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Biodegradation rates in adapted surface water can be assessed following a preadaptation period with semi-continuous operation

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Abstract

The paper presents a *semi-continuous preexposure procedure* (SCEP) for use with surface water batch simulation biodegradability tests at low test substance concentrations (0.1–100 μg/l). Simple one step batch tests are normally used first of all for determining "initial rates" characteristic of the water as sampled, as by contrast to "adapted" rates obtained as a result of exposure of the microbial community to the test compound. The aim of the SCEP is to facilitate this adaptation and to become able to estimate reproducible "adapted rates" representing a steady state situation. This is accomplished by maintaining the microbial diversity and a supply of test substance and natural substrates. Conducting a SCEP involves regular renewal of part (e.g. one third) of the test suspension (e.g. every two weeks) adding freshly collected natural water with test compound of the initial concentration. An example study was performed with aniline, 4-nitrophenol, 2,4-dichlorophenoxyacetic acid, 4-chloroaniline, and water from the urban river Mølleå. Following preadaptation lag phases were considerably reduced and much more reproducible than obtained with simple batch tests. In tests at 100 μg/l lag phases for aniline decreased from 5.2 to <1 day, 4-nitrophenol from 10 to <1 day, 2,4-dichlorophenoxyacetic acid from 24 to <1 day, and 4-chloroaniline from 88 to 9 days, respectively. Adapted rates obtained with the SCECP were roughly the same as final rates in simple batch tests with successful adaptation. The adapted rate constant is perceived as an inherent characteristic of the test compound at a specific concentration and under environmental influence (temperature, natural substrates, etc.) but with no simple links back to the original microbial population. By contrast, the initial rates in one step batch tests are determined also by the microbial population initially present in the water sampled.

Keywords: Lag phase; Kinetic; Trace concentration; Risk assessment; 14C

1. Introduction

Information on the biodegradability of chemicals in aquatic environments is needed for chemical risk assessments at all levels. Uses of biodegradability data ranges from advanced modelling of chemical fates to simple persistency ranking, or categorisation as "readily" and

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"not readily" biodegradable. Experimental protocols for biodegradability can be very different using a range of parameters to assess biodegradation. All aquatic batch tests do nevertheless seem to have one problem in common—that of variable lag phases and occasional failure of adaptation. Lag phases are poorly reproducible and can last for weeks or months (Spain, 1990; Painter, 1995; Ingerslev et al., 1998; Alexander, 1999; Ingerslev and Nyholm, 2000), and this variable duration makes planning of sampling for kinetic experiments practically impossible. False negative test results may also occur due to the circumstance that the simple batch system used has a limited lifetime and may deteriorate in time and lose its specific degradation ability. During a batch experiment the diversity of the microbial community may decrease with time due to various loss mechanisms and insufficient selection pressure or unfavorable growth conditions, e.g. due to depletion of the water sample of essential nutrients and primary carbon substrates (Painter, 1995). Adaptation to tolerate toxicity is an additional problem causing variable lag phases and non-reproducible test results (Nyholm, 1991; Painter, 1995).

To mitigate the problems of poorly reproducible lag phases in screening tests, preadaptation of the inoculum has been used by several investigators. A commonly used procedure proposed by Sturm (1973) involves the preadaptation of settled raw sewage, test compound, mineral medium, and yeast extract and harvesting an inoculum after 2 weeks. This procedure has been used mostly with surfactants. In a major pilot study of various tests to assess the biodegradability of industrial chemicals, Gerike and Fischer (1979, 1981) used the closed bottle test with inoculum prepared according to Sturm. The Sturm procedure was investigated with various inoculum sources by Nyholm et al. (1984) in a study of causes of variable screening test results with 4-nitrophenol. Standardized consensus procedures for preadaptation have not been agreed upon, however, and those commonly used have typically provided more favorable conditions for biodegradation than in the subsequent test. Even sludge adapted to high chemical concentrations in an SCAS test for inherent degradability has been used rather often as an inoculum in screening tests with much lower test substance concentration (e.g., Larson, 1983; Painter, 1995). This obviously represents an enhancement of degradation greater than obtainable under normal screening test conditions. During the last two decades regulatory authorities have generally disliked preadaptation as an option in biodegradation tests. Their argument was fear of the creation of "superbugs" or unnaturally "overadapted" inocula (Berg and Nyholm, 1996) that were anticipated leading to false positive test results. For screening tests there seems to be rather universal agreement that the inoculum must be used as collected or preconditioned at the most but not deliberately exposed to the test chemical prior to the test. This is part of the definition of ready biodegradability from precautionary principles.

In simulation testing where the objective is to mimic processes in the real world, adaptation must in some way be taken into account and allowed for, because in the real world adaptation is known to play a crucial role (Painter, 1995). To meet this need we suggest a generalised environmentally-realistic *semi-continuous preexposure procedure* (SCEP). The aim is: (1) to reduce the risk of false negative test results (e.g. by random failure of degradation of degradable compounds in our confined test system) and (2) to minimize the problem of variable duration of lag phases and thus optimize sampling schemes. The procedure has already been included as an option in a new generation of standardized simulation or kinetic tests with natural water and low test compound concentrations (ISO 14592-1, 2002; OECD 309, 2004). The SCEP mimics the processes conditions as found in the natural environment. Tests studies with continuous operation are practically impossible with the long hydraulic residence times needed to avoid washout of bacteria, and we therefore suggest semi-continuous operation combined with a batch test under identical conditions. The SCEP is carried out in practice by periodically renewing part of the test suspension with freshly collected surface water making up the replaced volume of water with test compound to the starting concentration, and in this way maintaining the system characteristics. Based on results from earlier investigations (Spain and Van Veld, 1983; Ingerslev and Nyholm, 2000) and from this study, it has been suggested to routinely replace one third of the volume every 2 weeks (ISO 14592-1, 2002; OECD 309, 2004). The replacement water should be freshly collected natural water from the same site as the original sample. Whilst performing a SCEP, the feasible duration of the batch test is extended from a few weeks to much longer time periods—infinite in principle. Once adaptation has taken place, semi-continuous operation is simply discontinued and the test system is now a batch system with time zero being the time of the last renewal.

2. Materials and methods

2.1. Sampling of river water

Surface water was collected from the lowland river MølleÅ near Copenhagen. The river is eutrophic and free from wastewater discharge, but receives occasional overflows from sewers during rainstorms. River water was collected weekly from 14 May to 15 June 1999. Water was sampled from the surface in the middle of the stream and was used on the day of collection. Upon arrival at the laboratory, the water was filtered through