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

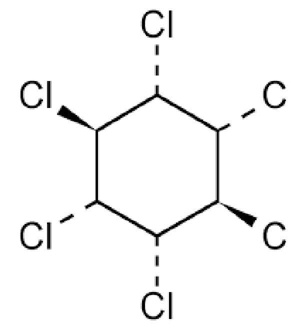
Luz A. Puentes Jácome, Line Lomheim and Elizabeth Edwards
University of Toronto, Toronto, ON, Canada

Wenjing Qiao and Shujun Ye
Nanjing University, Najing, China

Xianjin Tang
Zhejiang University, Hangzhou, China

Sarra Gaspard
Université des Antilles, Guadeloupe, France

γ-hexachlorocyclohexane (γ-HCH) or lindane



Aqueous solubility ~ 7 mg/L
Log K_{ow} ~ 3.7

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.**

HCH isomers and technical HCH (t-HCH)

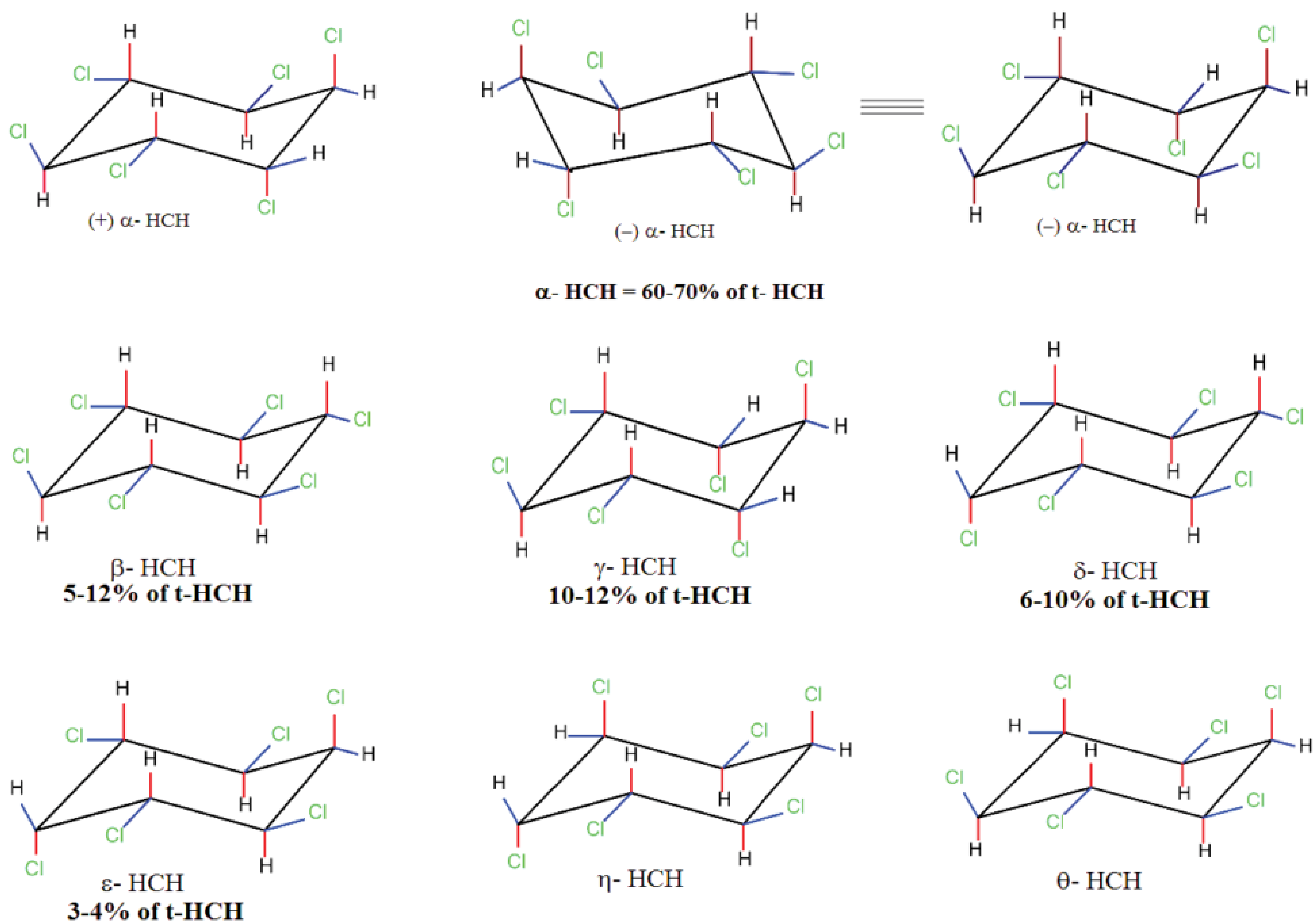
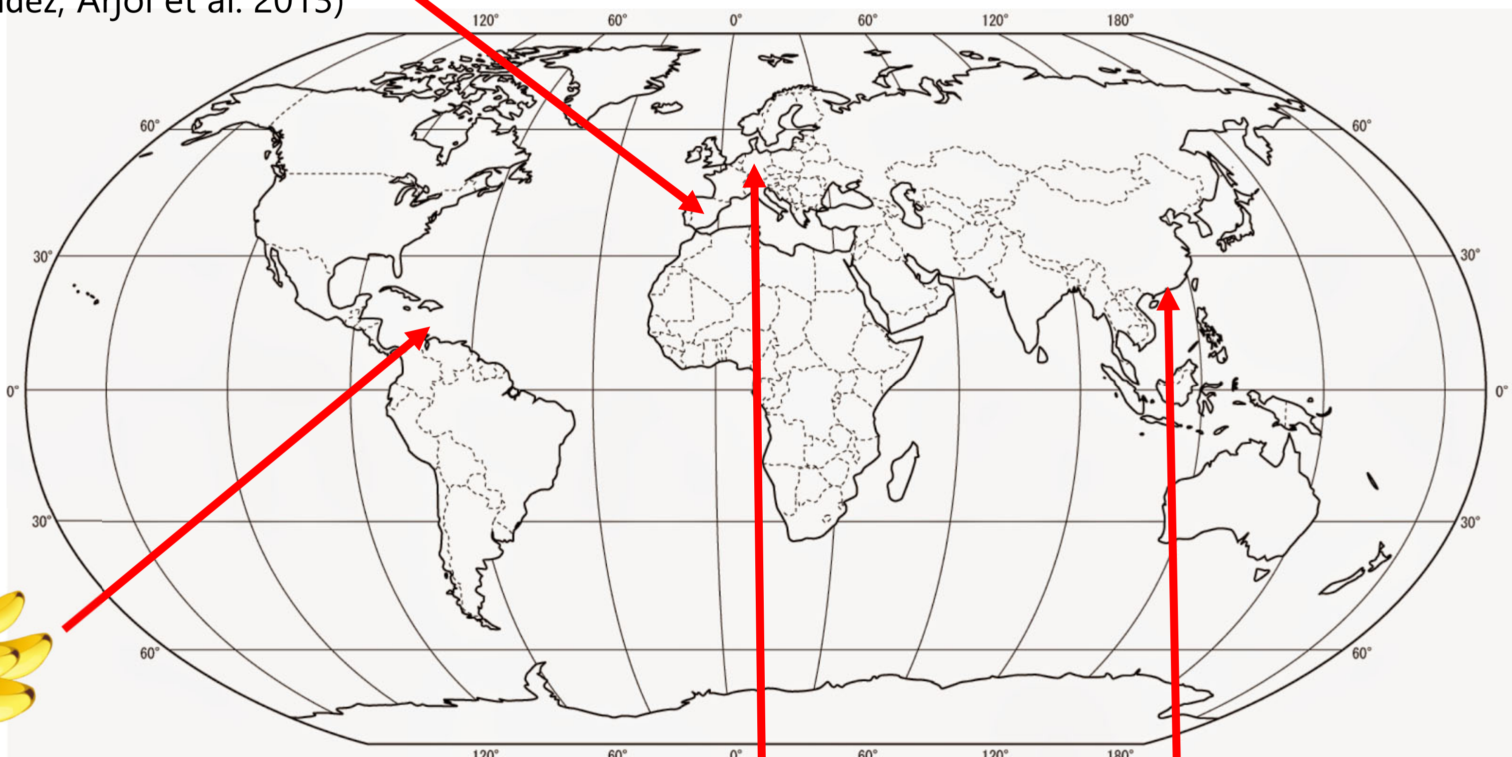


