Journal of Hazardous Materials 195 (2011) 378–382

Contents lists available at SciVerse ScienceDirect

Journal of Hazardous Materials

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

Influence of microbial adaption and supplementation of nutrients on the biodegradation of ionic liquids in sewage sludge treatment processes

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ARTICLE INFO

Article history:
Received 8 June 2011
Received in revised form 17 August 2011
Accepted 18 August 2011
Available online 24 August 2011

Keywords:
Ionic liquids
Biodegradation
OECD 301
Adaptation
Supplementation

ABSTRACT

As ionic liquids are winning more attention from industry as a replacement of more hazardous chemicals, some of their structures have the potential to become persistent pollutants due to high stability towards abiotic and biotic degradation processes. Therefore it is important to determine the hazard associated with the presence of ILs in the environment, for example biodegradation under real conditions. Standard biodegradation testing procedures generally permit pre-conditioning of inoculum but do not allow for pre-exposition to the test substance. These are usually conducted in a mineral medium which does not provide additional organic nutrients. Though very valuable, as a point of reference, these tests do not fully represent real conditions. In *in situ* conditions, for example in wastewater treatment plants or natural soils and water bodies, the presence of readily available sources of energy and nutrients as well as the process of adaptation may often alter the fate and metabolic pathways of xenobiotics. Our results have shown that these are the opposing processes influencing the biodegradation rate of ILs in sewage sludge. The results have significant practical implications with respect to the assessment of biodegradability and environmental fate of ILs and other xenobiotics in environmental conditions and their potential remediation options.

1. Introduction

Industrial development during the last decades resulted in increased pollution of the environment by xenobiotics. Due to this, the need for understanding the impact of toxic compounds on microbial populations and the catabolic degradation pathways of xenobiotics has arisen. Thus standardized biodegradability and toxicity test were developed to allow for classification of xenobiotics according to the environmental hazard they pose. Bearing in mind the definition of xenobiotics, as man-made chemicals foreign to organisms which inhabit the environment, their biodegradation rate in natural soils and waters is in most cases much lower than that of natural compounds. Nevertheless structural similarities to biomolecules can result in relatively high biodegradation rates if enzymes of low substrate specificity are present. Factors which may influence this rate, among others, include microbial adaptation and availability of additional nutrients [1].

Ionic liquids (ILs) as a non-conventional class of novel solvents are becoming increasingly important owning to a number of desirable characteristics including negligible volatility, non-flammability, high thermal stability, low melting point, broad liquid range and controlled miscibility with organic compounds or water [2–5]. The negligible volatility limits their impact on air quality, but their release to the environment may affect soil and water. Moreover, some IL structures have the potential to become persistent pollutants due to their high stability towards abiotic and biotic degradation processes. Therefore it is important to determine the hazard associated with the presence of ILs in the environment.

Adaptation is defined as a change in the microbial community that leads to an increase in the biodegradation rate, or maximal biodegradable concentration of a given xenobiotic as a result of previous exposure. Examples of such adaptation processes are *e.g.* rapid degradation of p-nitrophenol by aquatic microorganisms [6] and enhanced degradation rates after elongated exposure of subsurface soil communities to m-cresol, m-aminophenol and aniline [7]. Mechanisms of adaptation usually involve processes such as genetic mutation or horizontal gene transfer, induction of specific enzymes which enhance the degradative capacity of the entire community, and population change such as selective growth of certain strains [8]. All mechanisms may take place simultaneously, or one may dominate and exact prediction of which will occur is not possible [9]. Furthermore it should be noted, that adaptation does

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doi:10.1016/j.jhazmat.2011.08.053

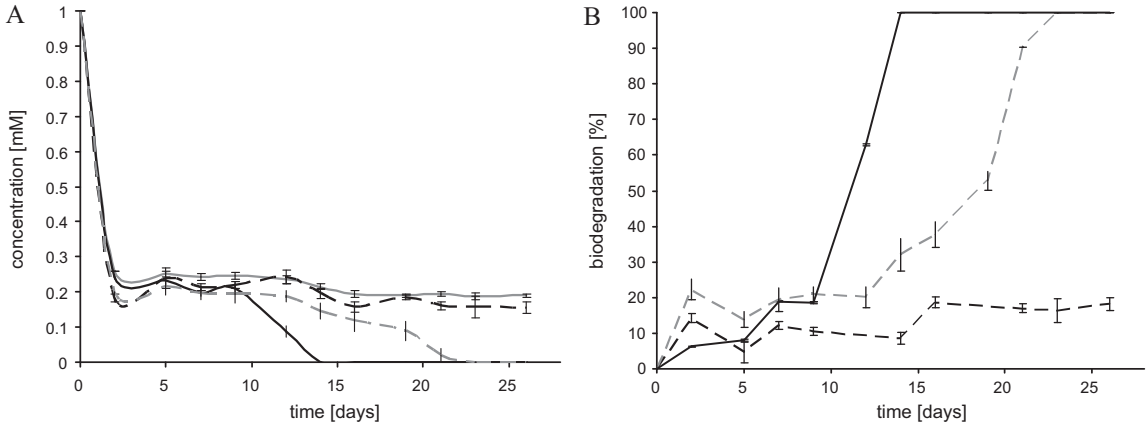

Fig. 1. (A) Biodegradation curves of [OMIM][Cl] as a sole source of carbon (—), with supplementation of glucose (- - -), with supplementation of synthetic feed (▭ ▭ ▭), sorption control (═). (B) Biodegradation (%) normalized for sorption to sewage sludge flocs of [OMIM][Cl] as a sole source of carbon (—), with supplementation of glucose (- - -), with supplementation of synthetic feed (▭ ▭ ▭).

