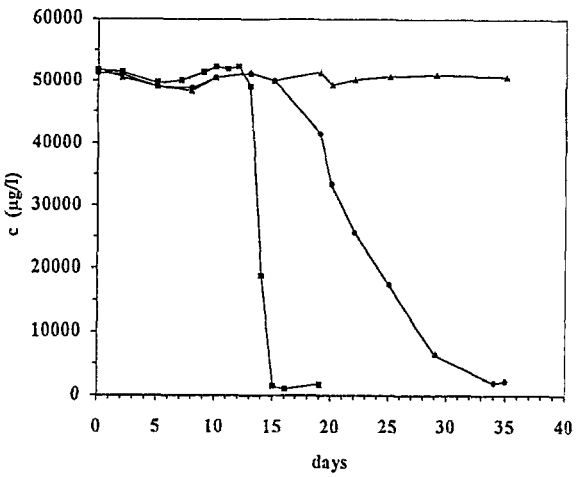

Fig. 3. Biodegradation of sulphanilic acid in the batch shake flask tests DOC-die-away test with activated sludge as inoculum (DAWT - ■ -), modified OECD screening test with effluent (MOST/E - ● -), and modified OECD screening test with effluent and pond water (MOST/EP - ▲ -) at a test concentration of 50000 μg/l using DOC measurement as analytical parameter.

estimated biodegradation rates of 675, 105 and <0.1 μg/h are obtained. With SAA at 50000 μg/l in the DAWT the criteria for ready biodegradability are fulfilled: DOC degradation >70% in <10 d of biodegradation phase.

An example of the river model is Fig. 5. The test concentration of 5000 μg/l DNP was calculated from the added stock solution and confirmed by the measured values in the first tray. The end concentration (<500 μg/l) was determined in the last tray (No.7) after complete biodegradation of the test compound (>15 d). If for example tray 3 is considered, no degradation took place until day 9 and full degradation from day 15 on. The period of substance existence in the dynamic system was therefore 15 d and the biodegradation degree >90%. The duration of the biodegradation phase was determined after full adaptation had occurred and intensive degradation had taken place. This is, for example, the case on day 16, when at the inlet of the first tray the test concentration of about 50000 μg/l was measured and on the same day in the third tray complete degradation (<500 μg/l) was observed. The mean hydraulic retention time in one tray was 3.4 h and in 3 trays therefore 10.2 h, which is defined as the biodegradation phase. These 10 h and the amount of biodegraded test compound (>4500 μg/l) were used to estimate the biodegradation rate of about 450 μg/h.

The test compounds, which were chosen because they fulfilled the requirements for these investigations, are in principle biodegradable. This has been reported for DNP by Wiggins & Alexander (1988) and Lenke et al. (1992) although DNP is a strong uncoupler of oxida-

tive phosphorylation. Biodegradability of NSA (Brilon et al. 1981, Zürrer et al. 1987) and of SAA (Pitter 1976, Thurnheer et al. 1986, Locher et al. 1989, Feigel 1990, Feigel & Knackmuss 1993) were also described. Our investigations using different concentrations and several analytical procedures indicated that all three of the test compounds are ultimately biodegradable. Depending on the test system and the test conditions degradation degrees >90% were obtained. A biodegradation degree of >70% DOC is, for example, a prerequisite for the criterion 'readily biodegradable' according to the OECD Guidelines (OECD 1993). Qualitative measurements of the radioactivity of biomass in samples taken from the glass beads after addition of radiolabelled substances showed that ^{14}C was incorporated into the biomass. We concluded from all this information that the test compounds can be considered to be mineralized even when a definitive measurement of, for example, CO_2production was not performed. The isolation and identification of metabolites were also outside the scope of these investigations.

In different environmental compartements and situations as well as in different test systems, varying results can be obtained for the same test compound. One reason for insufficient biodegradation could be toxicity of the test substances. The results of inhibition controls showed, however, that no inhibiting effects occurred under the test conditions used. An important reason for diverging results is the test concentration. Already at the usually very low concentrations of substances in surface waters such as rivers and lakes, which are in a range of some μg/l and less, completely different micro-organisms (oligothrophic or oligocarbophilic bacteria), metabolic pathways and kinetics will occur. This is in contrast to aquatic systems such as waste water treatment plants which contain eutrophic or saprophytic bacteria as a consequence of relatively high substrate concentrations in the range of some few mg/l. Simple batch test methods and summary parameters can normally only be used at test concentrations >10 mg/l DOC, exceeding significantly realistic environmental concentrations.