Figure 1 in Nayyar and Lar (2016), Hexachlorocyclohexane Contamination and Solutions: Brief History and Beyond. Emerging Model to Study Evolution of Catabolic Genes and Pathways. 10.4172/2155-6199.1000338.

HCH contamination is of global concern

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

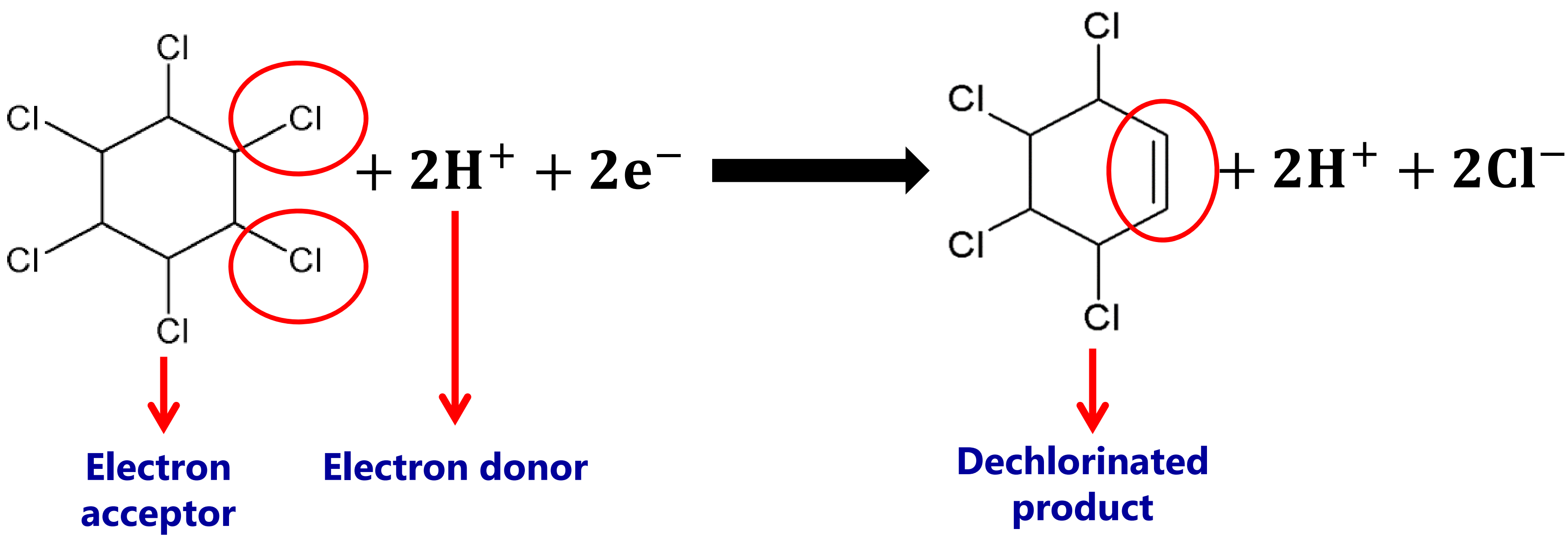


Guadeloupe and Martinique
Extensively used in banana crops
(Laquitaine, Durimel et al. 2016)

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



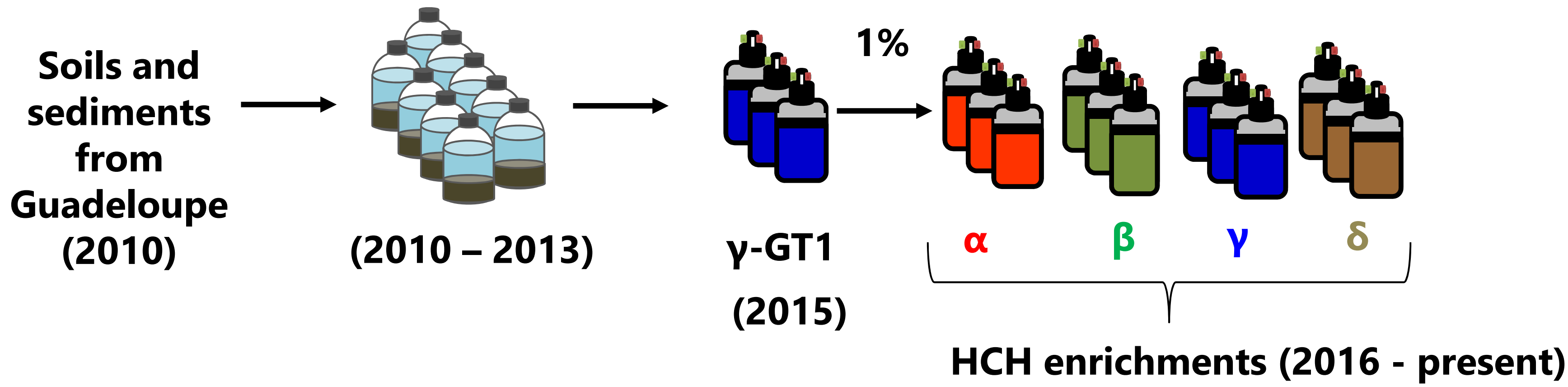
Dehalobacter **sp. E1 (metabolic tranformation of β-HCH)**

