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Biotransformation and inhibition effects of hexachlorocyclohexanes during biogas production from contaminated biomass characterized by isotope fractionation concepts

Shujuan Lian[a], Marcell Nikolausz[b], Ivonne Nijenhuis[a], Athaydes Francisco Leite[b], Hans Hermann Richnow[a,*]

[a] Department of Isotope Biogeochemistry, Helmholtz Centre for Environmental Research – UFZ, Permoserstraße 15, 04318 Leipzig, Germany
[b] Department of Environmental Microbiology, Helmholtz Centre for Environmental Research – UFZ, Permoserstraße 15, 04318 Leipzig, Germany

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ABSTRACT

Hexachlorocyclohexane (HCH) production for pesticides was banned by Stockholm Convention (2009) due to its harmful and adverse effects on the environment. Despite this measure, many areas contaminated with former HCH production-waste products still require management. As a potential solution contributing to clean-up of these sites, anaerobic digestion (AD) of pesticide-contaminated biomass to produce biogas is a promising strategy. High pesticide concentrations, however, may inhibit biogas production. Therefore, laboratory-scale batch reactors were set up to investigate biogas reactor performance in presence of HCH. Inhibitory effects on biogas yield was observed with concentrations of HCH ≥ 150 mg/L. Carbon isotope composition of methane ($\delta^{13}C_{CH4}$) showed significant fluctuation after an inhibition phase, indicating that HCH toxicity can affect the activity of acetoclastic methanogens. Furthermore, combined results of metabolites and carbon isotope fractionation factors (ε_c) demonstrated that α- and γ-HCH can be degraded to chlorobenzene and benzene via anaerobic reductive dechlorination.

1. Introduction

A huge amount of Lindane (containing 99% of γ- hexachlorocyclohexane (HCH)) was used as pesticide worldwide and the other HCH isomers as side-products of the synthesis are still found at high concentrations at contaminated fields (Quintero et al., 2005). Due to the inappropriate treatment of HCH containing wastes and the persistence of HCH residues in environment, these HCH wastes still cause serious environmental issues, related to their lasting toxic effects, and bio-accumulative and long range transport capacity (Pozo et al., 2009). Thereby, the Stockholm Convention in 2009 classified HCHs as persistent organic pollutants and banned the substance.

Quintero et al.(2006) demonstrated the total degradation of the HCH isomers and high degradation rates, especially for α- and γ-HCH, in anaerobic slurry reactor. Anaerobic systems have ultimate potential for the elimination of halogenated organics, as result from reductive dehalogenation reactions, which can be an energy yielding process under anoxic conditions (van Lier et al., 2001). Reductive dehalogenation is an electron transfer reaction that can release halogen as a halogenide ion and replace it by hydrogen. Therefore, anaerobic digestion (AD) systems have the potential to utilize contaminated biomass of plants used for phytoremediation of contaminated fields. Studies on reductive dehalogenation of HCH under anoxic conditions in other systems have already been published (Jagnow et al., 1977; Phillips et al., 2005). Only a few studies on degradation of HCH isomers were also carried out in bioreactors (Baczynski and Kurbiel, 2001; Bhatt et al., 2006). Quintero et al. (2005) and Robles-González et al. (2012) found chlorobenzene as metabolite in AD process.

Waste products from agriculture, industries and municipalities are often applied in AD and used as carbon sources for biogas production. The resulting digestate from biogas plants is commonly used as fertilizer containing carbon, nitrogen, and phosphorous-rich substances. However, the presence of remaining residues such as pesticides, pharmaceuticals, and other toxic chemical compounds in the digestate may contaminate the fields and watersheds (Sreekanth et al., 2009). Therefore, to prevent the deposition and accumulation of toxic chemical residues in the environment, an understanding of the microbiome capacity to degrade these chemicals in AD is crucial.

This study aimed at investigating the effects of HCH on biogas yield when the HCH-contaminated biomass used as the substrate of AD

* Corresponding author.
E-mail address: hans.richnow@ufz.de (H.H. Richnow).

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process, and deducing the influence of AD on this pesticide. Thus, a concept was developed based on stable isotope fractionation for the assessment of potential inhibition effects caused by the toxicity of HCHs on biogas production and identification of biotransformation mechanisms of HCH isomers during AD process via reductive dechlorination.

In order to identify the transformation of HCH in AD system, carbon isotope composition ($\delta^{13}C$) of HCH extracted from the digestate was measured by compound-specific stable isotope analysis (CSIA). Studies on stable isotope analysis concepts to trace the biotransformation of HCH in contaminated ground water have been published (Liu et al., 2017). Moreover, the carbon isotope fractionation factors (ε_c) of γ- and α-HCH were calculated to investigate the transformation mechanism in the bioreactor and compared with other HCH-degrading cultures (Badea et al., 2009, 2011; Bashir et al., 2013).

Values of $\delta^{13}C$ in CH_4 and CO_2 can allow interpretation of the predominant methane formation pathway in AD (Conrad, 2005; Nikolausz et al., 2013), and can be used as an indicator of pathway shift and for analysing the inhibition of methanogenesis during the biogas process (Lv et al., 2014; Leite et al., 2016). The observed apparent fractionation factor (α_C) has potential to characterise the predominant operation of acetoclastic or hydrogenotrophic methanogenesis, respectively (Whiticar et al., 1986).