The important influence of substance concentrations for biodegradation studies is described in reviews by Alexander (1994) or Pagga (1987). Correlations between test concentrations and biodegradation rates are reported for a number of substances showing a decrease in rates at lower concentrations (Boethling & Alexander 1979a, 1979b; Pfaender & Bartholomew 1982; Bartholomew & Pfaender 1983). Phthalates, for example, are biodegraded less at 20 μg/l than at 10 mg/l (Johnson & Heitkamp 1984), aniline less at 5 ng/l than at 500 μg/l (Subba-Rao et al. 1982), and Rubin et al. (1982) reported differences for phenol between <1 ng and 10 g/l. On the other hand Larson (1979) reported higher rates and degrees with detergents at 50–500 μg/l compared with the standard test concentrations of 10–20 mg/l.

Jannasch (1979) and Alexander (1985) stated that threshold concentrations exist below which biodegradation no longer occurs, or is very slow. Such thresholds were observed even in the case of easily biodegradable substances because under these conditions the bacteria lack sufficient substrate for maintaining energy metabolism, growth and reproduction. Threshold concentrations vary with growth substrate. For phenol and p-nitrophenol, for example, <1 ng/l (Rubin et al. 1982) and for 2,4-dichlorophenoxy acetic acid about 2 μg/l (Nesbitt & Watson 1980, Boethling & Alexander 1979a) were reported. Alexander (1994) defines a range of about 0.1–5 μg/l where such limitations were frequently observed.

Threshold concentrations may also be influenced by the water solubility of a substance (Thomas et al. 1986) or by substrates that are subject to cometabolism (Rubin & Alexander 1984, Schmidt &Alexander 1985, Alexander 1981). Cometabolism may be important in natural environments as bacteria may obtain enough energy and carbon from natural substrates and under these conditions may degrade problematic substances even at low concentrations. In the tests of the present investigations cometabolism is probably of minor importance because the test compounds were either the only source of carbon or the added effluent from the model wastewater treatment plant no longer contained easily degradable substances. The thresholds reported in literature are considerably lower than the lowest concentration used in this study (50 μg/l) and of course much lower than the standard batch test concentrations (about 10 mg/l DOC) and should not therefore be a reason for insufficient biodegradability within these investigations.

Predicted environmental concentrations (PECs) are used for ecological risk assessments of chemicals and compared with ecotoxicity data such as the predicted no-effect concentrations (PNECs), obtained with representative organisms such as bacteria, algae, daphnia or fish in standardized toxicity tests. The relationship PEC to PNEC indicates whether a certain substance is of environmental concern or not. The results of biodegradation tests and especially the measured end concentrations in aquatic simulation tests are useful