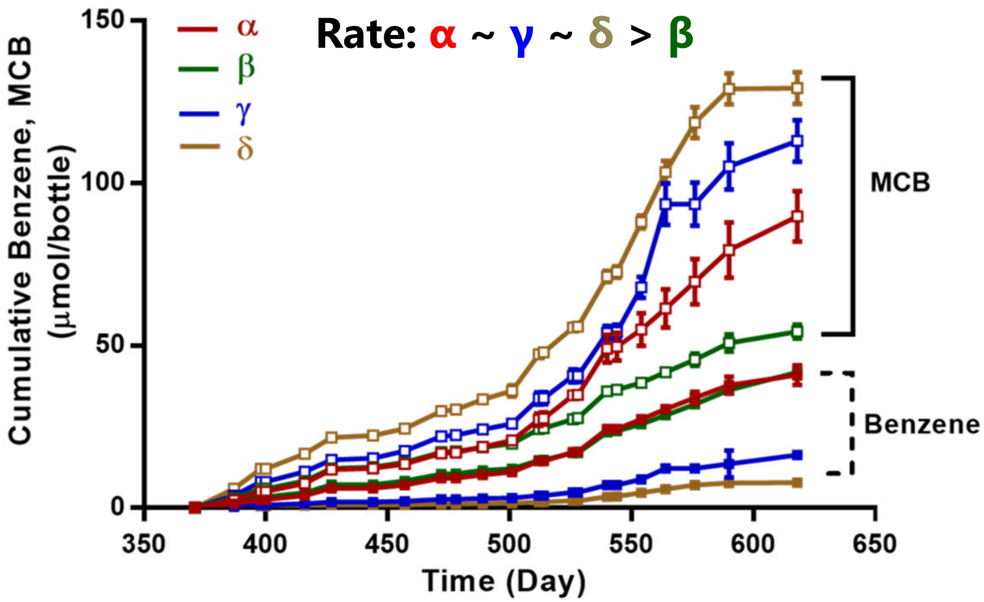
Clostridium **spp. (co-metabolic transformation of α-HCH and γ-HCH)**

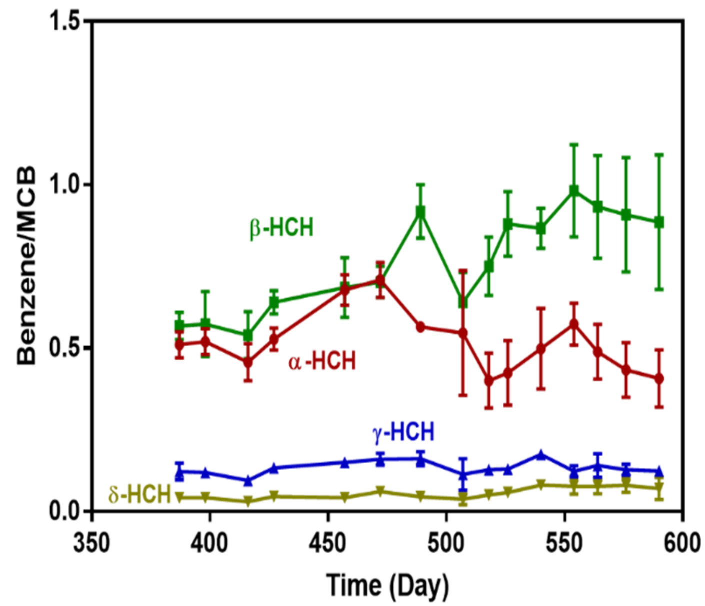
D. mccartyi **strains BTF08 and 195 (co-metabolic transformation of γ-HCH)**

Van Doesburg, Van Eekert et al. 2005; Elango, Kurtz et al. 2011; Maphosa, van Passel et al. 2012; Bashir, Kuntze et al. 2018.

The Guadeloupe transferred (GT) HCH enrichments

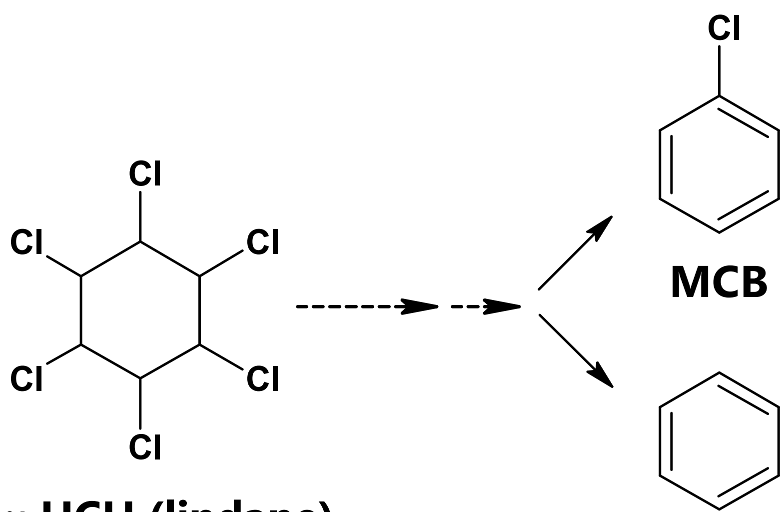






Qiao W., Puentes Jacome L.A., et al. 2019 (in preparation)

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



γ-HCH (lindane)

