A new concept to assess the environmental fate of chiral pesticides: Combined stable isotope and enantiomeric fractionation of alpha HCH

Silviu-Laurentiu Badea
PhD Student
Department of Chemistry
Silviu.badea@chem.umu.se

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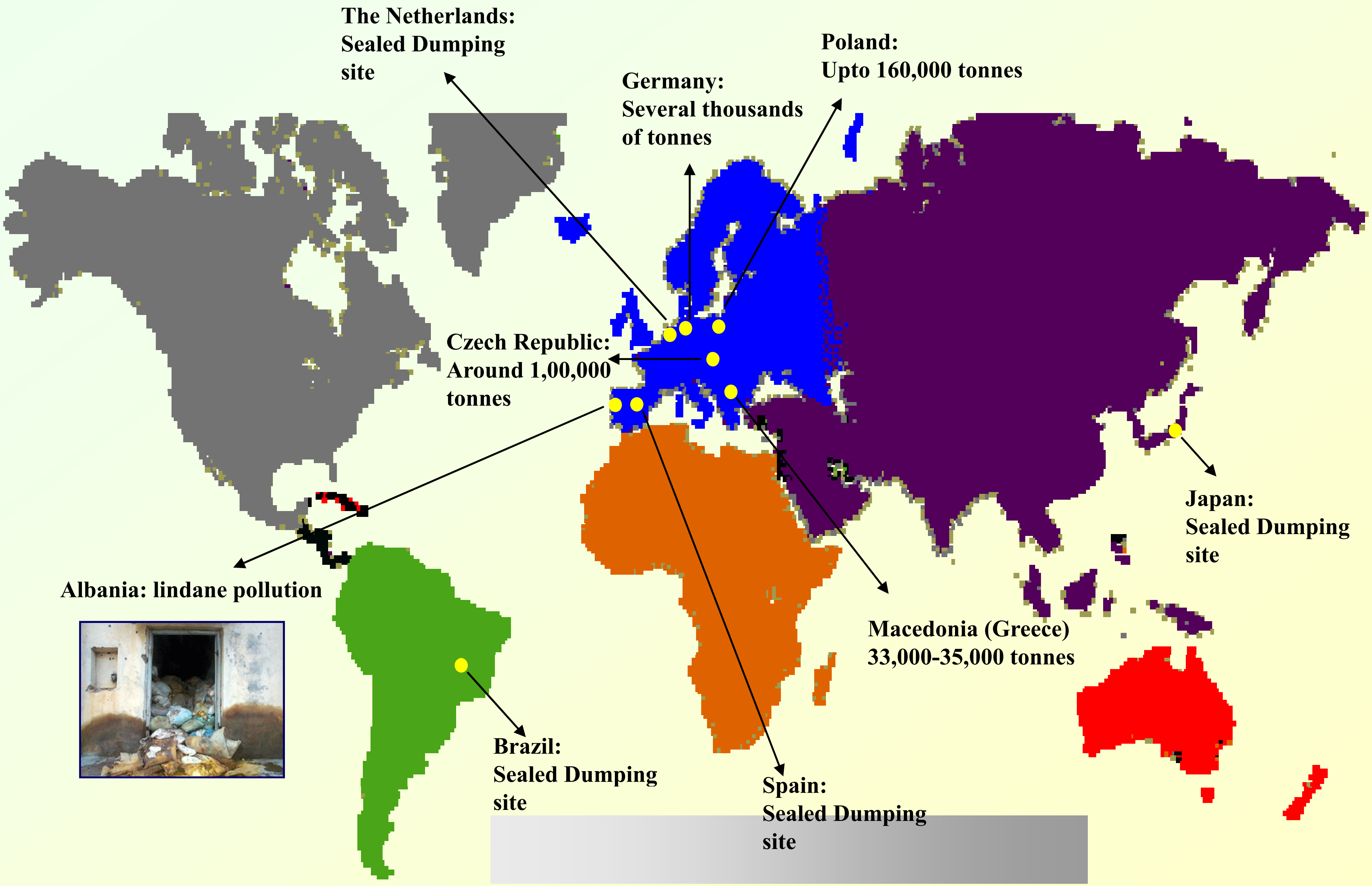
HCHs: A GLOBAL PROBLEM



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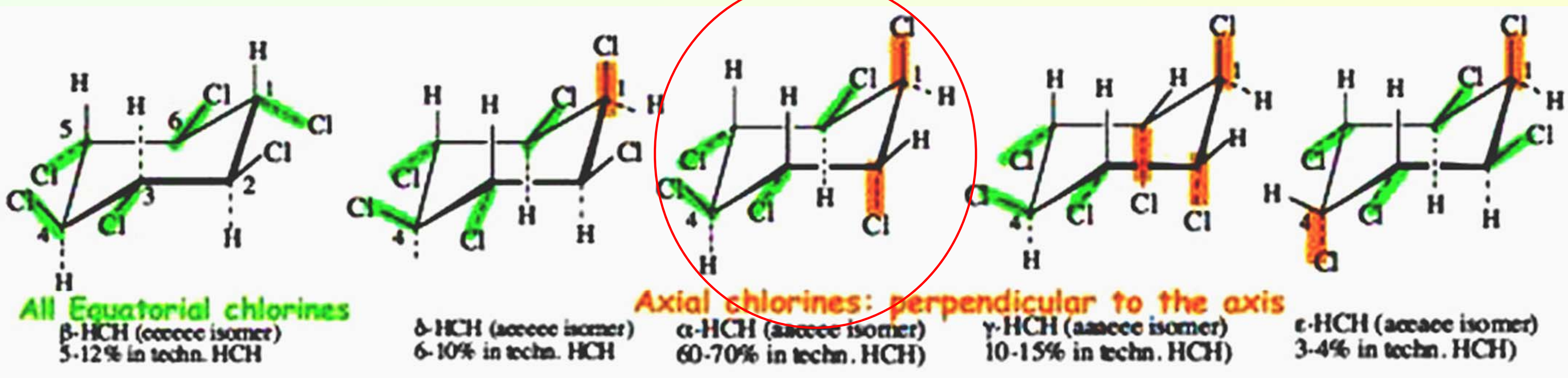
HCHs: A GLOBAL PROBLEM

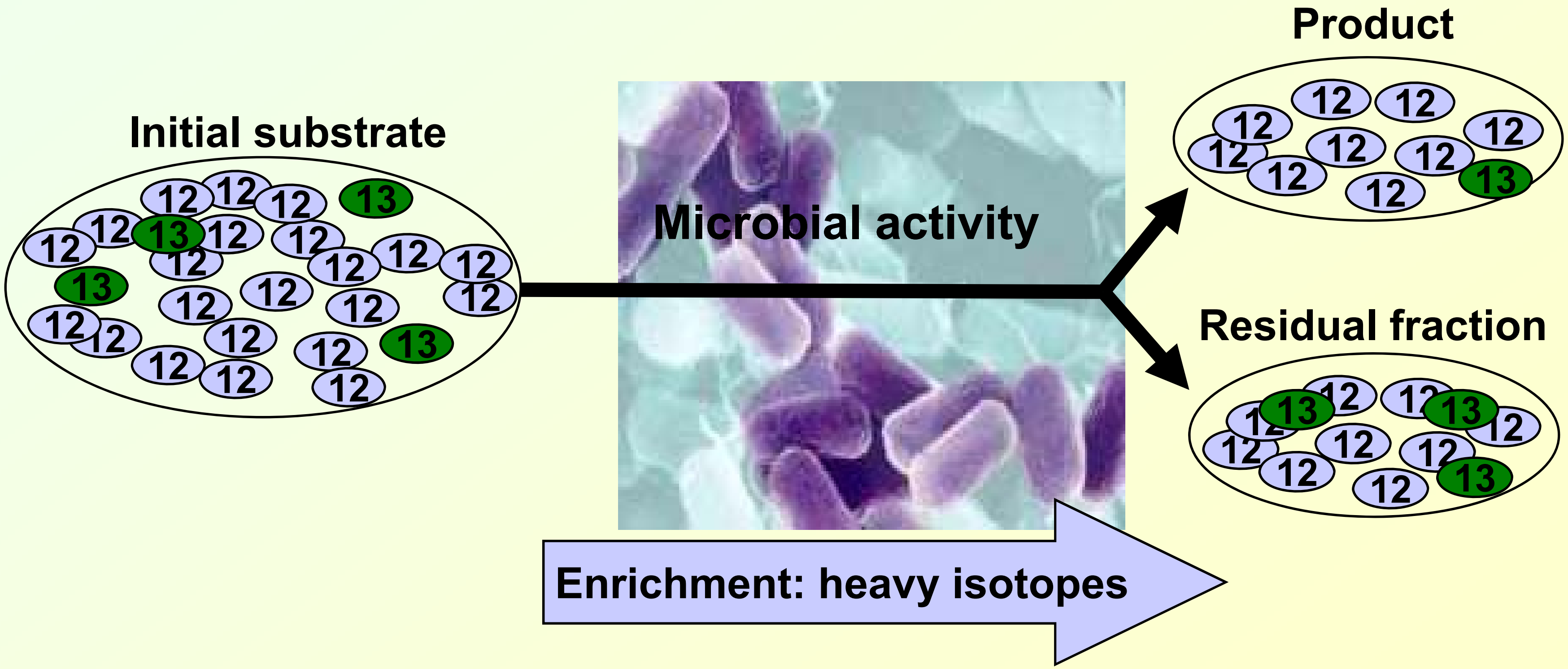
In the contaminates sites technical grade HCH contains mainly 5 isomers: α, β, γ, δ, ε