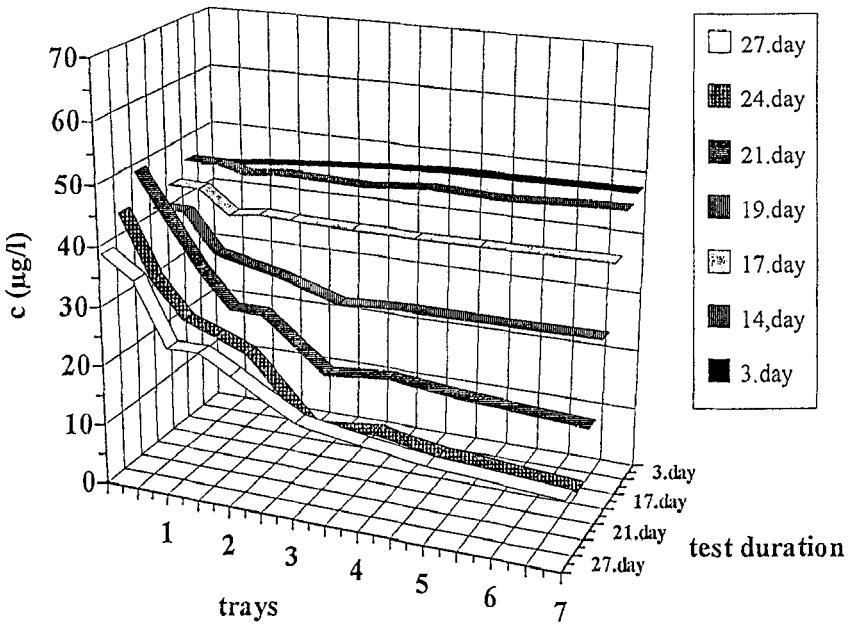

Fig. 4. Biodegradation of sulphanilic acid in the river model at a test concentration (c) of 50 µg/l using ^{14} C determination as analytical parameter.

information for obtaining reliable PECs. As expected the measured end concentrations depended on the analytical tools used. With radio-labelled substances reliable data between 2 and 11 µg/l were obtained. With capillary electrophoresis the end concentrations were <500 µg/l, which was the detection limit of the method. Using specific analytical techniques their accuracy and detection limits determine whether sensible information is obtained. The detection limit of DOC measurements is in a range of 500–1000 µg/l. Unspecific DOC concentrations are, however, influenced by organic substrate deriving from the effluent added to the test water and from the inoculum. Even when blank values were subtracted, DOC concentrations were in a range of 6000–12000 µg/l, indicating clearly that DOC is not a suitable tool for determining end concentrations of test compounds or to obtain any information on metabolites. DOC can on the other hand be used to compare the biodegradation degrees obtained with various test methods.

Biodegradation depends not only on the molecular structure of the test compounds but also on the properties of the test system, especially on the origin, quality, physiological status and concentration of the micro-organisms of the inoculum. Aniline as a reference substance was readily and completely biodegraded both in the batch tests and in the river model, indicating that in principle sufficient catabolic activity was present in all inocula. In cases where sufficient micro-organisms were available, as with the DAWT and the river model, degrees of biodegradation of >90% in periods between 6 and 19 days were obtained. Using lower quantities of bacteria (MOST/E and MOST/EP), in some cases biodegradation rates of >70% were obtained, and in other cases no or almost no biodegradation was recorded. The colony-forming units (cfu/ml) in the DAWT were 10^5-10^8 and in the MOST 10^2 - 10^5. The fact that the biomass in the river model and therefore the biodegradation potential was almost completely fixed to the glass beads and only a minor part was in suspension (10^4 - 10^6cfu/ml) is an important advantage of this test, compared with batch systems, where most bacteria are in suspension, and also represents better the natural occurrence of micro-organisms in rivers. These observations are confirmed in the literature e.g. by Wang et al. (1984) who reported an influence of the inoculum for 2,4-dichlorophenoxyacetic acid, which was degraded in tests with wastewater and eutrophic lake water but not with oligothrophic and mesotrophic lake water. Budack (1992) came to the conclusion to