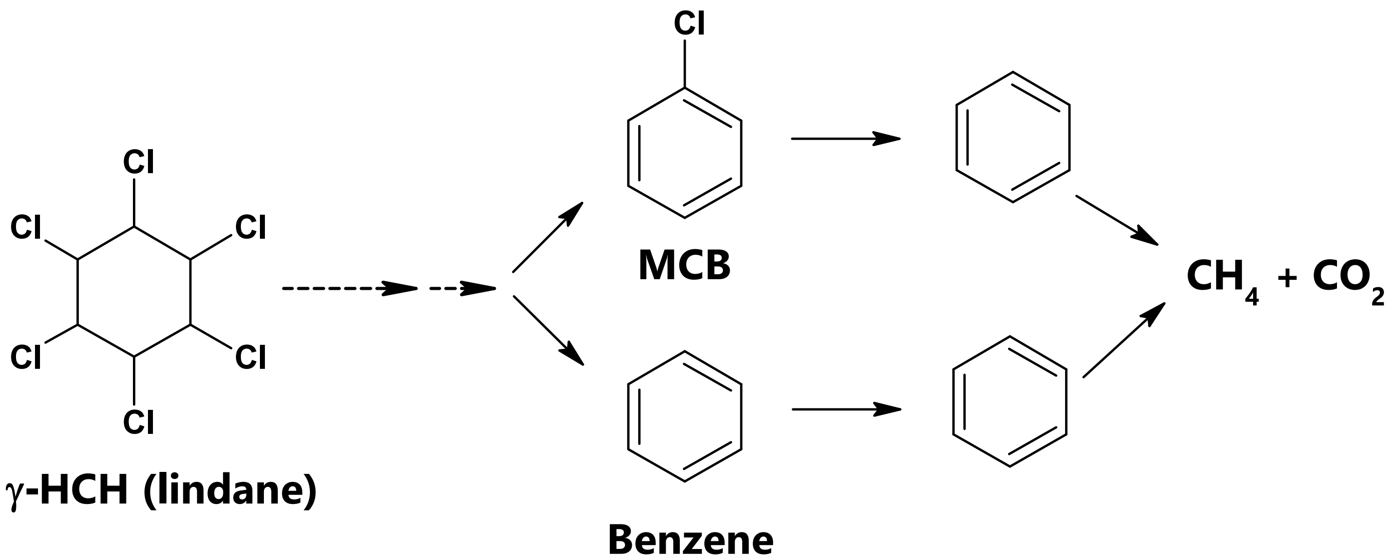
MCB

Benzene

carcinogen

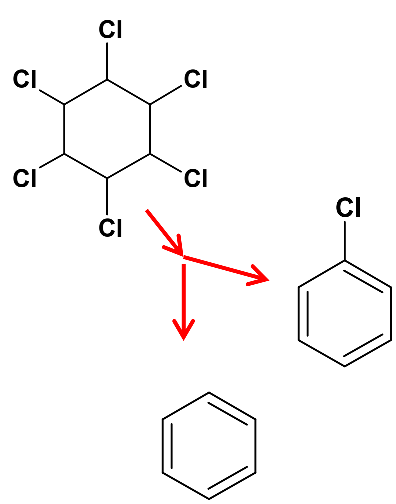
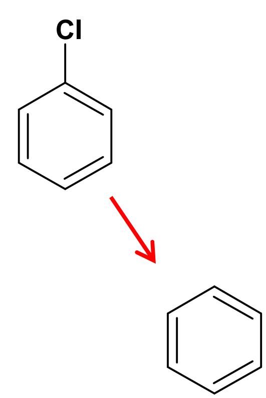
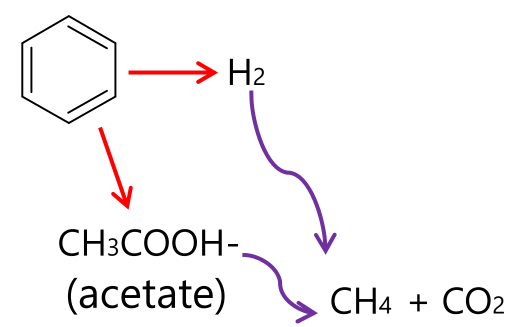
- **sediment microcosms** (Boyle, Haggblom, Young 1999)
- **co-cultures** (Van Doesburg, Van Eekert et al. 2005)
- **anaerobic sludge** (Elango, Kurtz et al. 2011)
- **DNAPL pools from landfill leachate** (Fernandez, Arjol et al. 2013; Santos, Fernández et al. 2018)
- ***D. mccartyi* strains 195 and 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?

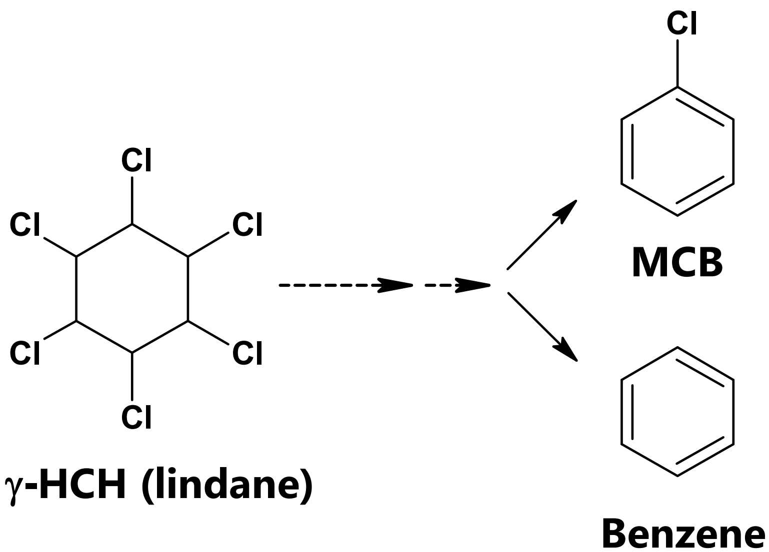


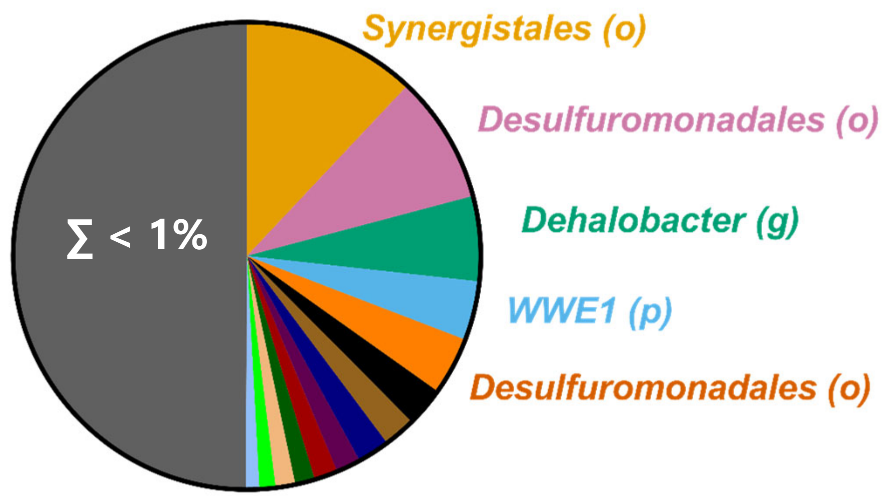
Microbial culture I + **Microbial culture II** + **Microbial culture III** = **Non-toxic end products**

Overview of anaerobic enrichment cultures

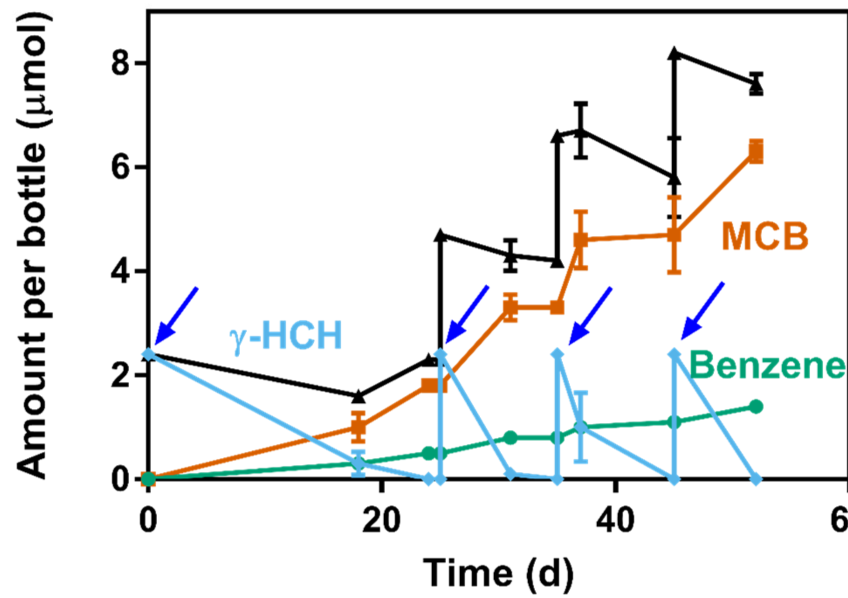
	Culture I	Culture II	Culture III
Source:	HCH-contaminated sediments from Guadeloupe	TCE-contaminated soils in southern Ontario (KB-1-derived culture)	Soil samples from an Oklahoma Oil Refinery
Electron acceptor:	HCH	Monochlorobenzene (MCB)	CO_2
Electron donor:	Ethanol	Methanol	Benzene
Catalyzed reaction:	HCH → MCB + Benzene 	MCB → Benzene 	Benzene → CH_4 + CO_2 