Alpha HCH is the only optical active isomer

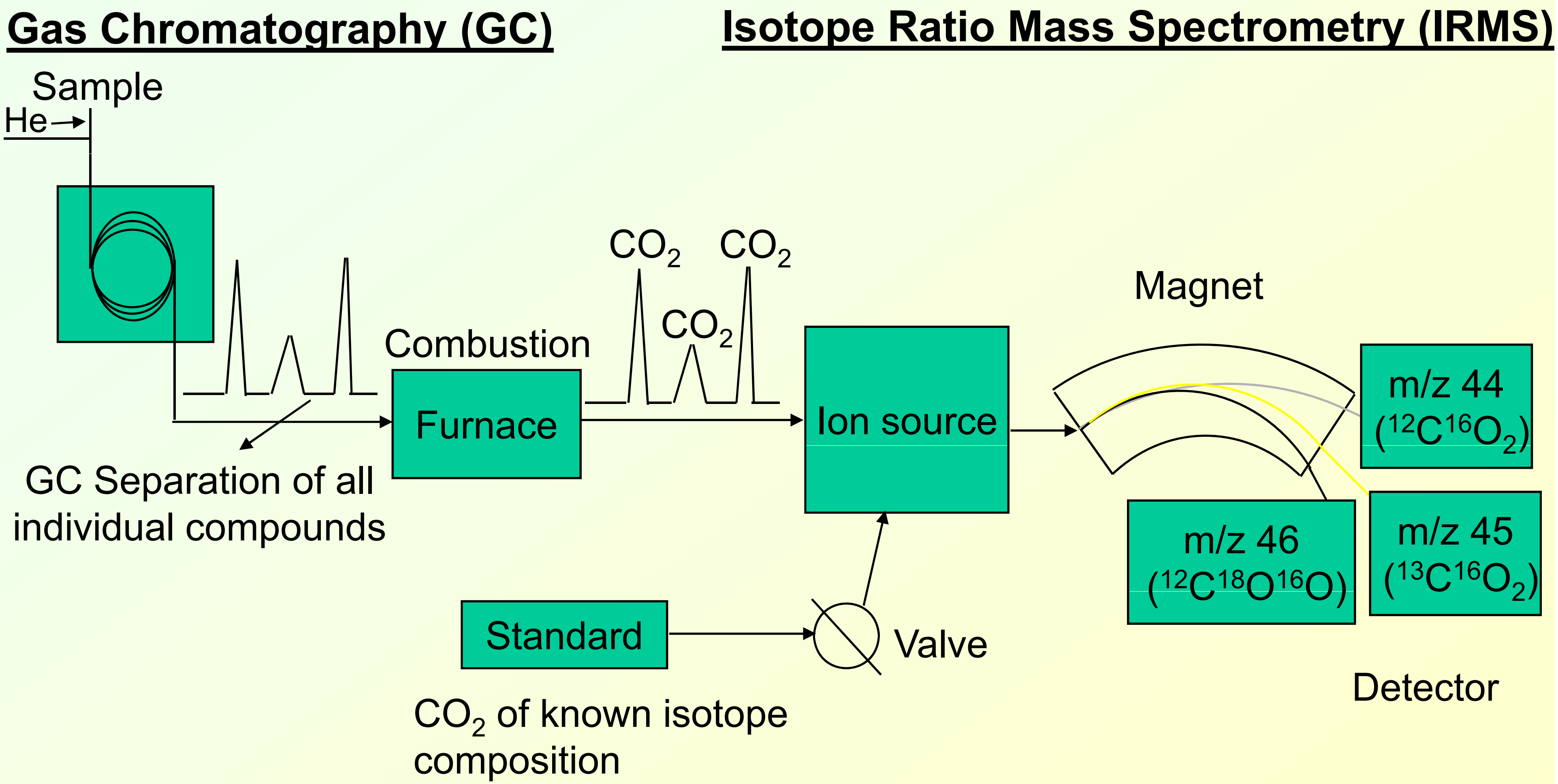
Stable isotope fractionation: a tool for the assessment of the environmental fate of HCHs

- Compound Specific Isotope analysis (CSIA): identification of biodegradation
- Preferential use of the lighter isotope during biodegradation



Ref.: Bloom et al., 2000; Hunkeler et al., 1999; Kirtland et al., 2003; Sherwood Lollar et al., 1999, 2001; Slater et al., 2001; Vieth et al., 2003

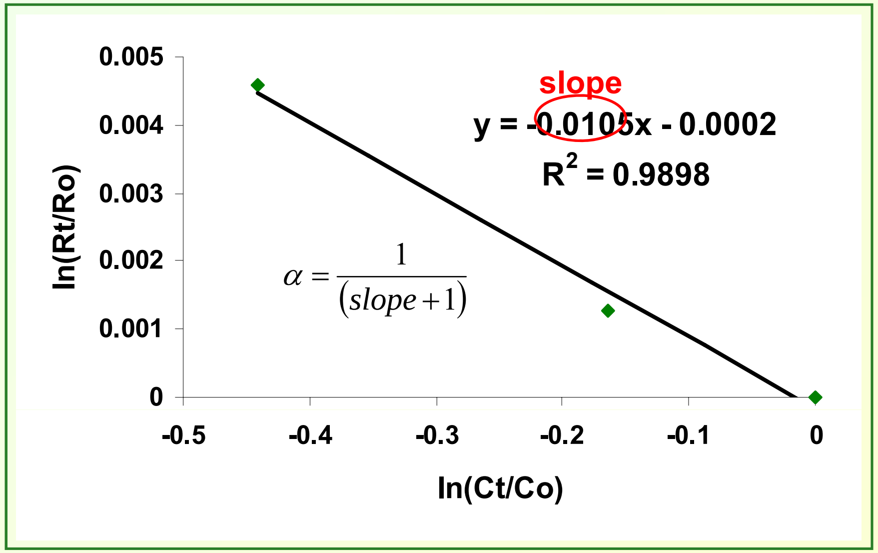
Compound-specific Stable Isotope Analysis (CSIA) of HCHs



Quantification of stable isotope fractionation

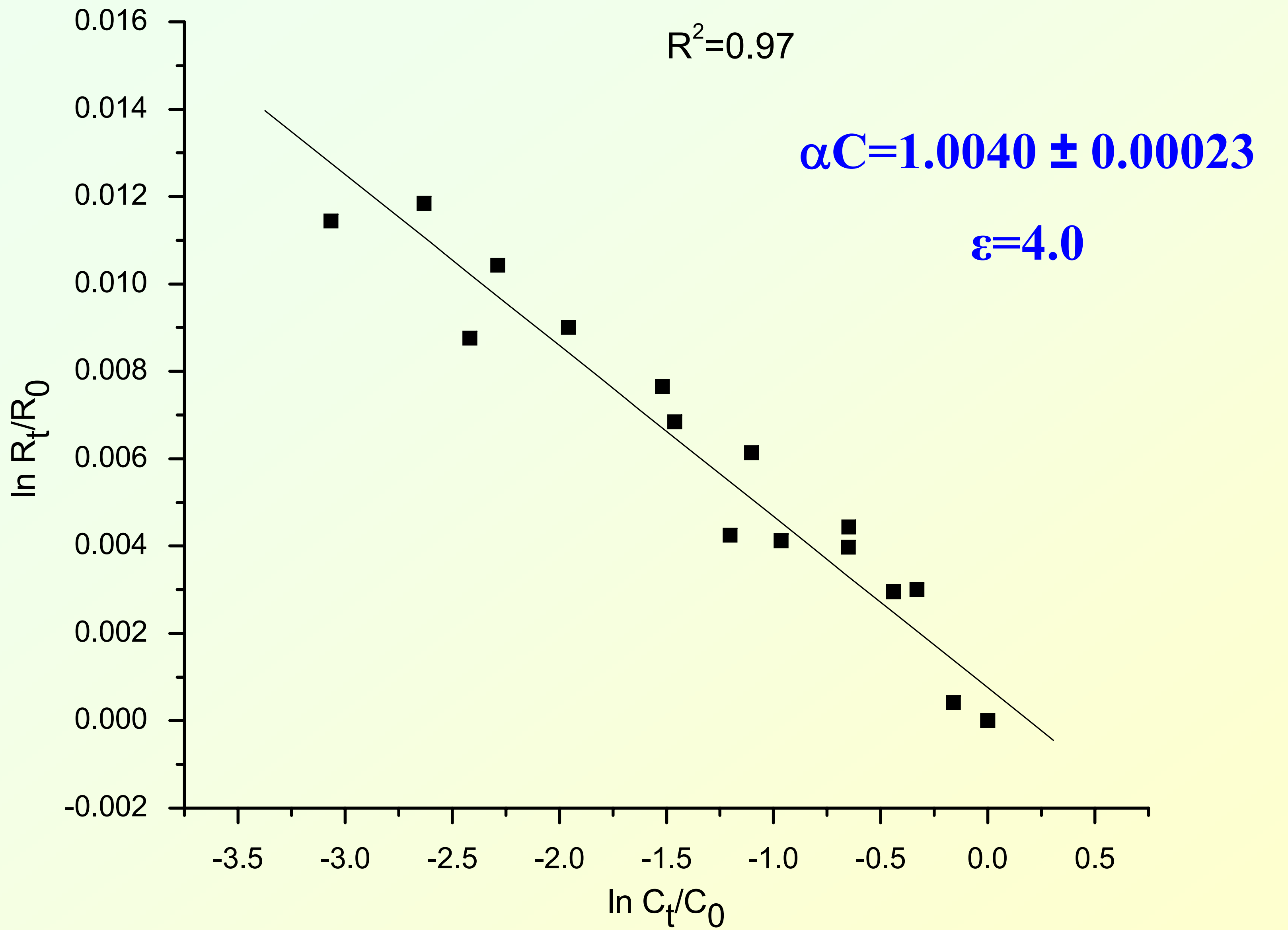
Rayleigh Equation:

$$\frac{R_t}{R_0} = \left(\frac{C_t}{C_0}\right)^{\left(\frac{1}{\alpha}-1\right)} \longrightarrow \ln\left(\frac{R_t}{R_0}\right) = \left(\frac{1}{\alpha}-1\right) * \ln\left(\frac{C_t}{C_0}\right)$$