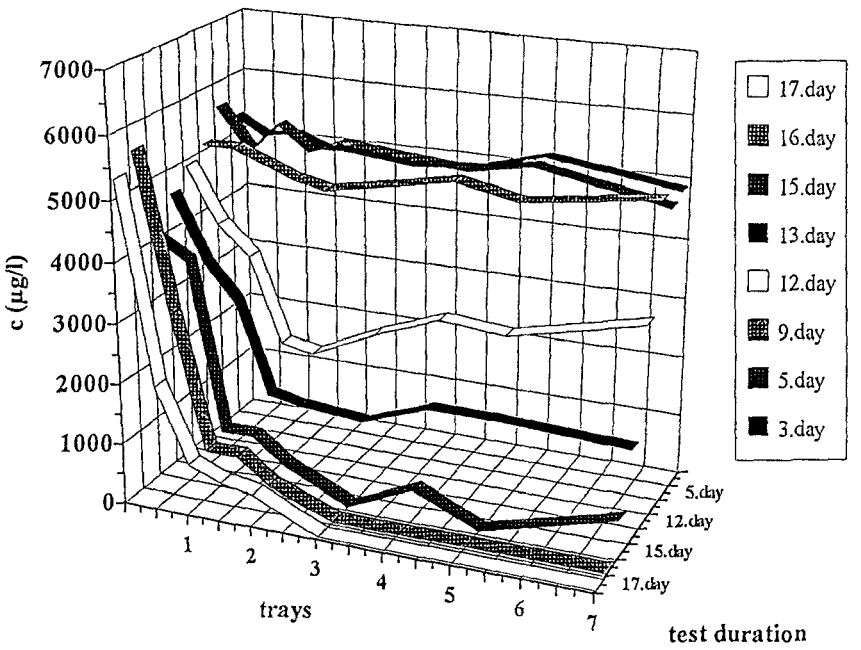

Fig. 5. Biodegradation of 2,4-dinitrophenol in the river model at a test concentration (c) of 5000 µg/l using capillary electrophoresis as analytical parameter.

use in batch tests activated sludge at concentrations of dry solids in the range of 1 to 5 mg/l, which corresponds to about 10^6 to 10^7 cfu/ml, instead of effluent from waste water treatment plants with considerably lower bacteria concentrations (about 10^3 cfu/ml).

A direct comparison of kinetic parameters in batch and continuous tests is not possible and no detailed determination or examination of biodegradation kinetics was performed. The estimated biodegradation rates in these investigations were just determined by the disappearance of the test compounds during the more or less linear biodegradation phase. They varied according to the concentrations of the test compounds (for 50 µg/l at about 1–10 µg/h, for 5000 µg/l at about 100–500 µg/h and for 50000 µg/l at about >1000 µg/h).

The periods of substance existence are only of interest when biodegradation occurred. They were between 6 and 30 days in the static systems and between 9 and 24 days in the river model and differed considerably even for the same substance, SAA showing the longest periods. This period is influenced by the duration of the lag phase, which depend on the availability of sufficiently adapted bacteria, and the duration of the biodegradation phases, which depend on the concentrations of the test compounds and of the inocula. The period of substance existence cannot be directly transferred from test systems to natural environments but could, however, be used to estimate time periods which are required in certain cases for complete biodegradation in natural environments, e.g. when a discontinuous discharge or shock load of a substance to a river occurs.

Information on the biocenotic quality of the river model increases the predictive value of test results considerably because the simulation character of the test system is improved and can be better compared to real environments. Such information may be obtained by analyses of the saprobic index which needs, however, expert knowledge and is time-consuming. An alternative with less expressiveness is the determination of parameters such as oxygen concentration, BOD_5 and ammonia concentration. At any tray and time the concentration of dissolved oxygen in the test water of the river model was > 8 mg/l. The mean BOD_5 was at about 2.5 mg/l and the mean NH_4^+-N concentration at about 0.5 mg/l. The determination of the saprobic index in the trays of the cascade was based on a total of 58 species from the taxonomic groups Chlorophyta, Ciliata, Diatomea, Euglenophyta, Rhizopoda, Rotatoria and Suctoria. After the addition of test compounds an effect on the number of indicator species was observed