not necessarily occur in every case. Aelion et al. did not observe any adaptation of subsurface microbial communities to chloro- and trichlorobenzene after an eight months adaptation period [7]. Similarly Nyhoim et al. did not note any increase in the biodegradation rate after pre-exposure of activated sewage sludge to aniline and pentachlorophenol [9]. The specific reason for this remains unclear though many theories exist. The most probable reasons include lack of complete enzyme systems within the population, accumulation of toxic degradation products, binding with enzymes causing inactivation or insufficient cell density of inoculum [10]. One of the few papers which discusses the adaptation of soil microorganisms to ionic liquids proposes that the electron-donor ability of the IL effect the biodegradability [11].

A number of research groups have performed biodegradation tests with alkyl substituted imidazolium cations using activated sewage sludge [12–15]. In most cases ILs were used as a sole source of organic carbon and organic nitrogen. This is especially important, because it should be remembered that in wastewater treatment plants or natural environments, other organic substrates are present, which might be preferentially degraded or co-metabolized with the primary contaminant resulting in lower biodegradation rates [16]. Romero et al. [17], discussed the biodegradability of imidazolium ILs in the presence of additional carbon source. It was found that the ILs tested were not biodegradable when D-glucose was available. However, ILs with no additional carbon were also not degraded (2–10%), which is in contrast to other research where, *e.g.* complete primary biodegradation of 1-methyl-3-octylimidazolium chloride [OMIM][Cl] was shown [12,18]. Results of Romero et al., though very interesting, should be treated with caution due to the very short duration of the test (five days) as well as lack of collaborating results in literature.

Standard biodegradation testing procedures generally permit pre-conditioning of inoculum (aeration in the presence of a mineral medium) but do not allow for pre-exposition to the test substance. The purpose of this is to provide repeatable results enabling comparison and standardization of biodegradation rates of different chemicals usually for regulatory purposes. Though very valuable, as a point of reference, these tests do not fully represent real conditions [19]. Therefore, to more accurately predict biodegradation under real conditions it is beneficial to take adaptation into account especially if biodegradation requires induction of specific metabolic pathways, *e.g.* aromatic ring break-down [6,20]. One of the few works which discuss the adaptation of microorganisms to ILs, conducted by Stolte et al., found a sixfold increase in the biodegradation rate of [OMIM][Cl] over a period of 31 days [12]. Additionally, Docherty et al. observed complete biodegradation of hexyl-methylimidazolium bromide after extending duration of the test and concluded that though IL could not be classified as readily biodegradable it is not expected to persist in the environment [20].

The aim of this paper is to describe the effect of additional substrates and pre-exposition of bacteria to IL on the rate of biodegradation, and thereby discuss the relevance of including pre-exposition in standardized tests.

2. Experimental methodology

2.1. Modified OECD 301A DOC Die-Away test – supplementation

The ionic liquid used in the test was [OMIM][Cl] provided by Merck KGA, Darmstadt, Germany. The sewage sludge (dry mass 6.5 g L^{-1}) was taken from the aeration chamber of the "Gdańsk – Wschód" municipal wastewater treatment plant, Gdańsk, Poland. Primary degradation was detected by direct determination of the substrate by HPLC – UV. Eight test flasks containing 0.5 L of sewage sludge flocs and mineral medium composed of: 8.5 mg L^{-1} KH_2PO_4, 21.75 mg L^{-1} K_2HPO_4, 22.3 mg L^{-1} $Na_2HPO_4{\cdot}2H_2O$, 1.7 mg L^{-1} NH_4Cl, 27.5 mg L^{-1} $CaCl_2$, 22.5 mg L^{-1} $MgSO_4{\cdot}7H_2O$ and 0.25 mg L^{-1} $FeCl_3$ dissolved in water were prepared as recommended by OECD procedure [21]. Subsequently, a solution of [OMIM][Cl] was added to yield the concentration of 1 mM and the amount of test solution was made up to 1 L. Each test concentration was conducted in duplicate. Two test flasks were additionally supplemented with glucose and two with synthetic sewage feed (16 g of peptone, 11 g of meat extract, 3 g of urea and 0.7 g NaCl dissolved in 1 L of water). Nutrients were added three times a week, 0.36 g and 2.5 mL, respectively. Also blank samples (without test substance) and chemically sterilized negative controls were prepared. All test vessels were aerated and analytical samples were collected in duplicate at specific time intervals. Mass loss due to evaporation was compensated at every collection interval.

2.2. Modified OECD 301A DOC Die-Away test – adaptation

The test vessels were prepared as previously described. Sewage sludge from the same source was used (dry mass 5.5 g L^{-1}). Increasing concentrations of [OMIM][Cl] (1 mM, 1.5 mM, 2 mM, 2.5 mM) were added every fortnight. The total time for the adaptation test was two months. All vessels were aerated and analytical samples were collected in duplicate at specific time intervals.