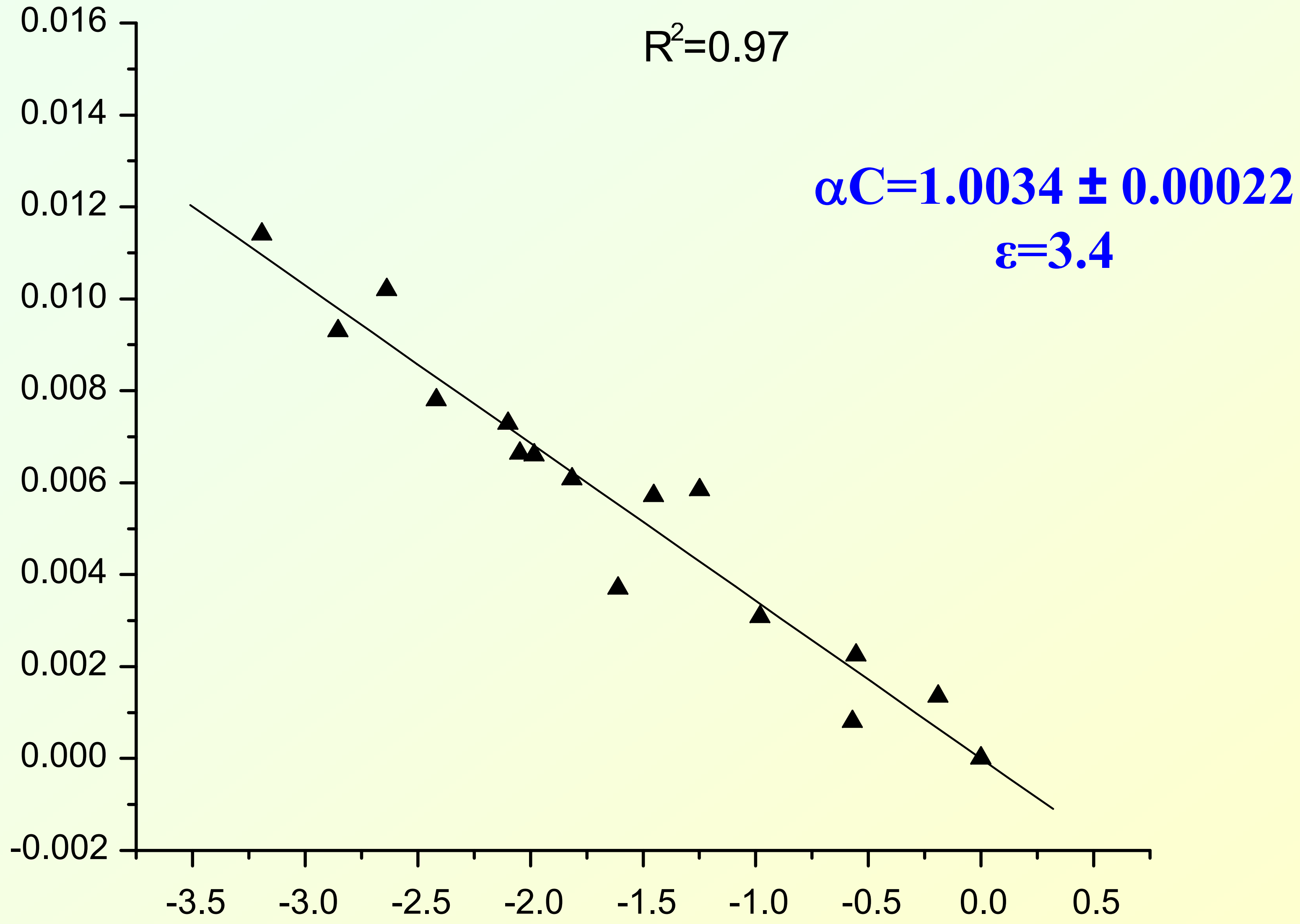


C_0 = initial concentration
C_t = concentration at time t
R_0 = initial isotope ratio
R_t = isotope ratio at time t
α = isotope fractionation factor

ε [‰] = $(\alpha-1)*1000$



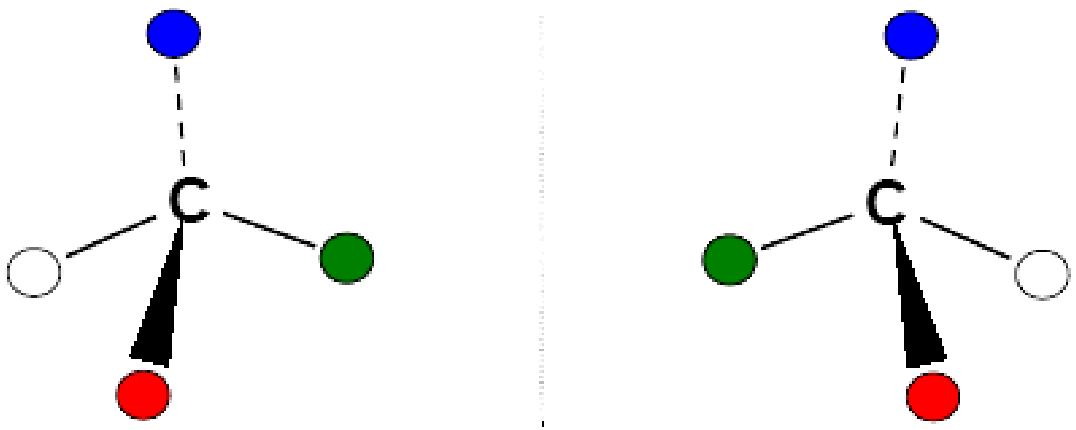
Quantitative assessment of isotope fractionation of γ HCH in the biodegradation experiments with *D. Gigas* (Badea et. al (2009), Environmental Science & Technology)



Quantitative assessment of isotope fractionation of γ HCH in the biodegradation experiments with *D. Multivorans* (Badea et. al (2009), Environmental Science & Technology)

Chirality of alpha HCH

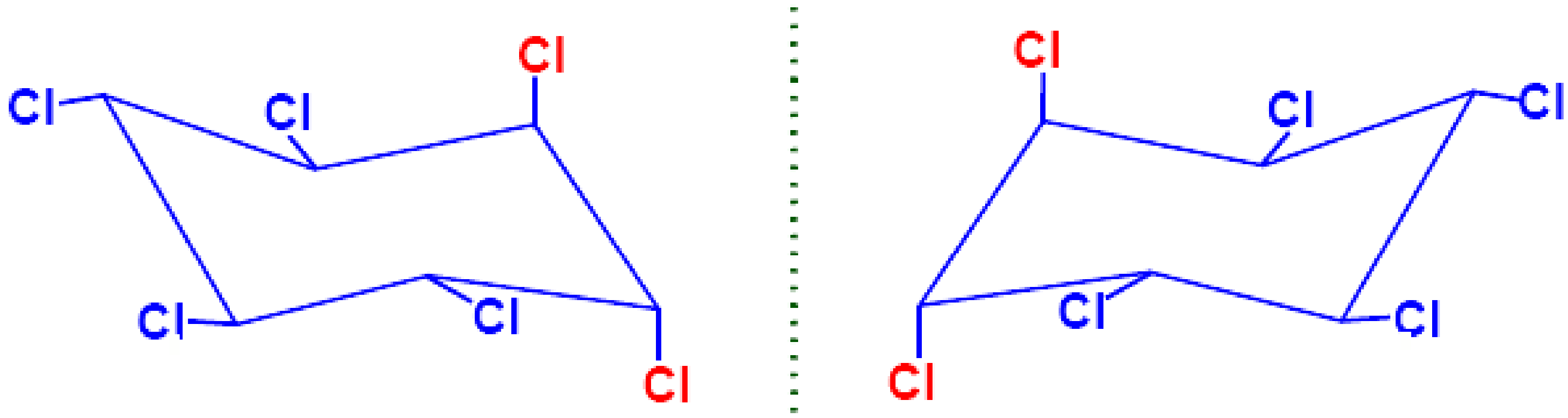
A chiral (handed) molecule cannot be superimposed on its mirror image



