Chemosphere 174 (2017) 716–721

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Biodegradation testing of chemicals with high Henry's constants – Separating mass and effective concentration reveals higher rate constants

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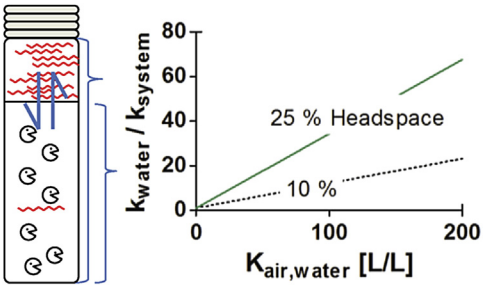
HIGHLIGHTS

- During biodegradation testing, volatile chemicals partition to the headspace.
- Combining partitioning and degradation models account for the partitioning effect.
- Water phase degradation rate constants are higher than test system rate constants.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 1 November 2016
Received in revised form
31 January 2017
Accepted 1 February 2017
Available online 3 February 2017

Handling Editor: I. Cousins

Keywords:
Biodegradation test
Volatile chemicals
Hydrocarbon
Rate constant
Headspace

ABSTRACT

During simulation-type biodegradation tests, volatile chemicals will continuously partition between water phase and headspace. This study addressed how (1) this partitioning affects test results and (2) can be accounted for by combining equilibrium partition and dynamic biodegradation models. An aqueous mixture of 9 (semi)volatile chemicals was first generated using passive dosing and then diluted with environmental surface water producing concentrations in the ng/L to μg/L range. After incubation for 2 h to 4 weeks, automated Headspace Solid Phase Microextraction (HS-SPME) was applied directly on the test systems to measure substrate depletion by biodegradation relatively to abiotic controls. HS-SPME was also applied to determine air to water partitioning ratios. Biodegradation rate constants relating to the chemical in the water phase, k_{water}, were generally a factor 1 to 11 times higher than biodegradation rate constants relating to the total mass of chemical in the test system, k_{system}, with one exceptional factor of 72 times for a long chain alkane. True water phase degradation rate constants were found (i) more appropriate for risk assessment than test system rate constants, (ii) to facilitate extrapolation to other air-water systems and (iii) to be better defined input parameters for aquatic exposure and fate models.

1. Introduction

Biodegradation is an important environmental fate process for most organic chemicals, and data describing biodegradation kinetics are thus needed for modeling and risk assessment purposes (Aronson et al., 2006). Outside of the regulatory systems,

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http://dx.doi.org/10.1016/j.chemosphere.2017.02.003
0045-6535/

estimation of kinetic degradation data from screening test data (Aronson et al., 2006) or via quantitative structure relationships has been attempted (Howard et al., 2005), however good quality kinetic data from laboratory based tests or field studies are called for (Aronson et al., 2006; Gouin et al., 2004).

Within the regulatory system biodegradability testing is required under the Registration, Evaluation, Authorisation and Restriction of Chemicals legislation in the European Union (European Parliament, 2006) and Toxic Substances Control Act in the United States (US Public law, 2002). At the screening level of environmental risk assessment or assessment of persistence, bioaccumulation and toxicity, the Ready Biodegradation studies, OECD series 301 (1992), are used. They include test methods without a headspace, which are appropriate for volatile chemicals, but are often conducted at very high test substance concentrations. These measure ultimate biodegradation on a pass/fail level. Half-lives or rate constants are assigned based on whether, or not, the chemical is assessed as Readily Biodegradable. Subsequent evaluations may require further assessment through simulation biodegradation studies that can deliver primary biodegradation half-lives or rate constants. Unfortunately, these methods are generally categorized as not applicable to volatile chemicals.

The first step of adapting test systems for volatile chemicals with high air to water partition ratios (i.e. Henry's constants) is a closed test design, which circumvents evaporative losses out of the test system. When testing chemicals at low concentrations, the dissolved oxygen in the environmental sample is sufficient for the degradation of the test chemicals, however, environmental samples contain natural organic matter which also consumes oxygen, and therefore a headspace can be needed to ensure aerobic conditions. A major fraction of volatile chemicals, will then reside in the headspace. During the degradation phase, partitioning between water phase and headspace will govern the distribution of chemicals in the test system, continuously replenishing the test chemicals degraded in the water phase. For volatile substances, there is then a mismatch between the effective concentration for the biodegradation in the water phase and the total mass distributed between water and headspace. In unsaturated soil, researchers have addressed the importance of the vapor phase as a reservoir and mass-transfer medium for volatile chemicals (Khan et al., 2016). However, in environmental surface water systems, volatilization would mostly act as a sink rather than a buffer for volatile chemicals, and therefore the water phase biodegradation is the relevant parameter when extrapolating to other test or environmental surface water systems.