Culture I, γ-HCH (lindane) is transformed to benzene and monochlorobenzene (MCB)

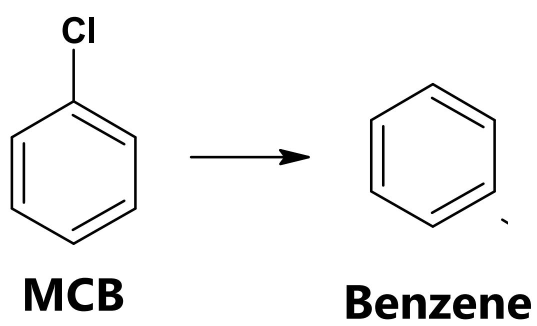


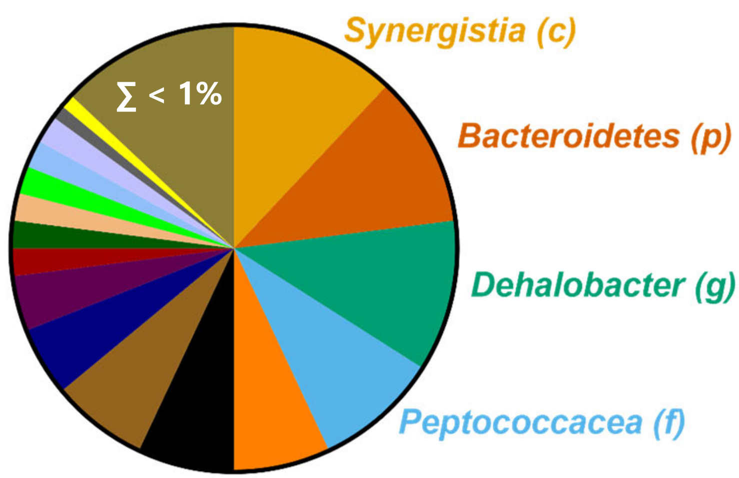


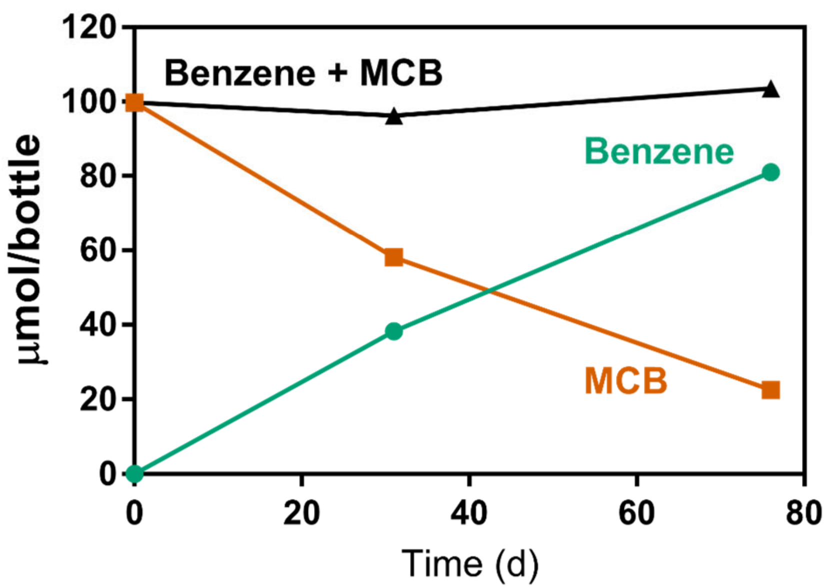
γ-HCH is completely transformed to benzene and MCB.