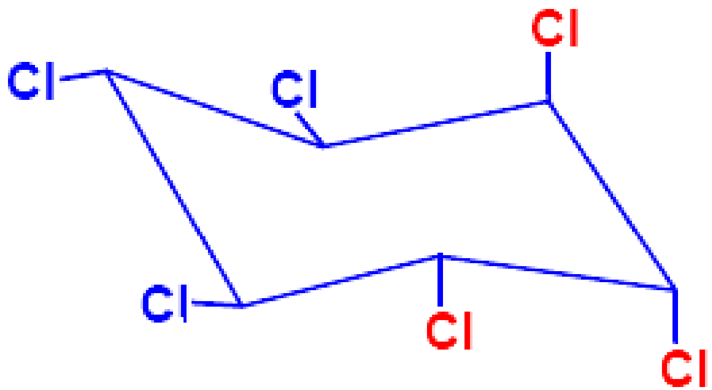
Types of chiral compounds:

1. The simplest chiral molecule has one asymmetric tetrahedral carbon atom (four different groups attached)

Types of chiral molecules
2. ring structures with no symmetry plane

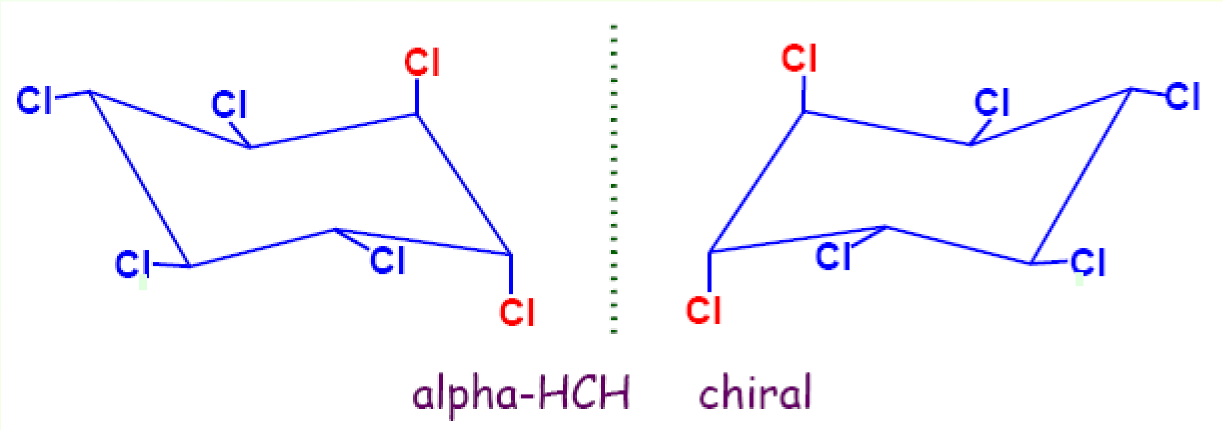


alpha-HCH chiral



gamma-HCH (lindane) achiral

Chirality of alpha HCH

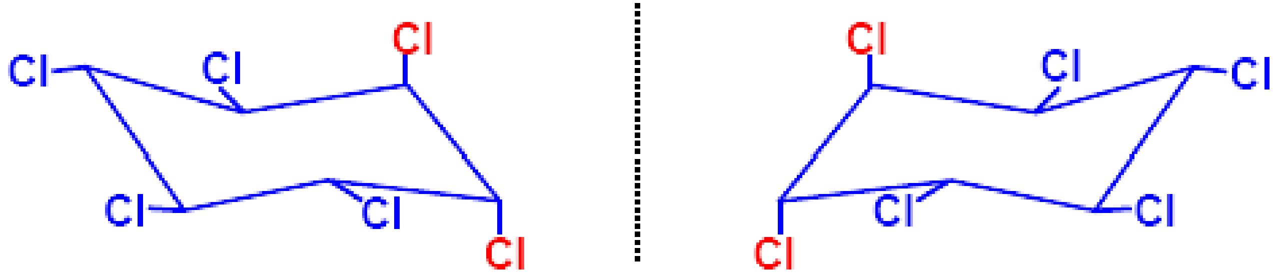


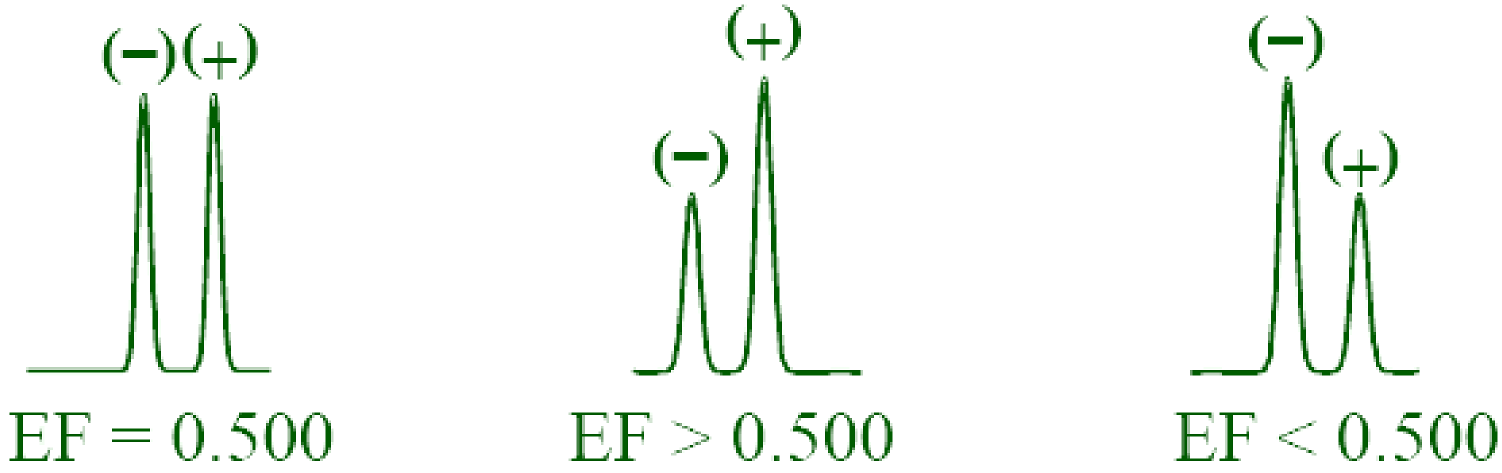
•Biodegradation: one enantiomer is usually preferentially degraded over the other

•Enantiomeric fraction (EF) of α-HCH

$$EF = (+)\ \alpha\text{-HCH} / [(+)\ \alpha\text{-HCH} + (-)\ \alpha\text{-HCH}]$$