The fact that the dissolved concentration rather than the total mass of test chemical may govern biodegradation rates has been realized earlier, especially for highly sorbing chemicals in test systems including sediments and soils (Reichenberg and Mayer, 2006). Monod kinetic parameters have in some cases been determined using sets of non-linear differential equations describing degradation and the distribution or rates of transfer between the air/water phase and unsaturated soil (Höhener et al., 2003; Ostendorf et al., 2007; Rein et al., 2007; Sleep and Mulcahy, 1998), soil-slurry (Woo et al., 2001), test vessel (Guha and Jaffé, 1996) or microcrystals (Adam et al., 2014). These studies employed quite extensive modeling efforts, which are not usually employed in legislative biodegradation testing.

Schirmer et al. (1999) suggested a simple approach assuming instantaneous partitioning between phases and describing the distribution in the test system by a mass distribution coefficient, defined as the ratio of the total mass in the test system to the bioavailable mass at equilibrium. A mass distribution coefficient of 1.86 was determined for *m*-xylene in their study (Schirmer et al., 1999). Later studies (Comber et al., 2012; Prince et al., 2008, 2007), using more volatile chemicals, did not take distribution to headspace into account, and thus biodegradation rates were likely underestimated.

The present study investigated the primary biodegradation of a mixture of 9 (semi)volatile chemicals in surface water. The chemicals were selected to cover different chemical structures of potential oil constituents (see Table 1), and the testing was conducted at low aqueous concentrations (ng/L – µg/L range) in order to obtain biodegradation results of high environmental relevance. We investigated (1) how partitioning between water phase and headspace affects test results and (2) how partitioning can be accounted for by combining an equilibrium partition model with a first order or logistic biodegradation model. The present study introduces a new experimental framework, where phase partitioning of the test chemicals was applied for the (i) conduct, (ii) analytical measurements and (iii) the assessment of the biodegradation tests: (i) Phase partitioning from a loaded silicone rod was used to generate defined composed mixtures of hydrocarbons at the beginning of the experiments. (ii) Phase partitioning into a thin silicone coating was the basis for the automated sampling directly in the test systems at the end of the experiment. (iii) Finally, the phase partitioning of the test substances between water and headspace was measured and then applied to distinguish the biodegradation kinetics in the test system (water and headspace) and the biodegradation kinetics in the water phase. The experimental and analytical procedure was designed to obtain very accurate and precise “relative concentrations”, as input for fitting the biodegradation kinetic model. This was obtained by incubation of test systems in gas tight 20 mL autosampler vials, which at the end of the experiment were measured directly by automated Headspace Solid Phase Microextraction, and by normalizing the Gas Chromatograpy (GC) response by measurements of abiotic control vials which had been incubated together with the samples and measured within the same analytical series.

2. Theory

Monod kinetics can be used to describe biomass growth on a single substrate (Simkins and Alexander, 1984). In a system including headspace, we propose to separate the total mass of the test substance in the test system (m_T) from the concentration in the water phase (C_w) determining the biodegradation rate, realizing that degradation takes place in the water phase.

At low substrate concentration (ng/L – µg/L range) and low initial biomass, monod based degradation kinetics can be simplified by the Logistic model (Simkins and Alexander, 1984), shown in Equation (1) for a test system with headspace.

$$\frac{dm_T}{dt} = -a_{water} V_w C_w (C_{w,0} + X_0 - C_w) \quad (1)$$

X_0 is the initial specific degrader population density divided by the yield (i.e. the amount of chemical needed to produce the initial specific degrader population density), a is the logistic rate constant, K_S is the half-saturation constant for growth of the degrading organisms and it is assumed that $C_{w,0} \ll K_S$.

For practical/regulatory purposes, this model is often approximated by a lag phase, t_{lag}, (during which biomass adapts/increases but no degradation takes place) followed by first order degradation (e.g. OECD 309, 2004).

The first order degradation after the lag phase in systems with a headspace is therefore described by equation (2).

$$\frac{dm_T}{dt} = -k_{water} V_w C_w \quad (2)$$

where k_{water} is the rate constant in the water phase and V_w is the volume of the water phase.