Biotransformation of γ-hexachlorocyclohexane (lindane) to non-toxic end products by sequential treatment with mixed anaerobic microbial cultures

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γ-hexachlorocyclohexane (γ-HCH) or lindane

1825: synthesis by M. Farady

1912: isolated by T. van der Linden

1942: insecticidal properties discovered

1950 – 2000: production of 600,000 t

1970: restricted use in the U.S. and other countries

2007: manufactured only in Russia and India, banned in the U.S.

2009: included in the Stockholm Convention POP list

2015: classified as carcinogenic

2019: aerobic degradation pathways and genes are relatively well understood; anaerobic biodegradation and the microorganisms involved are not well understood and anaerobic enzymes are unknown.

Aqueous solubility ~ 7 mg/L
Log Kow ~ 3.7
HCH isomers and technical HCH (t-HCH)

Figure 1 in Nayyar and Lar (2016), Hexachlorocyclohexane Contamination and Solutions: Brief History and Beyond. Emerging Model to Study Evolution of Catabolic Genes and Pathways. 10.4172/2155-6199.1000338.
HCH contamination is of global concern

**Sabiñánigo, Aragon (Spain)**
Generated 6800 t/year of solid HCH waste (1975–1988)  
(Fernández, Arjol et al. 2013)

**Guadeloupe and Martinique**
Extensively used in banana crops  
(Laquitaine, Durimel et al. 2016)

**Bitterfeld-Wolfen, Germany**
Produced 4200 t/y of lindane and technical HCH (1951–1982)  
(Popp, Brüggemann et al. 2000)

**Pearl River Delta, China**
China produced ~ 33% of the global HCH (Zhang, Parker et al. 2002)
HCH microbial reductive dechlorination

\[ \text{Electron acceptor} + 2H^+ + 2e^- \rightarrow \text{Electron donor} + 2H^+ + 2Cl^- \]

*Dehalobacter sp. E1* (metabolic transformation of β-HCH)
*Clostridium* spp. (co-metabolic transformation of α-HCH and γ-HCH)
*D. mccartyi* strains BTF08 and 195 (co-metabolic transformation of γ-HCH)

The Guadeloupe transferred (GT) HCH enrichments


Rate: α ~ γ ~ δ > β

HCH microbial reductive dechlorination often leads to the accumulation of toxic by-products

- **sediment microcosms** (Boyle, Haggblom, Young 1999)
- **co-cultures** (Van Doesburg, Van Eekert et al. 2005)
- **anaerobic sludge** (Elango, Kurtz et al. 2011)
- **DNAPL pools from landfill leachate** (Fernandez, Arjol et al. 2013; Santos, Fernández et al. 2018)
- **D. mccartyi strains 195 and BTF08** (Bashir, Kuntze et al. 2018)
- **enrichment cultures** (Qiao, Puentes Jacome et al. 2019 in preparation)
Is anaerobic biotransformation of γ-HCH to non-toxic end products possible?

γ-HCH (lindane) → MCB → Benzene → CH₄ + CO₂

Microbial culture I + Microbial culture II + Microbial culture III = Non-toxic end products
# Overview of anaerobic enrichment cultures

<table>
<thead>
<tr>
<th></th>
<th>Culture I</th>
<th>Culture II</th>
<th>Culture III</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Source:</strong></td>
<td>HCH-contaminated sediments from Guadeloupe</td>
<td>TCE-contaminated soils in southern Ontario (KB-1-derived culture)</td>
<td>Soil samples from an Oklahoma Oil Refinery</td>
</tr>
<tr>
<td><strong>Electron acceptor:</strong></td>
<td>HCH</td>
<td>Monochlorobenzene (MCB)</td>
<td>CO₂</td>
</tr>
<tr>
<td><strong>Electron donor:</strong></td>
<td>Ethanol</td>
<td>Methanol</td>
<td>Benzene</td>
</tr>
<tr>
<td><strong>Catalyzed reaction:</strong></td>
<td>HCH → MCB + Benzene</td>
<td>MCB → Benzene</td>
<td>Benzene → CH₄ + CO₂</td>
</tr>
</tbody>
</table>

![Chemical reactions](image)

Date: 2019-03-04
Culture I, γ-HCH (lindane) is transformed to benzene and monochlorobenzene (MCB).

γ-HCH (lindane) is completely transformed to benzene and MCB.
Culture II, dechlorination of MCB to benzene

Cl

\[ \text{MCB} \rightarrow \text{Benzene} \]

\[ \Sigma < 1\% \]

\begin{itemize}
  \item Synergistia (c)
  \item Bacteroidetes (p)
  \item Dehalobacter (g)
  \item Peptococcaceae (f)
\end{itemize}

![Graph showing dechlorination of MCB to benzene](image)

![Pie chart showing bacterial distribution](image)

![Bar chart showing gene copy number](image)
Culture III, benzene degradation under methanogenic conditions

\[ \text{Benzene} \rightarrow \text{H}_2 \rightarrow \text{CH}_3\text{COOH}^- \rightarrow \text{CH}_4 + \text{CO}_2 \]

- **CH\textsubscript{3}COOH\textsuperscript{-} (acetate)**
- **H\textsubscript{2}**
- **CH\textsubscript{4} + CO\textsubscript{2}**

**Pie Chart:**
- **Σ < 1%**
- **Candidatus marinimicrobica (p)**
- **Spirochaetaceae (f)**
- **Deltaproteobacteria (c) (ORM2)**

**Graphs:**
- Time (d) vs. Benzene (mg/L)
- Time (d) vs. Copies/mL Culture

- **ORM2**
  - Fed
  - Starved

2019-03-04
Sequential biotransformation of γ-HCH

Phase I
- Autoclaved (n=3)
- Active (n = 9)
  - ~ 3.8 mg γ-HCH/bottle
  - ~ 42 mg γ-HCH/L of culture
  - ~ 150 μmol γ-HCH/L of culture

Phase II
- Addition of culture II

Phase III
- Addition of culture III
Phase I: γ-HCH was transformed to MCB and benzene
Phase II: MCB was dechlorinated to benzene

Active bottles with culture II (n=5)

Controls without culture II (n=3)

Gene copies per mL

Bacteria  Dehalobacter
Phase III: benzene was biodegraded

Active bottles with culture III (n=2)

Control bottles without culture III (n=2)

Amount per bottle (µmol)

Time (d)

Benzene added

Deltaproteobacteria ORM2

Relative abundance

before inoculation

after inoculation

t = 337 d  
t = 340 d  
t = 412 d
Conclusions and Implications for HCH remediation

Our results indicate that successive or sequential bioconversion of γ-HCH all the way to non-toxic products is possible

- The process was accelerated using sequential addition of three specific anaerobic enrichment cultures.
- MCB and benzene can be biotransformed by anaerobic enrichment cultures.
- This approach is applicable to other HCH isomers, specially δ-HCH (low benzene to MCB ratio).
- Analogous to our experiments, field bioremediation approaches must be dynamic and should account for the spatial and temporal gradients in contaminated soils, sediments, and groundwater.
- Sequential bioaugmentation combined with active monitoring may be a suitable approach to tackle the world-wide HCH-contamination.
- Suitable technologies to deploy enrichment cultures in sediments need to developed and/or evaluated.
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