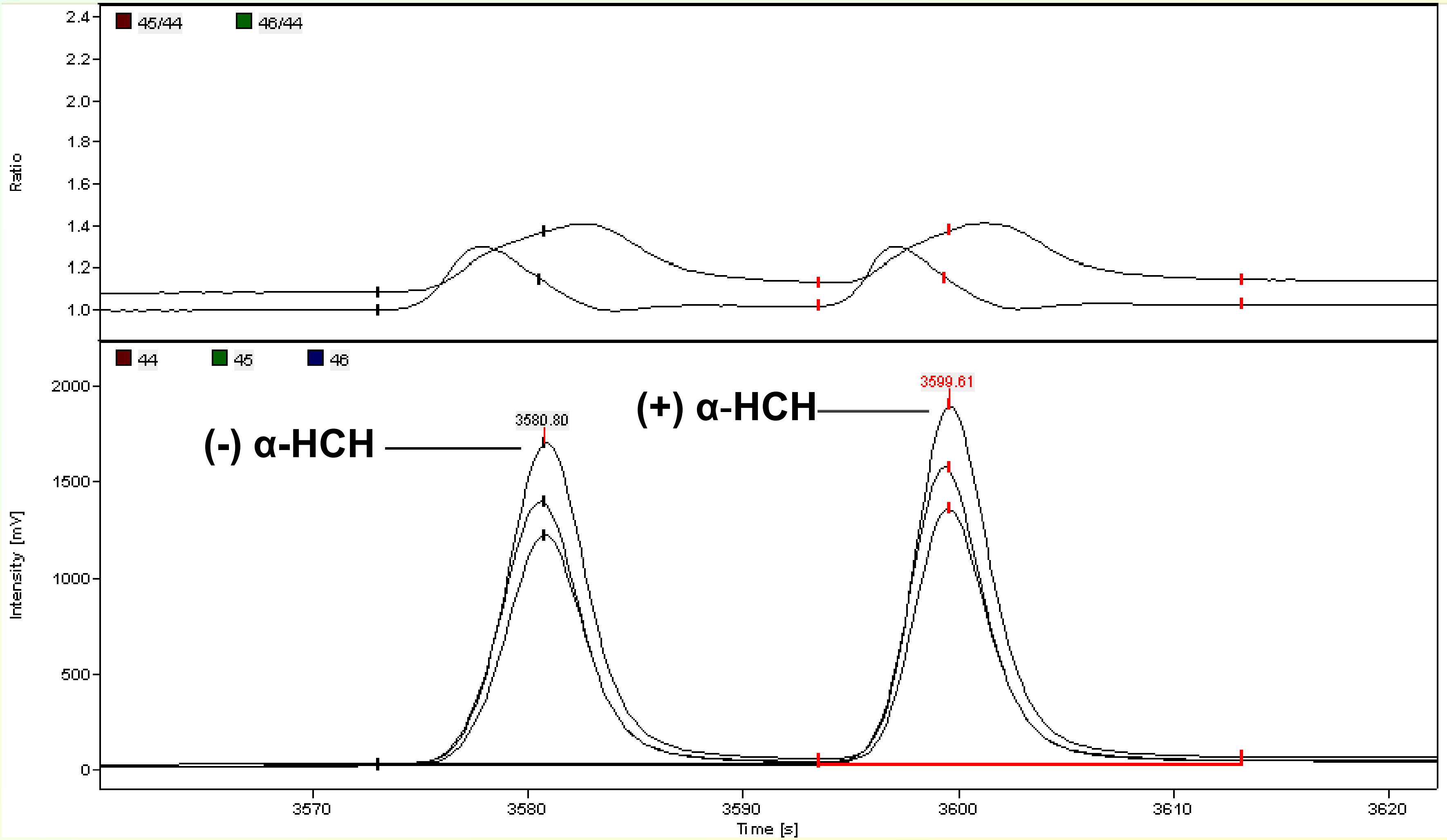
α-HCH is chiral



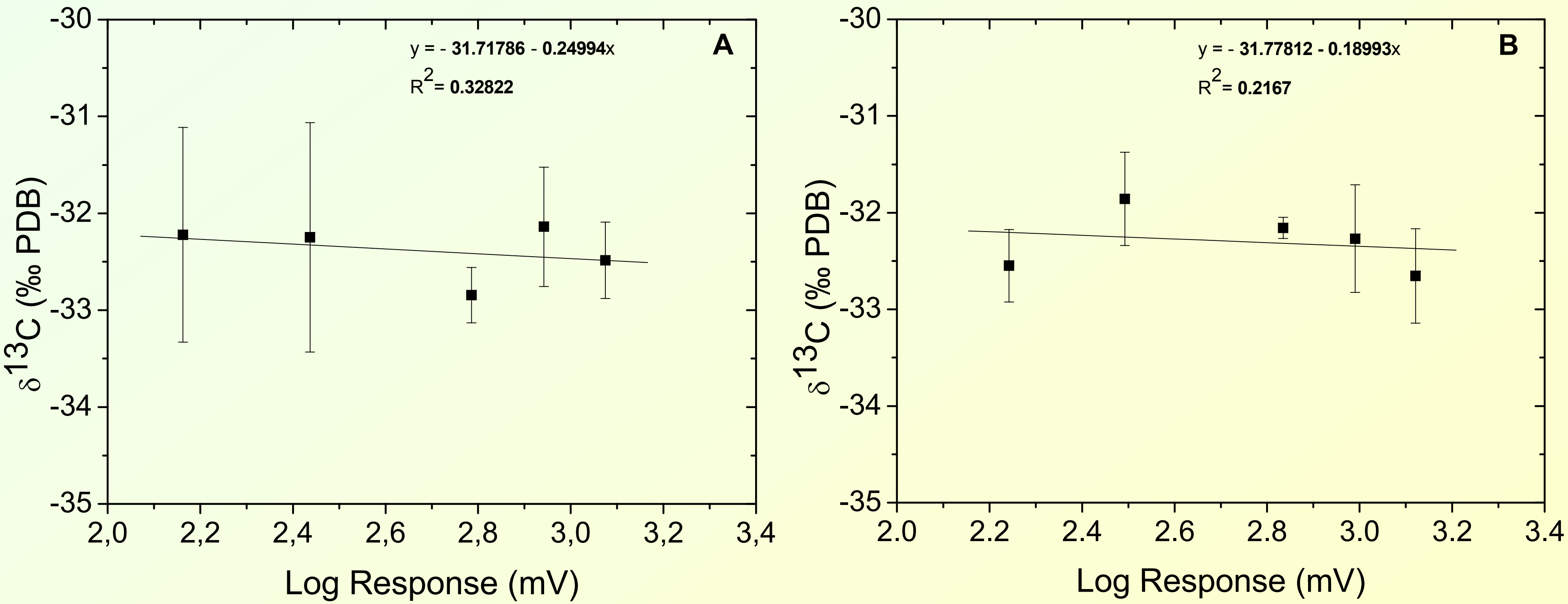


- Manufactured as a racemate
- Enantiomer fraction, EF = (+)/[(+) + (–)] = 0.500 (racemic)
- Preferential degradation of one enantiomer in water and soil leads to non-racemic composition

Stable Isotope Analysis of alpha HCH's enantiomers



The alpha HCH Enantiomers in a GC-C-IRMS Chiral Chromatogram



The plot graphs of the linearity test for (-) α HCH (A) and (+) α HCH (B).
The concentrations of the enantiomers pair were : 25, 50, 100, 150 and 200 µM

Experimental Approach

- Combined stable isotope and enantiomeric fractionation of alpha HCH with reference experiments with cultures obtained from culture collections (DSM) (Clostridium pasteurianum DSM 525, Desulfococcus multivorans DSM 2059 Desulfovibrio gigas DSM 1382) to obtain isotope fractionation factors for future field investigation
- Alpha HCH's enantiomers quantification (GC-MS Chiral Analysis with γ-DEX™ Chiral Column)
- Measurement of the isotope fractionation during degradation of HCH's enantiomers (GC-C-IRMS with β or γ-DEX™ Chiral Column)

