Fate of the insecticide chlordecone under methanogenic conditions

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Highlights

- A natural methanogenic biomass can remove 1 or 2 chlorine atoms from chlordecone
- Chlordecone dechlorination by methanogenic biomass is less efficient than adsorption
- Digester sludge is not a good source of chlordecone degraders for soil bioremediation

Keywords: mono-hydrochlordecone; chlordecol; 5b-hydrochlordecone; organochlorine; anaerobiosis

Introduction

Chlordecone (CLD) is an organochlorine insecticide of raw formula $C_{10}Cl_{10}O$, used from 1972 to 1993 (date of its ban) to control the banana weevil populations in French West Indies (FWI). By the time of writing, i.e. three decades after its ban use, CLD is still present in the FWI soils and has led to widespread contamination of the other FWI environmental compartments and associated food resources, resulting in a widespread impregnation of the West Indian population, with proven health consequences. Despite a series of measures to prevent CLD from reaching the consumer's plate, it continues to circulate actively at least among the population living in the most contaminated areas. A definitive solution to the problem would be to destroy the stock of CLD still present in soil. Of all the options available to achieve this goal, microbial degradation is generally an economically attractive option, as well as being potentially applicable *in situ*. Until the late 2010's, because of its structure, CLD was considered non-biodegradable but the work of Dolfing et al (2012) showed that there was no thermodynamic impediment to its partial transformation (e.g dechlorination) or ultimate mineralization, and that microorganisms capable of catalyzing these reactions should/could exist. Old literature data (Jablonski et al., 1996) suggested that all methanogens would be enzymatically equipped to attack CLD and that natural sources of these organisms such as anaerobic digesters could eventually be used for bioaugmentation in a soil remediation process. This possibility was reinforced by the monitoring of CLD concentrations at the inlet and outlet of 2 FWI industrial anaerobic digesters treating contaminated rum vinasses, which showed that 53 to 91% of the CLD present in the vinasses was not found at the outlet of the digesters and had apparently not accumulated in their biomass (Macarie et al., 2013). These conclusions remained fragile, however, due to i) the difficulty of quantifying biomass in reactors of several hundred m^3, ii) the low concentration of CLD in vinasses (a few µg/L), and iii) their complex composition, which make it impossible to track any transformation products, including chlorides. The objective of this work was to reproduce at lab scale the operation of an anaerobic digester fed with a simple culture medium containing CLD in order to make a precise CLD mass balance and confirm or not the capacity of any methanogenic mixed culture to degrade it.

Material and Methods

The experiments were done at ambient temperature with a 2 L glass UASB reactor covered by an aluminum film which was inoculated with a known quantity of granular methanogenic sludge from one of the two industrial reactors previously studied. The reactor was fed continuously for 81 days at a constant hydraulic retention time and volumetric loading rate through PTFE tubing with a simple low-chloride aqueous culture medium containing 2 mg CLD/L in dissolved form and the nutrients required for the methanization process (sources of C, N, P, S, trace metals). CLD was not added to the culture medium until one week after the start of the operation. Two categories of parameters were followed, those necessary to control the operation of the system and confirm the good functioning of the methanogenic population (COD inlet/outlet, pH, sludge redox potential, methane production, feed rate) and those necessary to evaluate CLD degradation (concentration of CLD and some of its known

degradation intermediates at the reactor inlet, outlet and in the reactor sludge and chloride concentration at the outlet). The quantification of CLD and known intermediates was done by GC-MS but also LC-MS for the reactor sludge. GC-MS was additionally used to search for the eventual presence of unknown transformation products. Chloride was determined by ionic chromatography

Results and Discussion

The parameters monitored confirmed that the system performed well under methanogenic conditions: very negative redox potential (EAg/AgCl = -345 to -434.2 mV) characteristic of this type of environment, total COD removal of 96.2 ± 2.5% coupled with a methane yield very close to the theoretical yield of 0.35 LCH_4/g $COD_{degraded}$. Remarkably, reactor performance remained stable after the addition of 2 mg CLD/L to the culture medium indicating the absence of CLD toxicity at this concentration for all the microbial partners required for the conversion of carbonaceous substrates to CH_4. In addition to CLD (2.21 ± 0.39 mg/l measured), chlordecol (17.2 ± 3.9 µg/l) and 5b-hydro-CLD (1.47 ± 0.1 µg/l) were detected in the culture medium. They correspond to impurities present in the analytical standard-grade CLD used to prepare it. A removal of over 99% of CLD and 5b-hydro-CLD and over 95% of chlordecol between the reactor inlet and outlet was obtained from the start of the CLD feed. This reduction was not associated with the accumulation of degradation intermediates in the liquid phase, with the exception of a monohydro-CLD (CLD-1Cl) different from 5b-hydro-CLD whose concentration however did not exceed 3.15 µg/l, representing barely 0.17 mol% of the CLD supplied to the reactor. Traces of 2 dihydro-CLD (CLD-2Cl) were also detected at the reactor outlet, but due to a lack of available analytical standards, they could not be quantified. Chloride analysis in the aqueous phase at the reactor outlet was found to be within the background noise of that of the low chloride content deionized water used (0.23 ± 0.13 mg/L) to prepare the medium, and well below that (1.43 mg Cl-/L) corresponding to 100% dechlorination of 2 mg CLD/L, thus demonstrating the absence of CLD transformation into more dechlorinated products not detected by GC-MS or its full mineralization. In order to reach a definitive conclusion on the fate of CLD in the reactor, its biomass was recovered and its CLD content quantified. Analysis on this matrix proved difficult, but finally, after much trial and error, the last method used (SPME[1]-GC-MS combined to standard addition on fresh biomass), showed that at least 69% of the CLD brought to the reactor had accumulated in its biomass, and that the CLD-1Cl also detected represented 4.3 mol% of the fed CLD.
Although the CLD mass balance did not close (25% of the CLD fed to the system was not recovered at the exit of the reactor, in its sludge or in the form of the transformation products quantified), the previous results show that the main mechanism of CLD removal in the reactor was due to adsorption on the biomass. The presence of CLD-1Cl and CLD-2Cl not detected at the entrance of the system confirms however that the methanogenic biomass used had the capacity to dechlorinate CLD, but that this capacity was very low despite massive inoculation of the reactor. They therefore suggest that the microbial group capable of dechlorinating CLD was probably not methanogenic and represented a very tiny proportion of the microbial consortium. Although the results suggest a slow capacity for enrichment of this population over time (CLD-1Cl concentration increased at the outlet from 0.031 µg/L on day 11 to 3.157 µg/L on day 81), they also show that the possibility of using digester sludge for bioaugmentation in a soil remediation process to achieve CLD degradation would not be viable.

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[1] *SPME: Solid Phase Micro Extraction*

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