Chemosphere 90 (2013) 512–520

Contents lists available at SciVerse ScienceDirect

Chemosphere

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

Evaluation of biodegradability of phenol and bisphenol A during mesophilic and thermophilic municipal solid waste anaerobic digestion using ^{13}C-labeled contaminants

Intissar Limam [a,b,c,*], Mohamed Mezni [c], Angéline Guenne [a], Céline Madigou [a], Mohamed Ridha Driss [b], Théodore Bouchez [a], Laurent Mazéas [a,*]

[a] Hydrosystems and Bioprocesses Research Unit, IRSTEA, 1 rue Pierre-Gilles de Gennes, CS 10030, F-92761 Antony Cedex, France
[b] Applied Analytical Chemistry Research Unit 05/UR/12-03, University of Sciences of Bizerte, 7021 Jarzouna, Tunisia
[c] Methods and Analysis Techniques Laboratory LR 10 INRAP 03, National Institute of Research and Physicochemical Analysis, 2020 Sidi Thabet, Tunisia

HIGHLIGHTS

- ▶ Phenol is biodegradable during mesophilic and thermophilic MSW anaerobic digestion.
- ▶ In mesophilic condition, phenol biodegradation occurs through the benzoic acid pathway.
- ▶ At 55 °C no metabolites (except acetate) were observed as mineralization is instantaneous.
- ▶ Bisphenol A appears not to be biodegradable during MSW anaerobic digestion.

ARTICLE INFO

Article history:
Received 4 April 2012
Received in revised form 2 August 2012
Accepted 8 August 2012
Available online 15 September 2012

Keywords:
Municipal solid waste
Anaerobic digestion
Phenol
Bisphenol A
Stable carbon isotopic tracing
NMR spectroscopy

ABSTRACT

In this paper, the isotopic tracing using ^{13}C-labeled phenol and bisphenol A was used to study their biodegradation during anaerobic digestion of municipal solid waste. Microcosms were incubated anaerobically at 35 °C (mesophilic conditions) and 55 °C (thermophilic conditions) without steering. A continuous follow-up of the production of biogas (CH_4 and CO_2), was carried out during 130 d until the establishment of stable methanogenesis. Then $^{13}C_{12}$-BPA, and $^{13}C_6$-phenol were injected in microcosms and the follow-up of their degradation was performed simultaneously by gas chromatography isotope-ratio mass spectrometry (GC–IRMS) and gas chromatography mass spectrometry (GC–MS). Moreover, Carbon-13 Nuclear Magnetic Resonance (^{13}C-NMR) Spectroscopy is used in the identification of metabolites. This study proves that the mineralization of phenol to CO_2 and CH_4 occurs during anaerobic digestion both in mesophilic and thermophilic conditions with similar kinetics. In mesophilic condition phenol degradation occurs through the benzoic acid pathway. In thermophilic condition it was not possible to identify the complete metabolic pathway as only acetate was identified as metabolite. Our results suggest that mineralization of phenol under thermophilic condition is instantaneous explaining why metabolites are not observed as they do not accumulate. No biodegradation of BPA was observed.

1. Introduction

The methanization of municipal solid waste (MSW) is growing popular as this waste treatment technique allows for the production of renewable energy through the valorization of biogas to electricity and heat. Moreover, the solid residue called digestate can be used either directly or after composting it as soil amendment. As MSW contains several genotoxic and reprotoxic compounds (Robinson et al., 2001; Hiroshi et al., 2002; Öman and Junestedt, 2008), it is important to study the fate of those pollutants during anaerobic digestion in order to promote technologies that may facilitate their biodegradation into less toxic compounds.

In such a complex environment, the mere observation of the reduction of pollutant concentration is not sufficient to deduce the removal of the toxic effects. Indeed, some abiotic processes such as sorption and dilution, which do not remove pollutants, can be responsible for such local observation. Moreover, when

* Corresponding authors. Address: Hydrosystems and Bioprocesses Research Unit, IRSTEA, 1 rue Pierre-Gilles de Gennes, CS 10030, F-92761 Antony Cedex, France. Tel.: +216 98680732; fax: +216 71537688 (I. Limam), tel.: +33 140966061; fax: +33 140966270 (L. Mazeas).