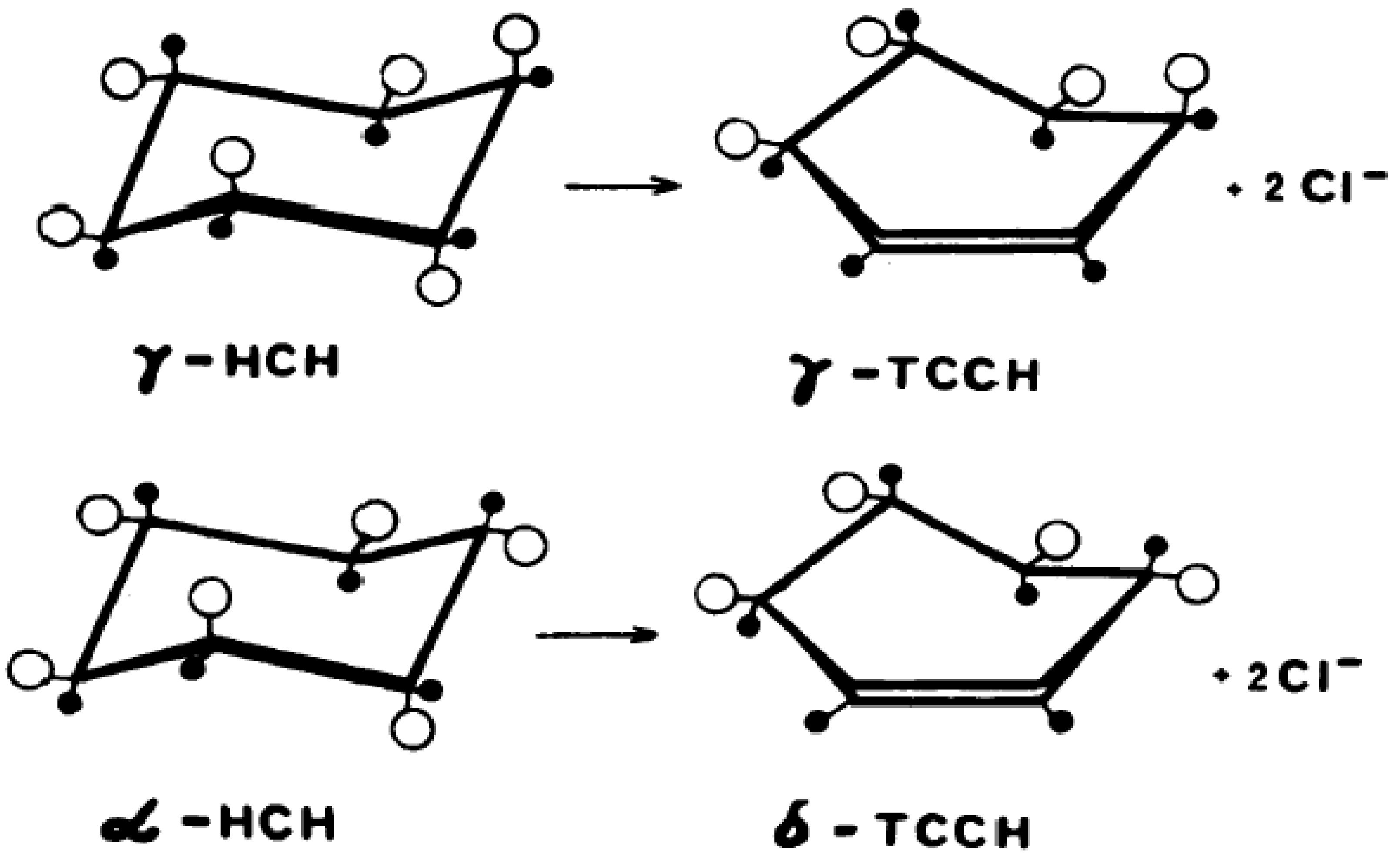
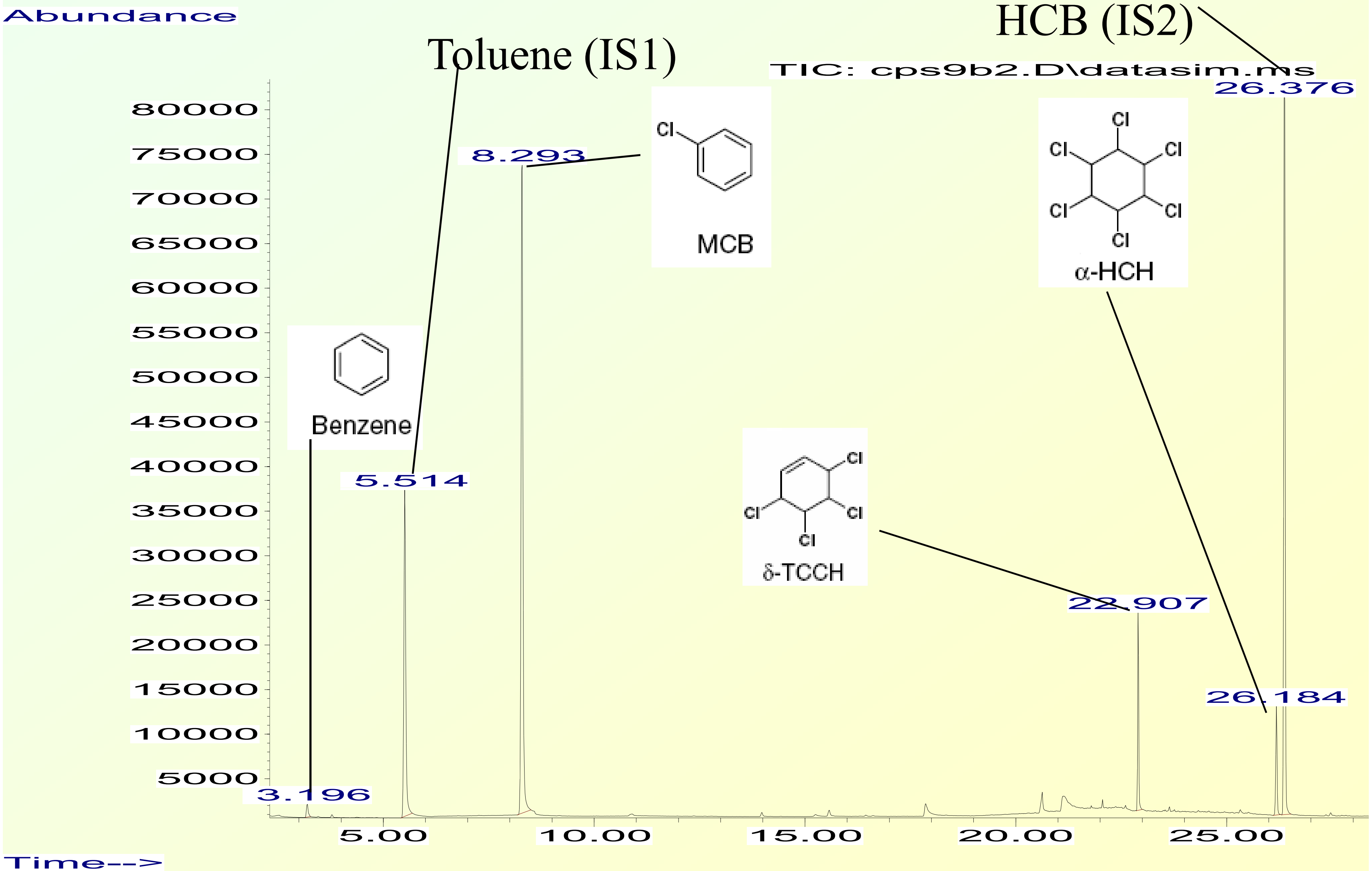
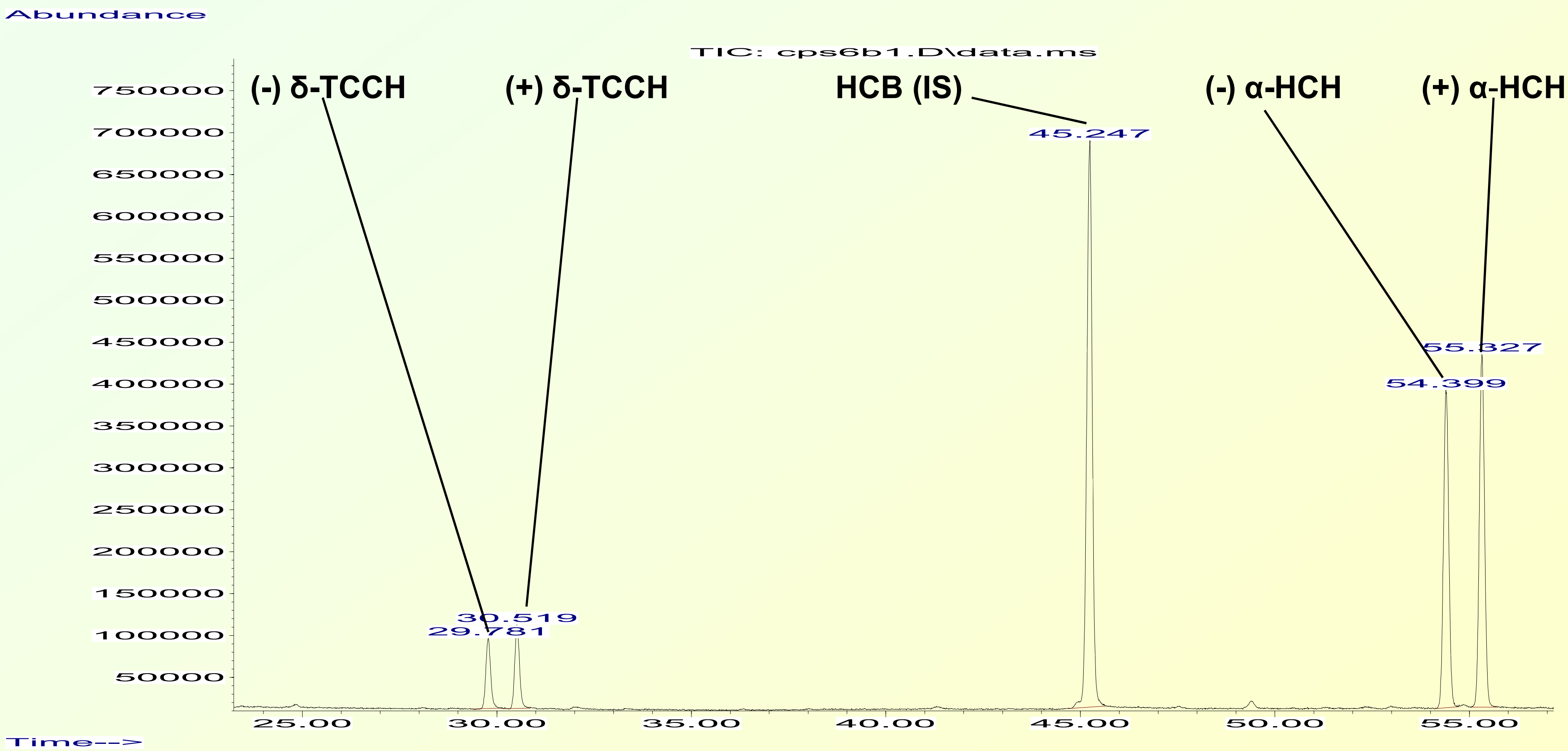


FIG. 1. *Degradation of the α- and γ-isomers of HCH to the δ- and γ-isomers of TCCH by C. sphenoides. Symbols: Chlorine* (○); *hydrogen* (●).

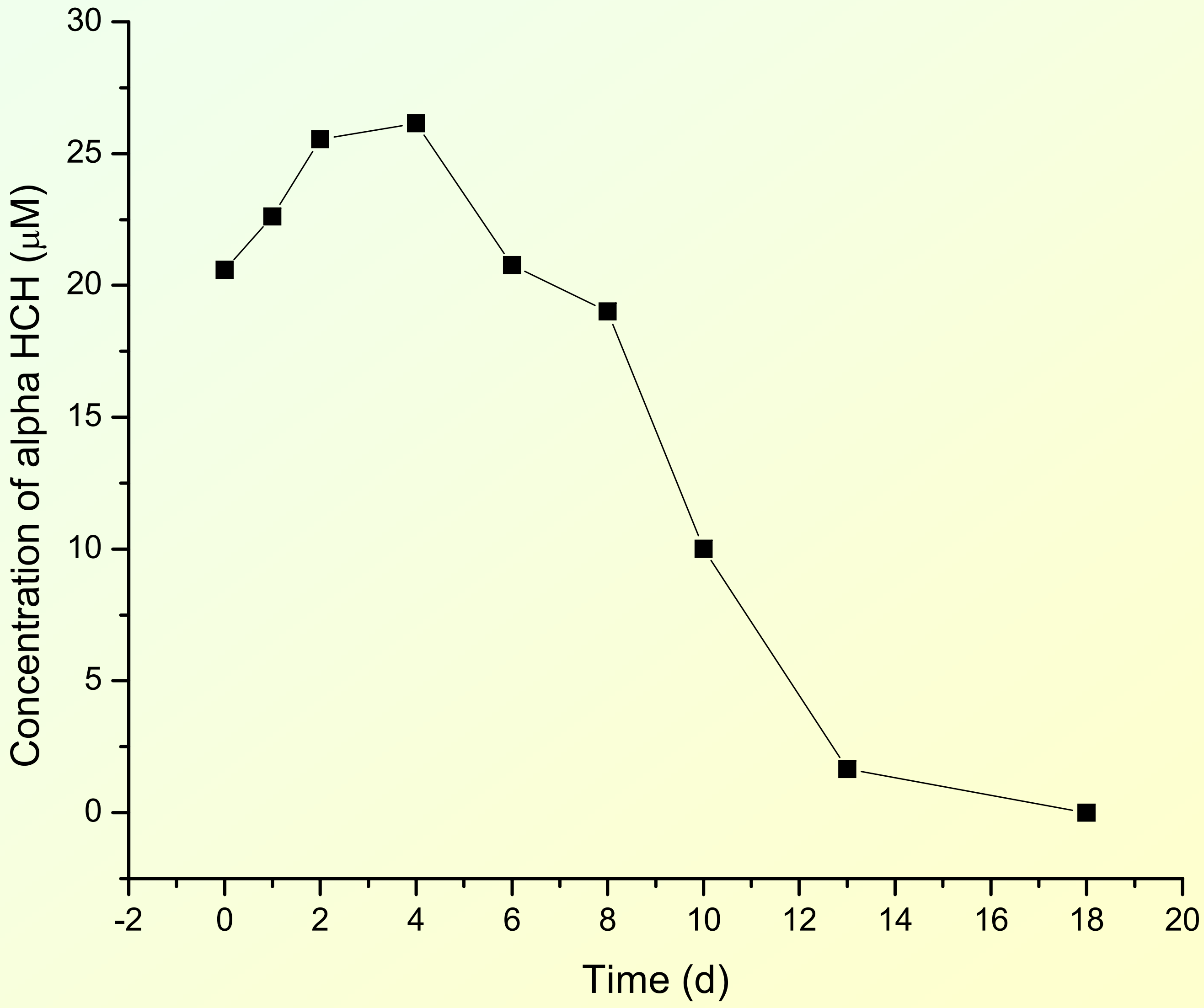
Detected metabolites in the Biodegradation experiments with *Clostridium Pasteurianum* DSM 525