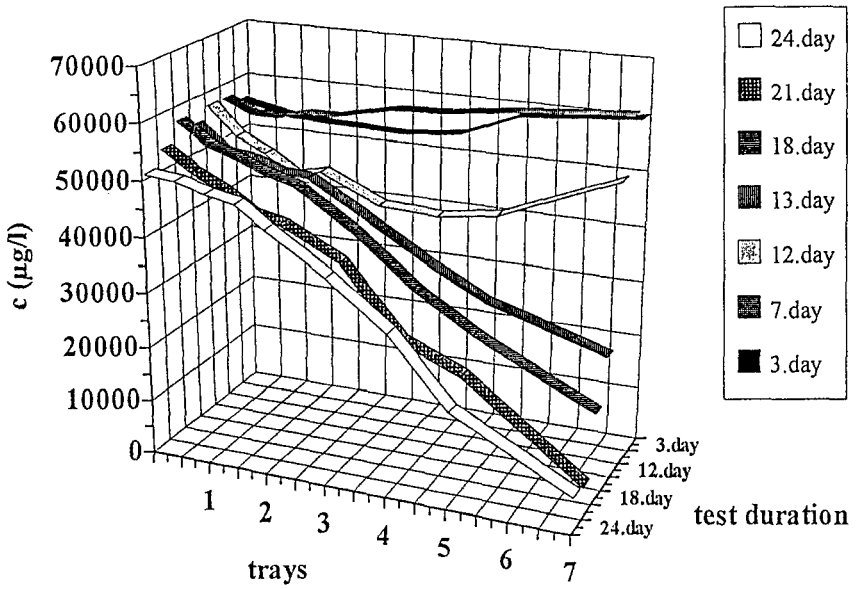

Fig. 6. Biodegradation of naphthalene-1-sulphonic acid in the river model at a test concentration (c) of 50000 µg/l using DOC measurement as analytical parameter.

in some cases. 5000 and 50000 µg/l DNP reduced for example the number of species from 25 to 15 after one week, and addition of 50000 µg/l NSA from 20 to 14, addition of SAA had no effect. One week later the original number of indicator species was almost completely restored. The mean saprobic index was at about 2.5 of a classification system, where 1 indicates best and 4 worst ecological quality. From this data the biological water quality of the river model was classified as II-III (critically polluted) according to the German river classification system. This quality class is found in many "low land rivers" which are intensively anthropogenically used. In such rivers sufficient bacteria, immobilized on stones and plants, are available, resulting in rather high biodegradation potentials.

The accuracy and reproducibility of results from biodegradation tests are in many cases low, as is usual with biological and ecological studies. Both depend on many factors such as the test system, the inoculum, the test concentration and the analytical methods. A statistical treatment of the measured biodegradation data is only sensible when sufficient values are available. A practical consequence is that not too high demands should be made on the numeric accuracy of test results. In many cases it is sufficient to know if biodegradability exceeds a certain limit value (e.g. >70% DOC removal as a criterium for ready biodegradability) or if the test results are given in 10% ranges. A summary of the results derived from the different test systems and a classification of biodegradability of the test compounds is given in Table 3.

Conclusions

Aquatic batch tests are frequently used to determine the biodegradability of substances. These tests have, however, no simulation character. Their disadvantage is the rather high concentration of the test compounds and in some cases the lack of sufficient inoculum. For certain investigations suitable and practicable dynamic simulation tests, such as the river model, are required.

Batch tests and the river model were used to perform biodegradation studies with DNP, NSA and SAA. The known biodegradability of these test compounds was confirmed in the different test system, depending on their type and the test conditions. The DAWT produces reliable test results, which can be compared with results of the river model. The MOST is in many cases, especially with a low-concentration inoculum, not a suitable test method for predicting biodegradability in surface waters and under no circumstances in wastewater treatment plants.

Table 3. Summary of conclusions from the tests on biodegradability of the test compounds 2,4-dinitrophenol (DNP), naphthalene-1-sulphonic acid (NSA) and sulphanilic acid (SAA) in the batch shake flask tests modified OECD Screening test (MOST), DOC die away test (DAWT) and in the dynamic river model (RIVER).

Biodegradability criteria	Test compound	MOST	DAWT	RIVER
Ultimate biodegradation	DNP	yes	yes	yes
Biodegradation degree (%)		90	90	90
End concentration (μg/l)		20	20	2
Biodegradation completed after days		10–20	10	10
Classification of biodegradability		readily biodegradable	readily biodegradable	biodegradable
Ultimate biodegradation	NSA	no	yes	yes
Biodegradation degree (%)		30–80	90	80
End concentration (μg/l)		10–20	5	5
Biodegradation completed after days		15–30	5–20	15–20
Classification of biodegradability		moderately biodegradable	readily biodegradable	biodegradable
Ultimate biodegradation	SAA	yes	yes	yes
Biodegradation degree (%)		90	90	80
End concentration (μg/l)		10	5–10	10
Biodegradation completed after days		20–30	15	20–25
Classification of biodegradability		readily biodegradable	readily biodegradable	biodegradable

The river model produces reliable test results of high predictive value in an environmentally realistic range of test concentrations. In the here presented version it is suitable for test substances which are not volatile and not sorbing significantly on biomass. Specific analytical techniques or radio-labelled compounds are required to perform investigations at low test concentrations. DOC measurements, which are influenced by the test water and the inoculum, are important and helpful in standard batch tests to determine the ultimate biodegradability of various compounds but are not suitable for the river model.