2. Materials and methods

2.1. Experimental setup

2.1.1. Automatic methane potential test system (AMPTS) for inhibition effect

An automatic methane potential test system (AMPTS II, Bioprocess Control Sweden AB, Sweden) was used to measure the methane yield under different concentration levels of mixed pure γ-HCH and muck sample (5 mg/L, 50 mg/L and 150 mg/L for each chemical) in triplicate following the AMPTS manual guideline. Mixtures of 28.5 g maize silage and 371.5 g inoculum (Section 2.3) were added to 640 mL bottles with a 240 mL headspace. Accordingly, positive controls (PC set) were set up by maize silage and inoculum, without HCHs added. Triplicate negative controls (NC set), only with the inoculum, were filled up by distilled water to 240 mL headspace. Thus, 15 bottles were setup in total. The headspaces were flushed with nitrogen for providing anoxic conditions. Batch reactors conducted at mesophilic temperature (39 °C) were stirred at 80 rpm for 1 min and followed with 1 mL pause cyclically. A CO_2-trap unit with a 3 M NaOH solution was connected before the gas volume measurement. The produced methane was automatically recorded by the device and corrected to standard temperature (273.15 K) and pressure (101.32 kPa), then reported as normalized milliliters. The daily methane production was lower than 5% of the total methane production after 36 days, when the experiment was stopped. Biogas samples were periodically collected in 10 mL gas-tight vacuumed vials from the headspace of the AMPTS in triplicate and stored for further analyses.

Experiments were conducted to assess the inhibitory effect of potential HCH metabolites on the methane production. PC set and NC set were set up with benzene and chlorobenzene as described above. Benzene (517 μM) or chlorobenzene (517 μM) was added separately to the AD system in triplicate experiments in amounts equal to the experiments with high concentration of HCH (150 mg/L is equal to 517 μM).

2.1.2. Batch experiments I for assessment of transformation pathway

Chemical pure γ- and α-HCH were used to study the HCH transformation pathway, separately, via batch experiments in 120 mL vials with 70 mL headspace closed with Teflon™-coated butyl rubber septa and crimped. The headspace was flushed with nitrogen for 5 min before the bottles were crimped. The substrate and inoculum source as well the ratio of digestate to maize silage were under the same conditions as AMPTS test (inhibition test). Initial concentration of HCH (each isomer) was set as 87.25 mg/L (300 μmol/L) in the bottle. Twelve parallel bottles were prepared to allow for stopping the transformation reaction at different time points during AD, and for extraction of HCH and metabolites. Positive and negative controls were also set in 50 mL valid volume as same as described above. Gas composition and production were measured as described previously, (Porsch et al., 2015).

2.1.3. Batch experiments II for stable carbon isotope fractionation of γ- and α-HCH in AD

In order to derive carbon isotope fractionation factors (ε_c) Eq. (5), 21.81 mg/L (75 μmol/L) for γ and α-HCH (chemical purity) were added respectively into 120 mL bottles with 50 mL biogas digestate. The bottles were prepared the in same way as described above in Section 2.1.2. Fifteen parallel bottles for each isomer were prepared for sampling at different time points and extents of reductive dechlorination. In addition, triplicate killed controls with sterilized digestate and HCH were conducted. In the sterilized control, no metabolites were detected, proving that biotransformation only occurred in active biogas digestate.

2.2. Chemicals

Waste residues, HCH muck, made up from > 90% (weight) white crystals was taken from a dump site in Bitterfeld (Germany), where high concentration of Lindane by-products was found. The distribution of α-, β-, γ-, and δ-HCH in these crystalline materials was around 85%, 10%, 1%, and 0.4%, respectively. The $\delta^{13}C$ of α-HCH was in the range from $-27.5 \pm 0.2‰$ to $-29.8 \pm 0.3‰$ (Ivdra et al., 2017; Liu et al., 2017). Hexachlorobenzene (HCB) (Lot 60119, analytical purity of 99%), α-HCH (chemical purity of 99%) and γ-HCH (chemical purity of 99%) were obtained from Sigma-Aldrich (Munich, Germany). Anhydrous Na_2SO_4 (extra pure) and hydrochloric acid (HCl) were purchased from Merck (Darmstadt, Germany).

2.3. Substrate and inoculum

Maize silage (Total solids (TS) = 35.65%, Volatile solids (VS) = 34.50%) was used as substrate at an inoculum to substrate ratio of 2:1. The digestate taken from a pilot-scale biogas plant operating with maize silage as main substrate was sieved (1 cm) and degassed before using it in batch reactors as inoculum. Then TS (6.84%) and VS (52.90%) of the inoculum were measured for calculating the amount for experimental setup.

2.4. Analytical methods

2.4.1. Basic parameters

TS and VS were determined according to the standard methods (Verein Deutscher Ingenieure, 2006). The pH values of digestate were measured before and after setup (WTW pH 3310 equipped with SenTix 41 electrode, Germany). Concentrations of HCH and metabolites in digestate were measured to calculate the transformation rate. Extraction with organic solvent for HCH and metabolites is described in Supplementary material provided in this paper. An Agilent 6890 series gas chromatograph (Agilent Technologies, Germany) equipped with a flame ionization detector (FID) was used to determine the concentration of HCH and its metabolites. The compounds were separated in a HP-5 column (30 m × 320 μm × 0.25 μm, Agilent 19091J-413, USA) with helium flow of 1.5 mL/min as the carrier gas.

2.4.2. Stable isotope compositions

The $\delta^{13}C$ of gas samples was analyzed using a GC–C–IRMS system consisting of a gas chromatograph (HP 6890 series; Agilent Technology, Santa Clara, CA, USA) coupled with an IRMS (Finnigan MAT253; Thermo Finnigan, Bremen, Germany) via a combustion interface (Feisthauer et al., 2010). For the GC separation of CH_4 and CO_2, gas