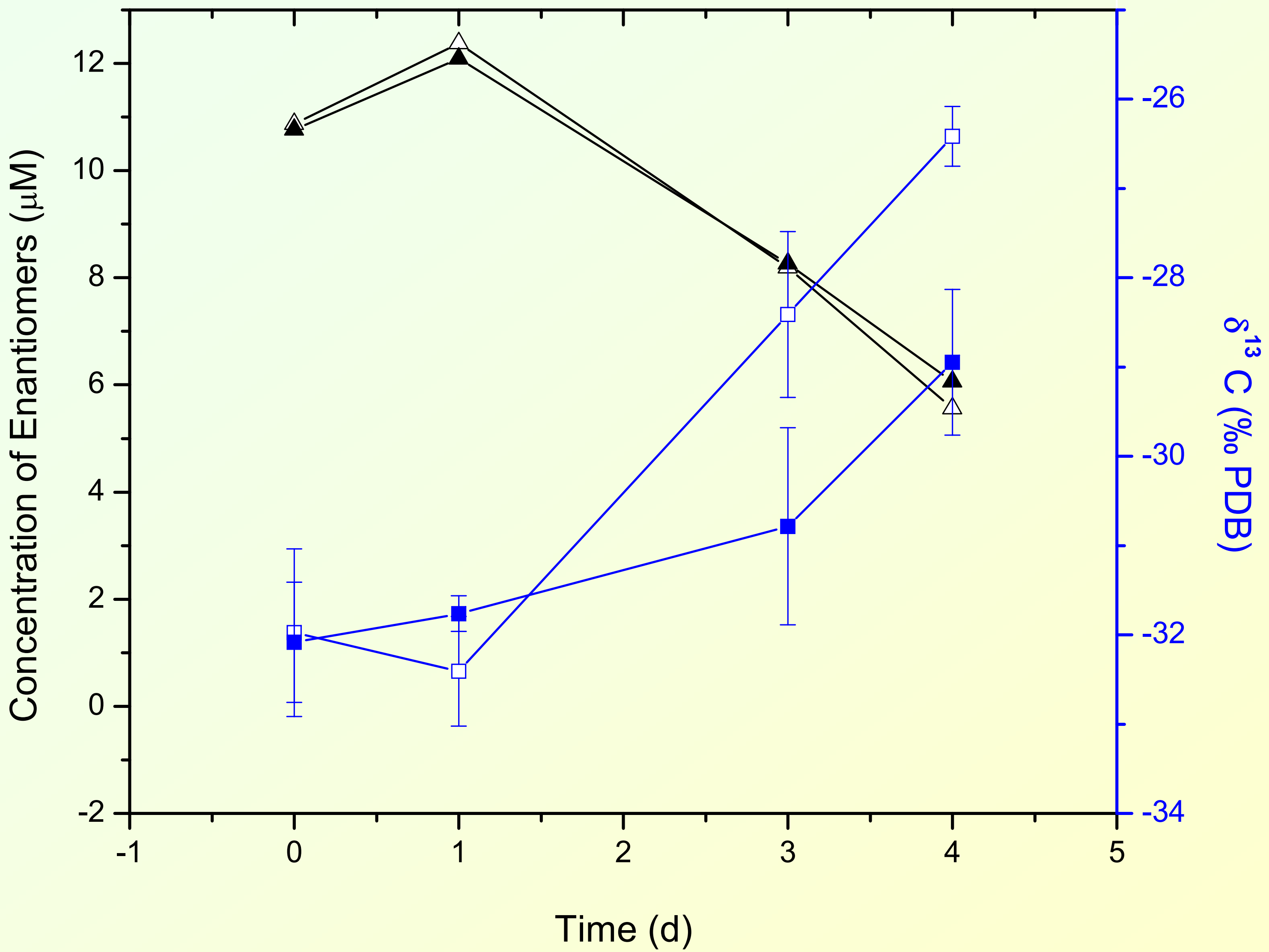




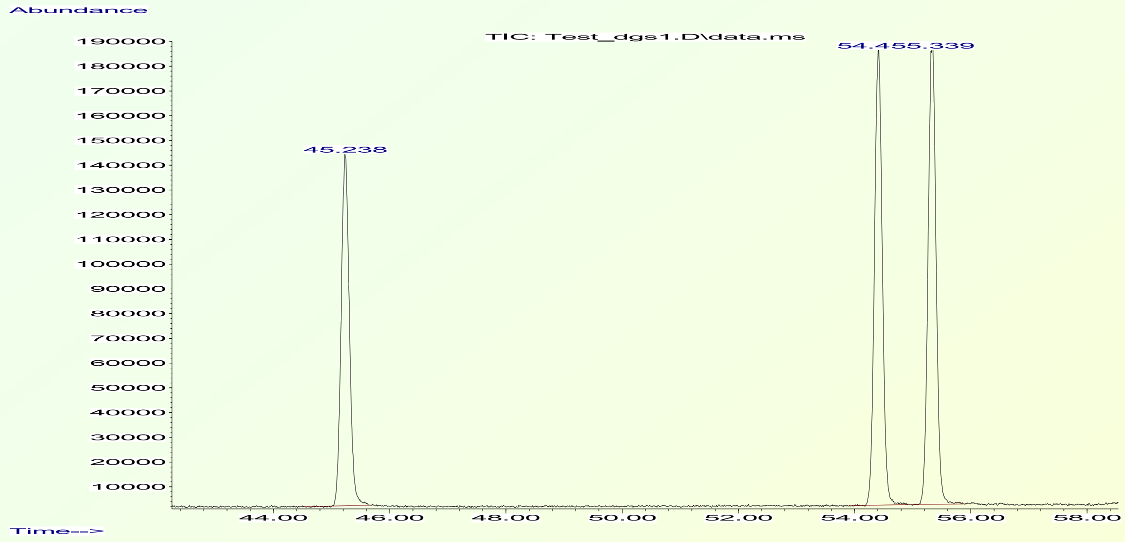
The Chiral GC-MS separation of alpha HCH and their metabolites