Even if this is not a simple test - the river model can be standardized and may be used as a tool to investigate substances on a high simulation level and to compare the results of simple batch tests to increase their predictive value.

Acknowledgments

We thank J. Jatzek for biocenotic investigations, U. Weberruß for capillary electrophoresis, and K.W. Maichel, Mosbacher and A. Seher for handling and analyses of radio-labelled substances.

References

Alexander M (1981) Biodegradation of chemicals of environmental concern. Science 211: 132–138

Alexander M (1985) Biodegradation or organic chemicals. Environ. Sci. Technol. 18: 106–111

Alexander M (1994) Biodegradation and Bioremediation. Academic Press, San Diego

Bartholomew GW & Pfaender FW (1983) Influence of spatial and temporal variations on organic pollutant biodegradation rates in an estuarine environment. App. Environ. Microbiol. 45: 103–109

Boethling RS & Alexander M (1979a) Effect of concentration of organic chemicals on their biodegradation by natural microbial communities. Appl. Environ. Microbiol. 37: 1211–1216

Boethling RS & Alexander M (1979b) Microbial degradation of organic compounds at trace levels. Environ. Sci. Technol. 13: 989–991

Brilon C, Beckmann W & Knackmuss HJ (1981) Catabolism of naphthalenesulfonic acids by Pseudomonas sp. A3 and Pseudomonas sp. C22. Appl. Environ. Microbiol. 42: 44–55

Budack AM (1992) Einfluß der Inokulumskonzentration auf das Abbauergebnis in Testverfahren auf leichte biologische Abbaubarkeit gemäß OECD 301. Diploma thesis Technical University of Darmstadt, Germany

DIN - Deutsches Institut für Normung, Berlin - Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung (German standards on water quality) - DIN 38 406/5 Ammonium-Stickstoff; DIN 38 409/3 Gesamter organisch gebundener Kohlenstoff; DIN 38 409/51 Biochemischer Sauerstoffbedarf in n Tagen nach dem Verdünnungsprinzip; DIN 38 410/1 Allgemeine Hinweise, Planung und Durchführung von Fließgewässeruntersuchungen; DIN 38 410/2 Saprobienindex.

EEC- Directives, Annex to 92/69/EEC and 88/302/EEC. Official Journal of the European Communities L383 A and L133, Luxemburg (1988 and 1992)

Feigel BJ (1990) Synergistischer Abbau von 4-Aminobenzolsulfonat durch Hydrogenophaga palleronii und Agroberterium radiobacter. Thesis, University of Stuttgart

Feigel BJ & Knackmuss HJ (1993) Syntrophic interactions during degradation of 4-aminobenzenesulfonic acid by two species bacterial culture. Arch. Micorbiol. 159: 124–130

International Organization for Standardization (ISO), Geneva - Standard methods for the evaluation in an aqueous medium of the ultimate aerobic biodegradability of organic compounds - ISO 7827 Method by analysis of dissolved organic carbon (DOC); ISO 9408 Method by determining the oxygen demand in a closed respirometer; ISO 9439 Method by analysis of released carbon dioxide; ISO 11733 Activated sludge simulation test

Jannasch HW (1979) Microbial ecology of aquatic low habitats. In: Strategies of Microbial Life in Extreme Environments. Life Sciences Research Report 13: 243-260. Dahlem Konferenzen, Berlin

Johnson BT & Heitkamp MA (1984) Environmental and chemical factors influencing the biodegradation of phthalic acid esters in freshwater sediments. Environ. Poll. (Series B) 8: 101–118

Larson RL (1979) Estimation of biodegradation of potential of xenobiotic organic chemicals. Appl. Environ. Microbiol. 38: 1153–1161