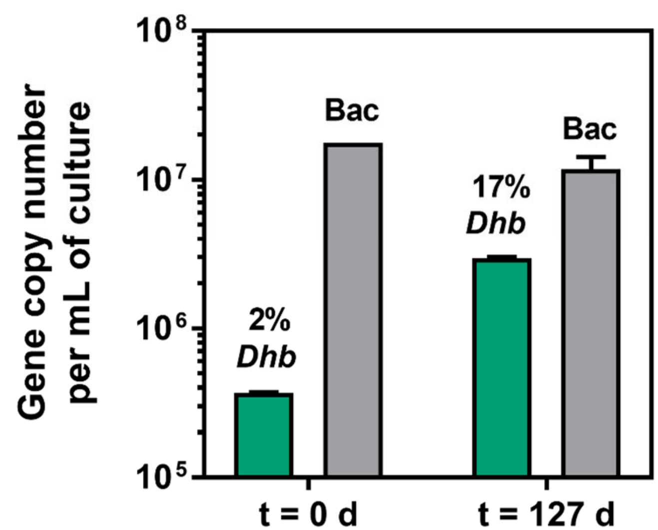


Culture II, dechlorination of MCB to benzene



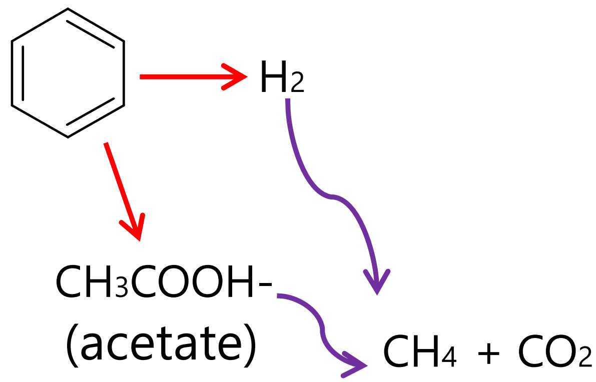


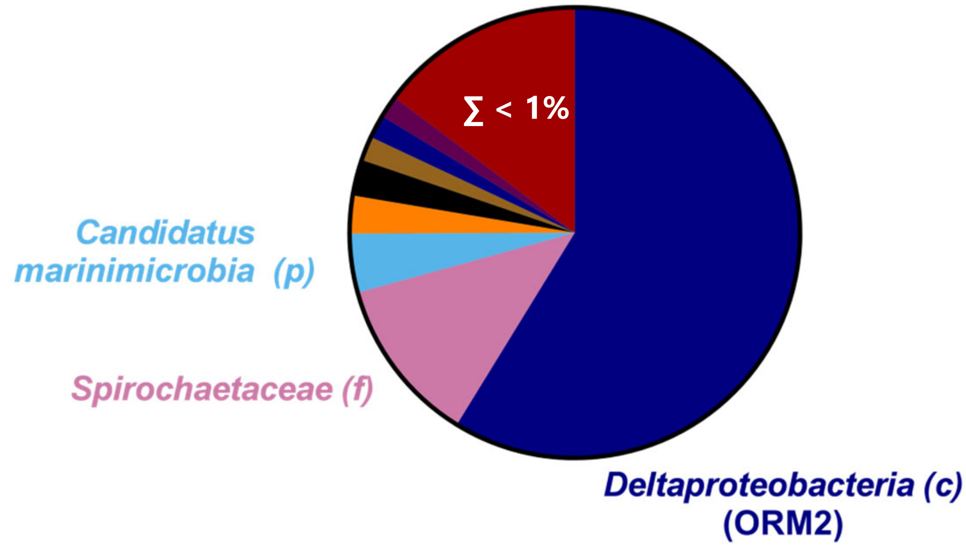


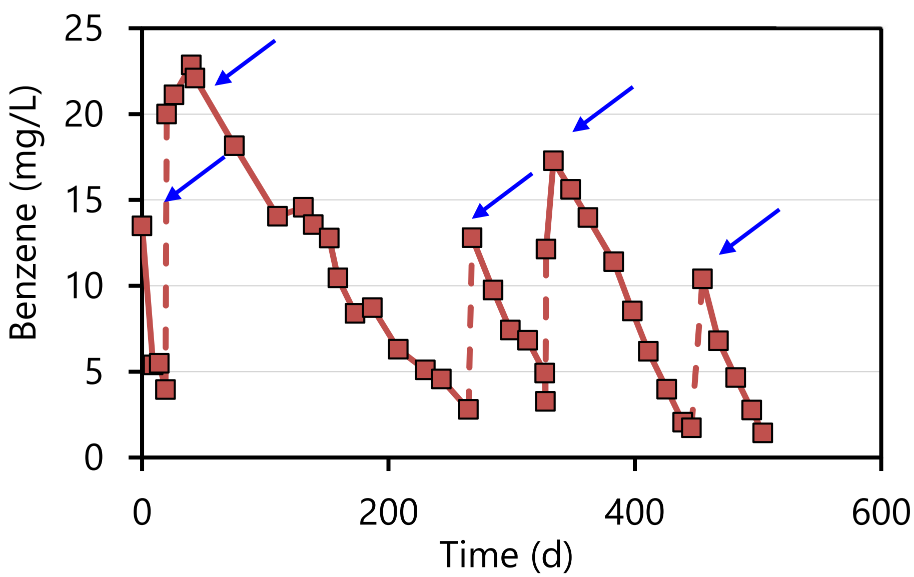


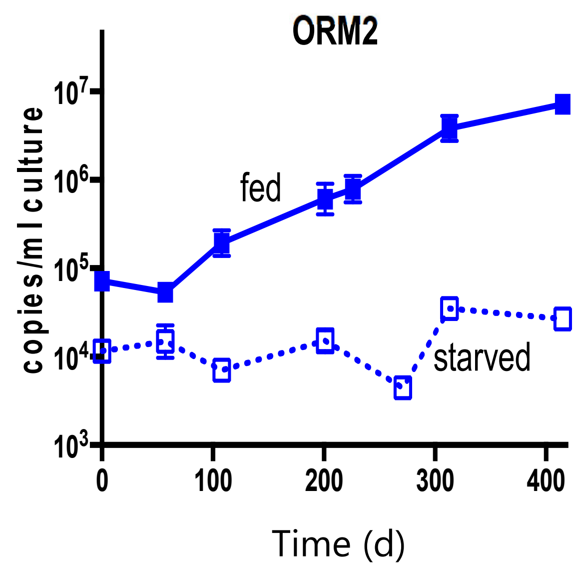
Bacteria (Bac)
Dehalobacter **(*Dhb*)**