E-mail addresses: intilim@yahoo.fr (I. Limam), Laurent.mazeas@irstea.fr (L. Mazéas).

0045-6535/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.chemosphere.2012.08.019

biodegradation does not lead to the complete degradation of the compounds into CH_4 and CO_2, some metabolites can be produced. Those degradation products can be more toxic than the initial pollutants.

Different approaches have been used to study the biodegradation of organic pollutants in complex ecosystems. The use of heavy stable isotope enrichment (Hunkeler et al., 2002; Meckenstock et al., 2002) is one of those approaches. Another technique is the characterization and identification of the functional microbial population involved in biodegradation (Amann et al., 1995; Radajewski et al., 2000; Heinaru et al., 2005; Hendrickx et al., 2006). As recently illustrated in the literature, isotopic tracing using ^{13}C-labeled pollutants represents a promising methodology to study the fate of a specific pollutant in a complex environment (Morasch et al., 2007).

Phenol and bisphenol A (BPA) are two of the main pollutants present in MSW. BPA has elicited great interest because of its endocrinal disrupting activity (Fromme et al., 2002; Oehlmann et al., 2008; vom Saal and Myers, 2008). This industrial chemical is largely used in the manufacture of polycarbonate plastics, epoxy resins, powder paints, and other products (Staples et al., 1998). In addition to being the precursor of BPA, phenol is used in the production of a variety of resins, including phenolic, epoxy, polycarbonate and polyamide, for various applications (ASTOR, 1989). In the natural environment, phenol is present for example, in olive mill wastewater with a high polluting risk as well as high antibacterial activity (Bleve et al., 2011). Phenol and its vapor are corrosive to the eyes, the skin and the respiratory tract, even at low concentrations. Phenol is a mutagenic product and its adverse effects on health are well known (Sax, 1983; Calabrase and Kenyon, 1991).

Bisphenol A in spiked river water samples was rapidly biodegraded under aerobic conditions (>90%), while a decrease of BPA concentration under anaerobic conditions was hardly found (<10%) over 10 d (Kang and Kondo, 2002a). Moreover, in anaerobic marine sediments, BPA was not biodegraded even after 3 months of incubation. These results suggest that anaerobic bacteria have little or no ability to degrade BPA (Voordeckers et al., 2002; Kang et al., 2006). Bisphenol biodegradation by microorganisms is also influenced by temperature and microbe counts (Kang and Kondo, 2002b). The biodegradation of BPA during MSW anaerobic digestion has not been studied yet.

Phenol is biodegradable under mesophilic conditions, through the benzoic acid degradation pathway proposed by different authors (Knoll and Winter, 1989; Sharak Genthner et al., 1991; Karlsson et al., 2000; Agarry, 2008). On the contrary, under the thermophilic conditions Leven et al. have observed that phenol was not biodegradable during anaerobic digestion (Levén and Schnürer, 2005). Nevertheless, in some other anaerobic environments a pathway through cyclohexanone and caproic acid has been suggested (Fang et al., 2006).

In this study, some ^{13}C enriched phenol or bisphenol A have been introduced during mesophilic and thermophilic anaerobic incubations of reconstituted MSW. A coupled molecular and isotopic approach was used to monitor the mineralization kinetic of those compounds and to identify the potential accumulated metabolites.

2. Materials and methods

2.1. Chemicals

The ethyl acetate, methanol, acetone, acetic anhydride, potassium bicarbonate, mercuric chloride and sodium chloride were obtained from Supelco (St. Quentin Fallavier, France). All the solvents and reagents were of analytical grade and ultra-pure water from Milli-Q system (Eschborn, Germany) was used throughout the experiments. The $^{13}C_6$-phenol (99%) and $^{13}C_{12}$ – bisphenol A (Rings-$^{13}C_{12}$, 99%) were purchased from Cambridge Isotope Laboratory (Andover, USA).