Lenke H (1990) Mikrobieller Abbau von Nitrophenolen: 2,4-Dinitrophenole und 2,4,6.Trinitrophenol. Thesis, University of Stuttgart

Lenke H, Pieper DH, Bruhn C & Knackmuss H.J (1922). Degradation of 2,4-dinitrophenol by two Rhodococcus erythropolis strains, HL 24–1 and HL 24–2. Appl. Environ. Microbiol. 58:2928–2932

Locher HH, Thurnheer T, Leisinger T and Cook AM (1989) 3-Nitrobenzene-sulfonate, 3-Aminobenzenesulfonat, and 4-Aminobenzenesulfonat as sole carbon sources for bacteria. Appl. Environ. Microbiol. 55:492–494

Nesbitt HJ & Watson JR (1980) Degradation of the herbicide 2,4-D in river water I. Description of Study Area and Survy of Rate Determining Factors. Water Res. 14: 1683–1688 II. The role of suspended sediment, nutrients and water temperature. Water Res. 14: 1689–1694 (1980)

OECD - Guidelines for Testing of Chemicals of the Organisation for Economic Cooperation and Development, Paris (1993) - OECD 301A DOC Die-Away Test; OECD 301B CO_2-Evolution Test; OECD 301E Modified OECD Screening Test; OECD 301F Manometric Respirometry Test; OECD 303A Aerobic Sewage Treatment Test.

Pagga U (1987) Biologischer Abbau von Stoffen bei geringen Konzentrationen. Z.Wasser-Abwasser-Forsch. 20:101–107

Pagga U (1985) Stoffprüfungen in einem Kläranlagenmodell - Abbaubarkeits- und Toxizitätstests im BASF-Toximeter. Z.Wasser-Abwasser-Forsch. 18:222–232

Pfaender FK & Bartholomew GW (1982) Measurement of aquatic biodegradation rates by determining heterotrophic uptake of radiolabeled pollutants. Appl. Environ. Microbiol. 44: 159–164

Rubin HE, Subba-Rao RV & Alexander M (1982) Rates of mineralization of trace concentrations of aromatic compounds in lake water and sewage samples. Appl. Environ. Microbiol. 43: 1133–1138

Rubin HE & Alexander M (1984) Effect of nutrients of the rates of mineralization of trace concentrations of phenol and p-Nitrophenol. Environ. Sci. Technol. 17: 104–107

Schmidt SK, Alexander M & Shuler ML (1985) Predicting threshold concentrations of organic substrates for bacterial growth. J. theor. Biol. 114: 1–8

Seel H (1993) Frage nach der Übertragbarkeit von Abbauergebnissen aus standardisierten Methoden auf ein Flußmodell. Diploma thesis Fachhochschule für Technik und Wirtschaft Reutlingen, Germany

Scholz N & Müller FJ (1992) The Riverine Biocenosis Model (Aquatic Stair Case Model). Chemosphere 25: 563–579

Strotmann U, Schwarz H & Pagga U (1995) The CO_2/DOC-combination test - a new method to determine the biodegradability of chemical compounds. Chemosphere 30: 525–538

Subba-Rao RV, Rubin HE & Alexander M (1982) Kinetics and extent of mineralisation of organic chemicals at trace levels in freshwater and sewage. Appl. Environ. Microbiol. 43: 1139–1150

Thomas JM, Yordy JR Amador JA & Alexander M (1986) Rates of dissolution and biodegradation of water-insoluble organic compounds. Appl.Environ.Microbiol. 52: 290–296

Wang YS, Subba-Rao RV & Alexander M (1984) Effects of substrate concentration and organic and inorganic compounds on the occurrence and rate of mineralization and cometabolism. Appl. Environ. Microbiol. 47: 1195–1200

Wiggins BA & Alexander M (1988). Role of chemical concentration and second carbon source in acclimation of microbial communities for biodegradation. Appl.Environ.Microbiol. 54:2803–2807

Zürrer D, Cook AM & Leisinger T (1987) Microbial desulfonation of substituted naphthalenesulfonic acids and benzenesulfonic acids. Appl. Environ. Microbiol. 53: 1459–1463