Culture III, benzene degradation under methanogenic conditions

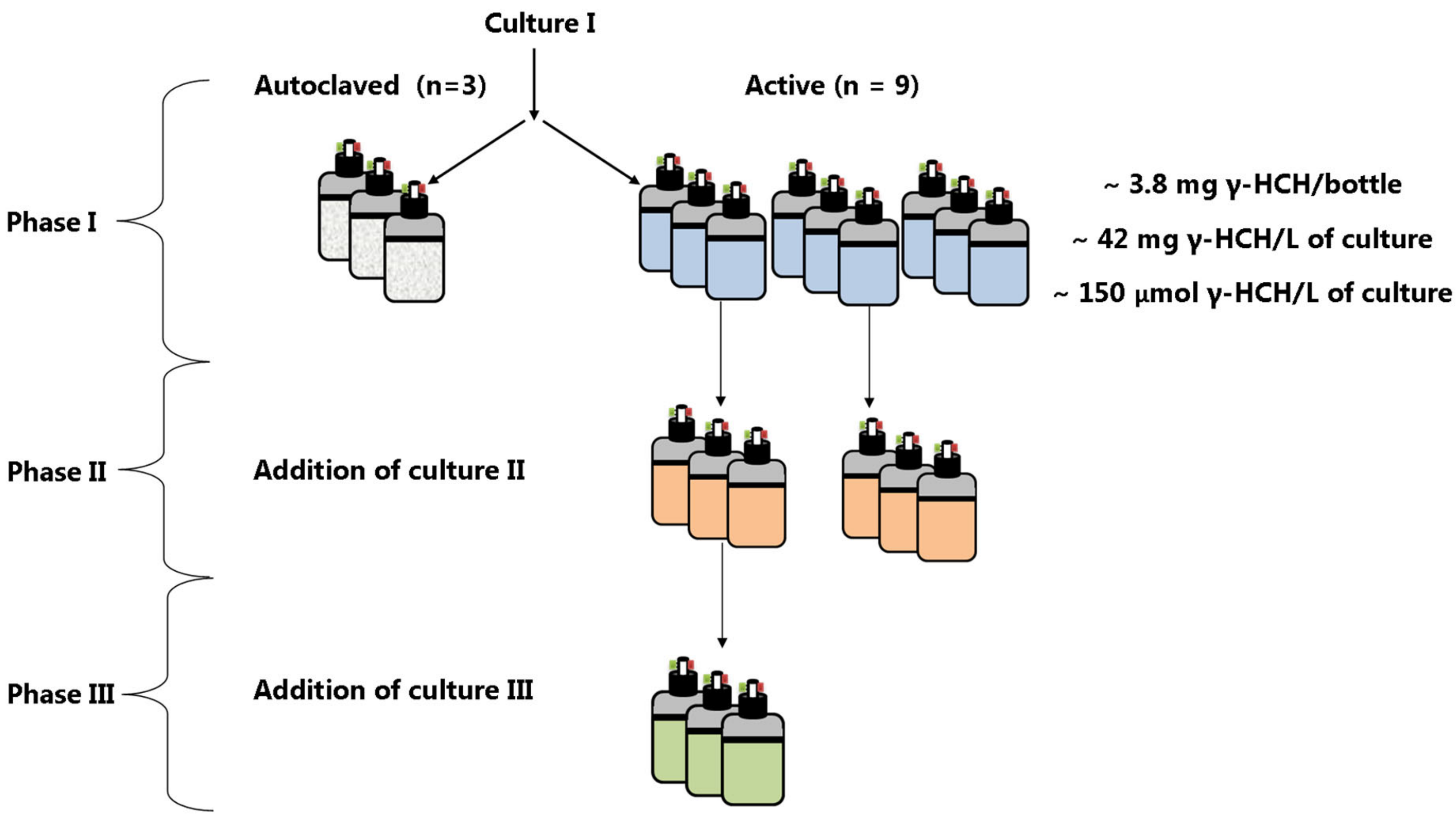




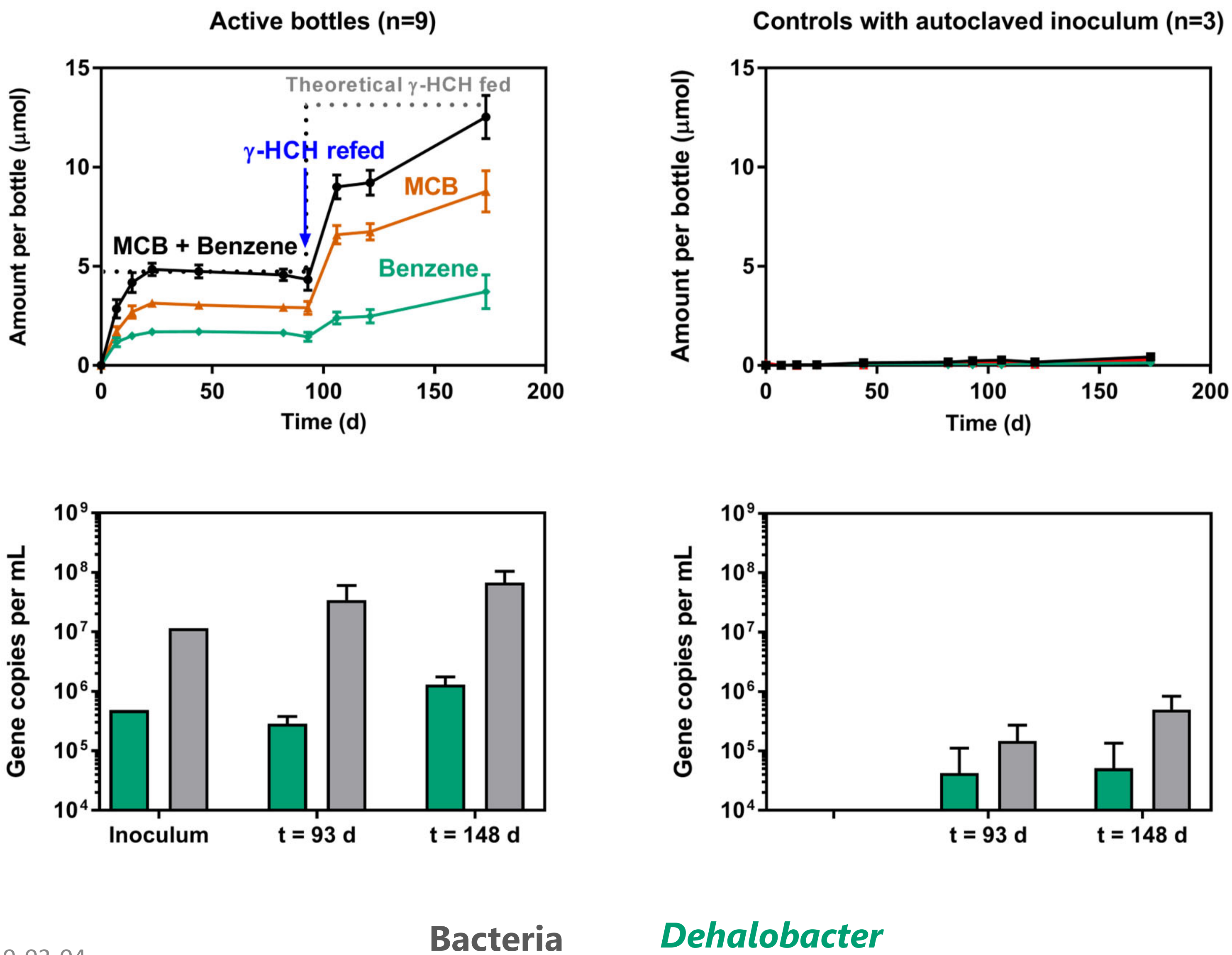




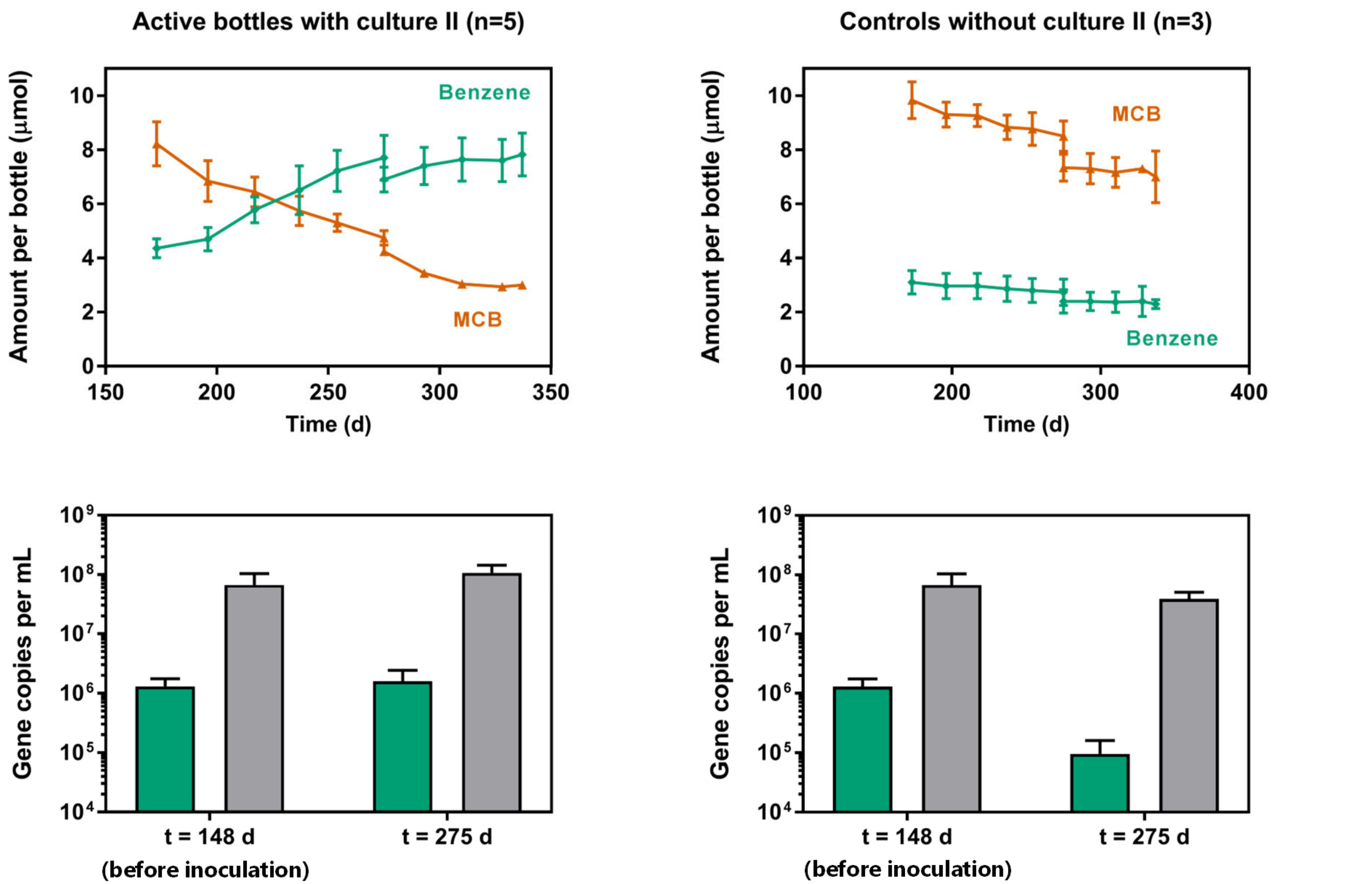
Sequential biotransformation of γ-HCH



Phase I: γ-HCH was transformed to MCB and benzene

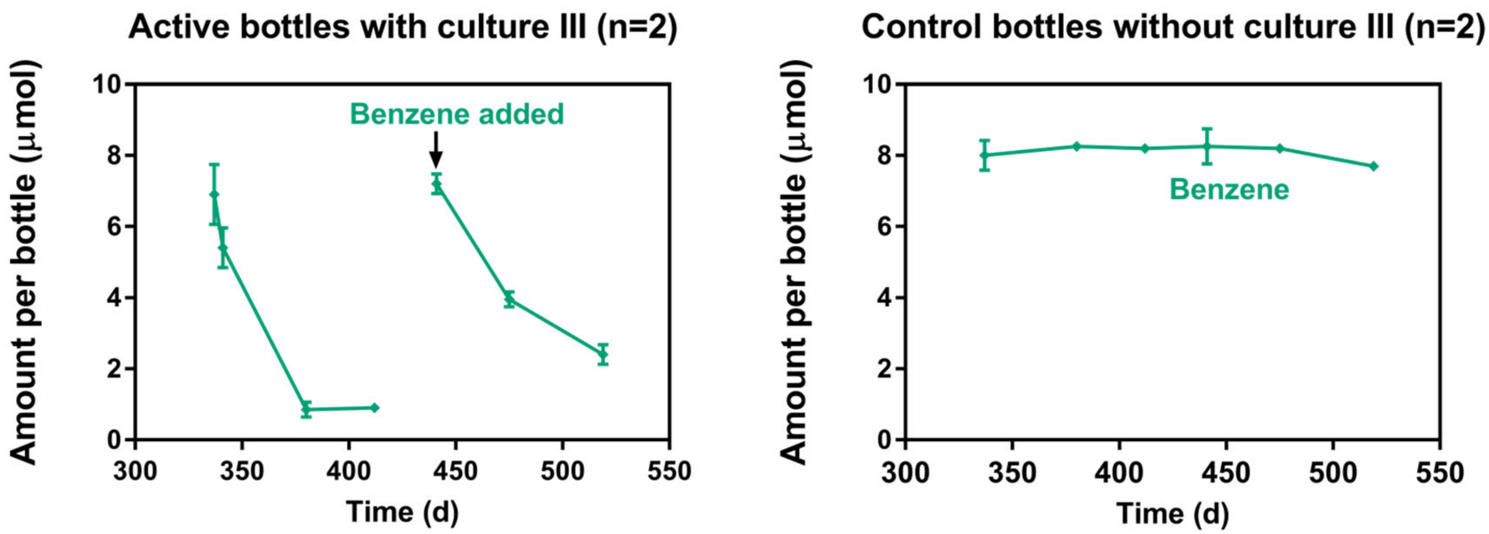