2.2. Inocula preparation

Two types of inocula were used in this study. The first type was prepared by the centrifugation of 10 fractions of 200 mL of leachate from a MSW landfill. The second type was prepared by the centrifugation of six fractions of 50 mL of an anaerobic sludge from a municipal wastewater treatment plant (MWTP). The centrifugation was carried out at 4 °C, during 10 min at 8500 rpm (AllegraTM X-22R Centrifuge, Beckman Coulter, France). Finally, 4 MSW inocula were used for the $^{13}C_6$-phenol degradation study; 6 MSW inocula and 6 MWTP inocula were used for $^{13}C_{12}$-bisphenol A degradation study.

2.3. Microcosm studies

Experimental microcosms were incubated in duplicate for phenol and triplicate for BPA at mesophilic as well as thermophilic conditions, as described previously (Qu et al., 2009). The first set of microcosms (set I) consisted of 10 glass bottles of 330 mL (Fischer Scientific Bioblock, Illkirch, France) filled with 210 mL of $NaHCO_3$ buffer solution at pH = 8.3 and 10 g of reconstituted French Solid Waste (MODECOM Composition). Then, the prepared MSW inocula were added simultaneously to the four bottles devoted to $^{13}C_6$-phenol and the six bottles devoted to $^{13}C_{12}$-bisphenol A.

$^{13}C_6$-phenol and $^{13}C_{12}$-bisphenol A were injected in the microcosms after 130 d of incubation when the stable methanogenesis phase was established. We added 1.5 mg of $^{13}C_6$-phenol to each microcosm devoted to phenol. We also added 1.2 mg of $^{13}C_{12}$-bisphenol A to each microcosm devoted to BPA. Consequently we obtained the initial dissolved concentrations of 7142 $\mu g\,L^{-1}$ for $^{13}C_6$-phenol and 4800 $\mu g\,L^{-1}$ for $^{13}C_{12}$-bisphenol A. Eventually, two microcosms were incubated for phenol and three microcosms for BPA at 35 °C. The same was done at 55 °C. For each temperature, one control microcosm is left after being prepared by adding 300 mg of $HgCl_2$ diluted in 15 mL sterilized water. This quantity is over the recommended concentration to ensure a complete inhibition of the microbial activity in the control microcosm (Tuominen et al., 1994).

Microcosms were then incubated in the dark at 35 °C and 55 °C under methanogenic conditions during 100 d in the case of $^{13}C_6$-phenol and 327 d in the case of $^{13}C_{12}$-bisphenol A. During this period, biogas (CH_4 and CO_2) isotopic composition was measured by Gas Chromatography–Combustion–Isotopic Ratio Mass Spectrometry (GC/C/IRMS). Moreover, a quantitative analysis of $^{13}C_6$-phenol and $^{13}C_{12}$-bisphenol A was performed by Gas Chromatography–Mass Spectrometry (GC/MS).

Only in the case of BPA, after the follow-up of this first set (set I) of the experiment (130 d: methanogenesis stabilisation and 200 d: $^{13}C_{12}$-bisphenol A degradation with the MSW inoculum), a second experimental period (set II) was put out. This second set is performed by incubating six glass bottles of 150 mL filled with 50 mL $NaHCO_3$ buffer solution at pH = 8.3 and containing the prepared MWTP inocula. After a period of 10 d these microcosms were stabilized and no biogas production was observed. An amount of 10 mL of microcosm leachate containing non-degraded bisphenol A from set I was introduced in only four bottles (the two remaining bottles were control microcosms). At both temperatures, we monitored the biogas isotopic composition.

For all incubations, in order to be in anaerobic conditions, glass bottles were closed with a screw cap and a septum (Fischer Scientific Bioblock, Illkirch, France) and an atmosphere of Nitrogen was