Alpha HCH´s Biodegradation by *C. Pasteurianum*

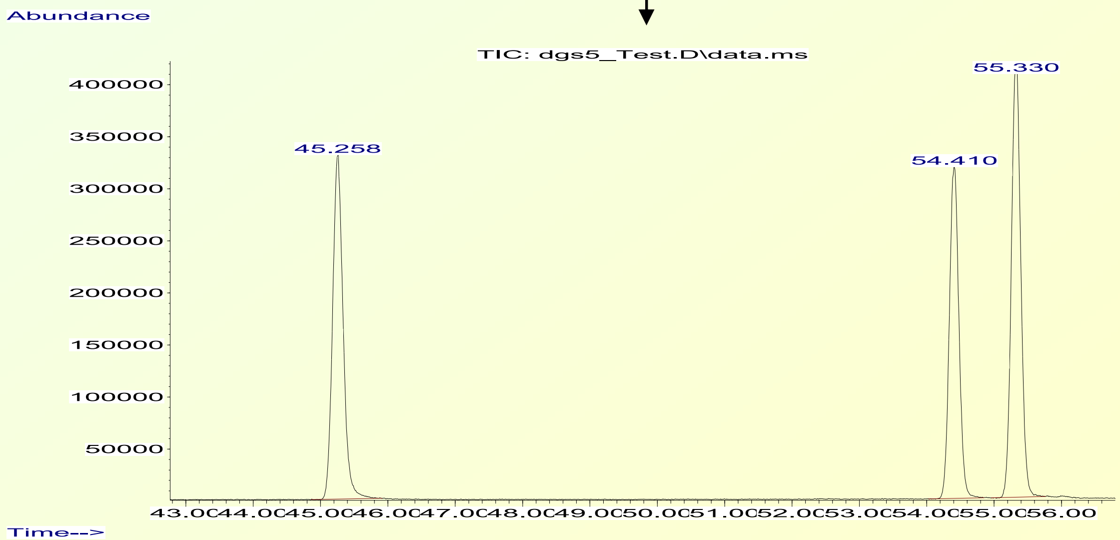




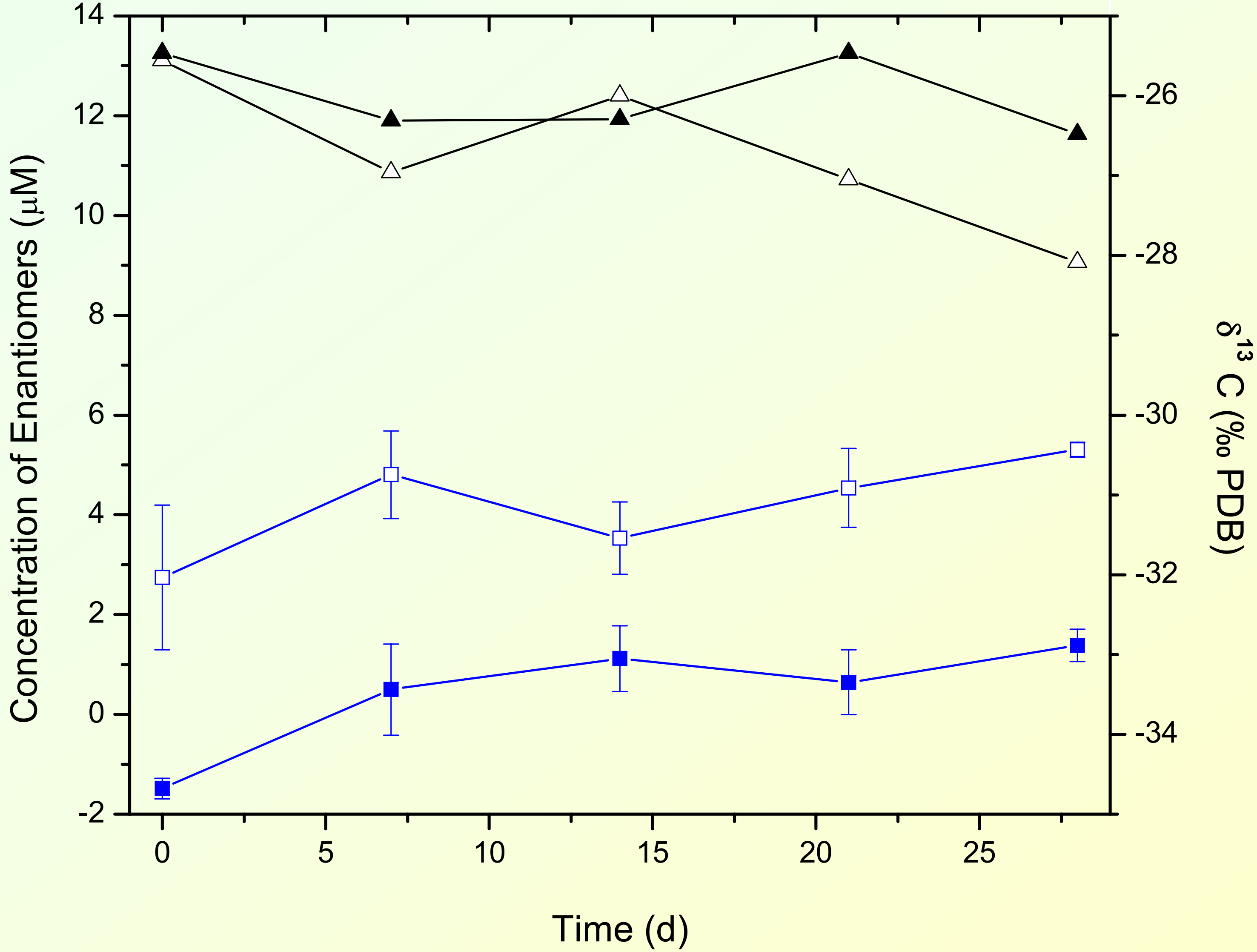
Change in concentration (Δ) and carbon isotope composition (□) of (-) alpha HCH, in concentration (▲) and carbon isotope composition (■) of (+) alpha HCH during reductive dechlorination by *C. Pasteurianum*



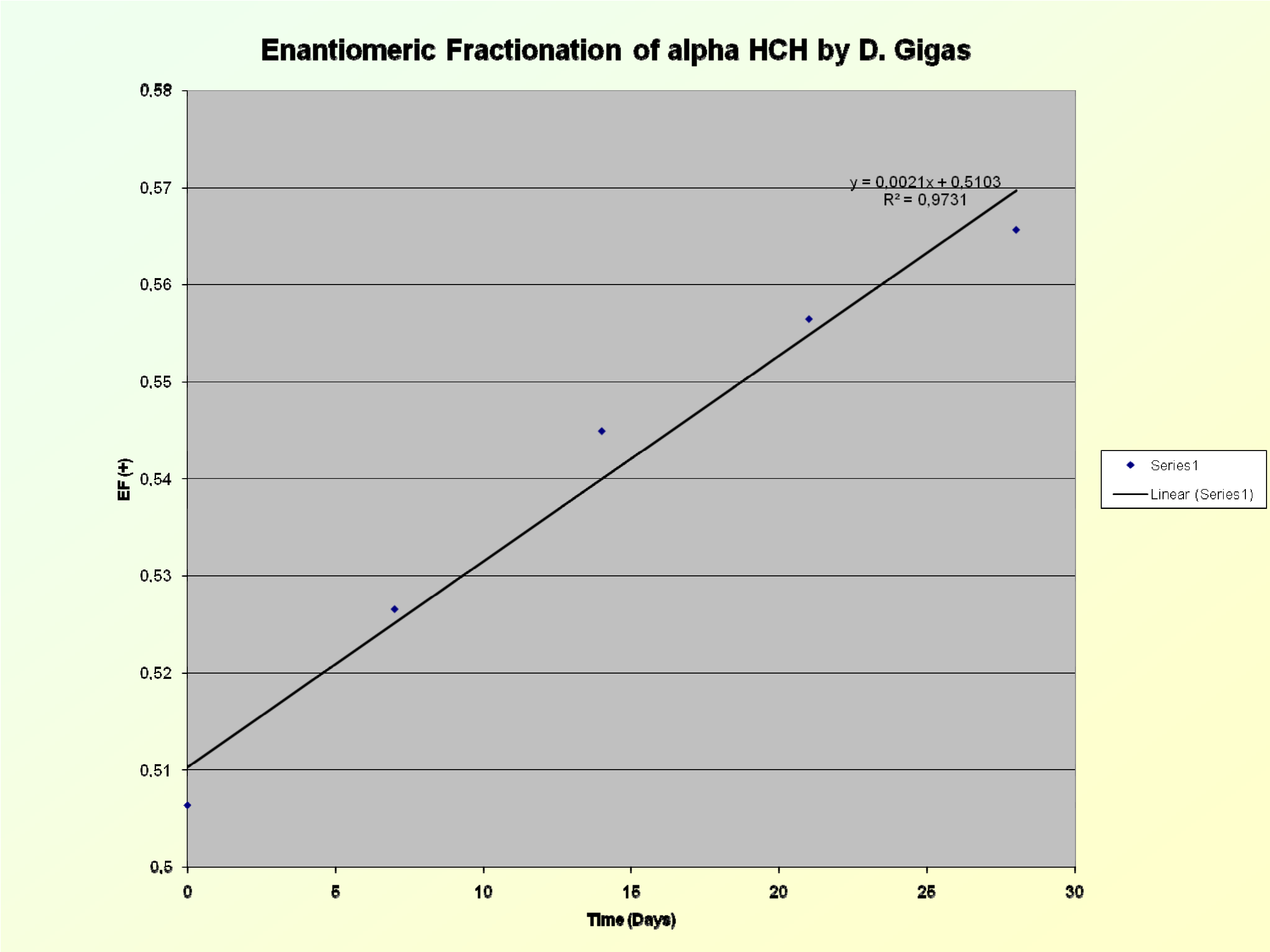
28 Days of Incubation



Enantioselective Transformation of alpha HCH by D. Gigas



Change in concentration (Δ) and carbon isotope composition (□) of (-) alpha HCH, in concentration (▲) and carbon isotope composition (■) of (+) alpha HCH during reductive dechlorination by *D. Gigas*


Enantiomeric Fractionation of alpha HCH by D. Gigas
0,58
0,57
0,56
0,55
0,54
0,53
0,52
0,51
0,5
EF (+)
0
5
10
15
20
25
30
Time (Days)
y = 0,0021x + 0,5103
R² = 0,9731
Series1
Linear (Series1)

Conclusions

- The α HCH transformation by Clostridrium Pasteurianum is associated with a low enantioselectivity with a specific isotope fractionation pattern for the enantiomers
- The α HCH transformation by Desulfovibrio Gigas is highly enantioselective even at a low extent of biotransformation
- Benzene, chlorobenzene and, the intermediate, δ-tetrachlorocyclohexene (chiral) were detected as microbial metabolites of alpha HCH transformation
- Combined Stable Isotope and Enantiomeric Fractionation of alpha HCH can be a new concept in Biogeochemistry of POPs
- More experimental data are needed to developed this approach

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