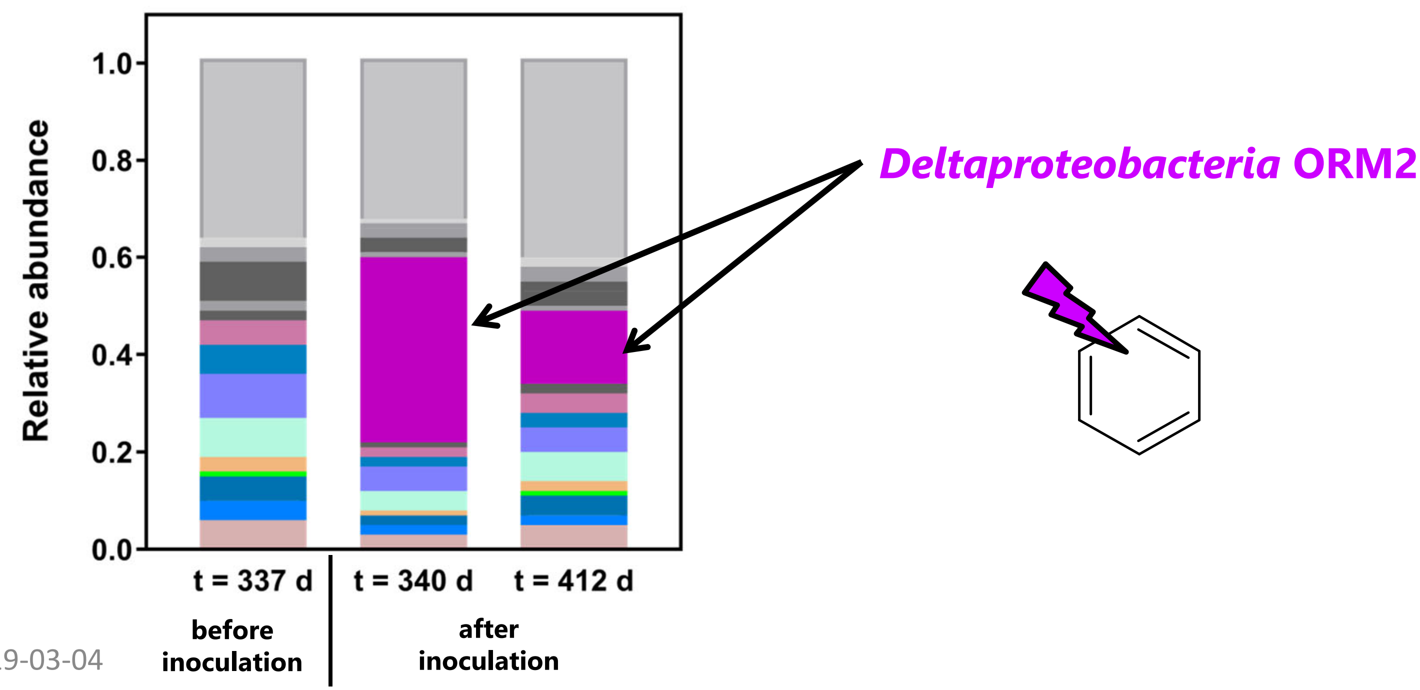
Phase II: MCB was dechlorinated to benzene



Bacteria *Dehalobacter*

Phase III: benzene was biodegraded





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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luz.puentesjacome@mail.utoronto.ca, elizabeth.edwards@utoronto.ca