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# How to verify that natural attenuation is bioremediation?

**Cédric Malandain**

**Senior Project Manager**  
**[www.enoveo.com](http://www.enoveo.com)**



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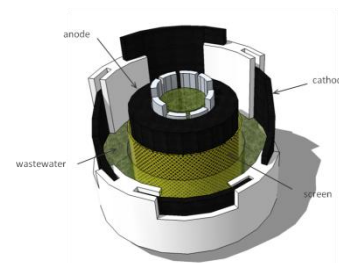
- **Company created in May 2008**
- **4 partners:**
  - *Post-doc, Molecular microbiology*
  - *PhD, Environmental Microbiology*
  - *Chemist*
  - *Professor, Environmental Microbiology and Bio-Engineering*
- **3 employees :**
  - *Msc, Project Engineer, Applied microbial ecology*
  - *Msc, Sales Engineer, Applied microbial ecology*
  - *PhD, Senior Project Manager, Environmental and molecular microbiology*
- **Offices in the USA (Berkeley, CA) and in Brasil (Florianopolis)**
- **Development and application of innovative technologies for diverse environmental problems. *Pilots***
- **Expert consulting in environmental microbiology and chemistry, and environmental bioprocess engineering – analyses, design and implementation.**



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- **Polluted soils and sites:**
  - Evaluation of biodegradation potential
  - Consulting
- **Industrial, urban and agricultural wastewater:**
  - Identify dysfunction
  - Water quality monitoring
  - Wastewater valorisation
- **Bioprocess**
- **Drinkable water**
- **Technological state of the art**
- **Environmental or industrial forensics:**
  - Identification of pollution source and/or origin
  - Degradation product prediction
  - Microbial contamination assessment

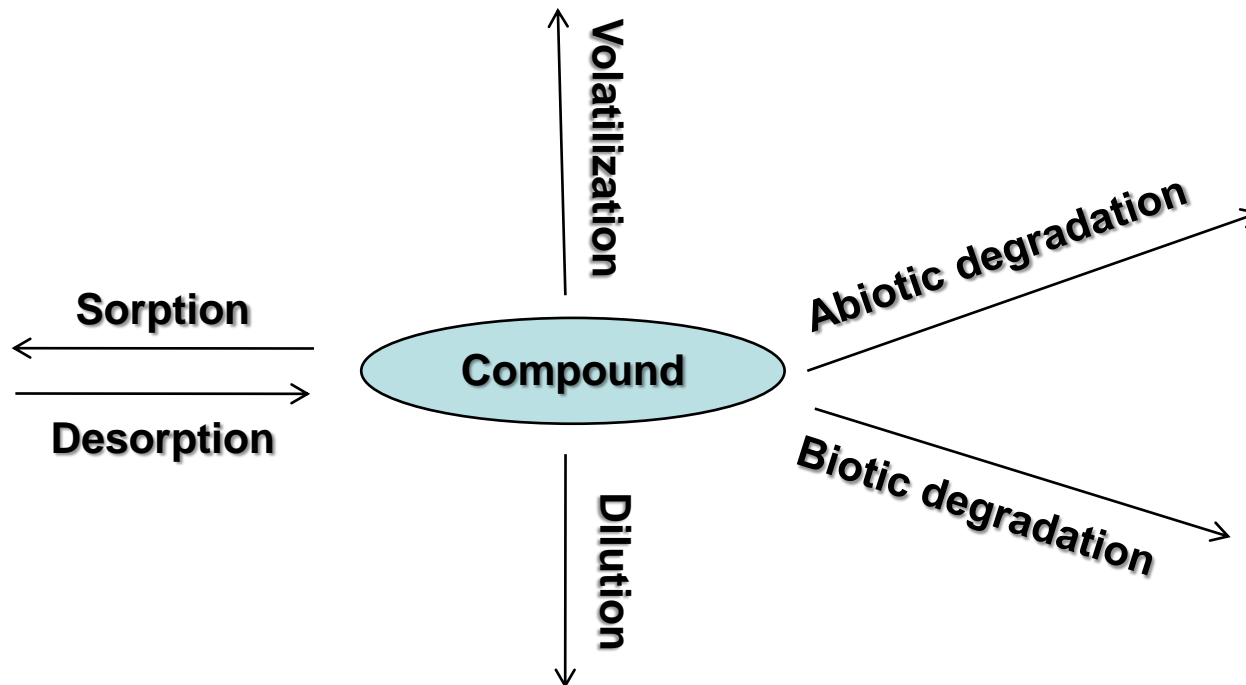




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# Natural Attenuation processes

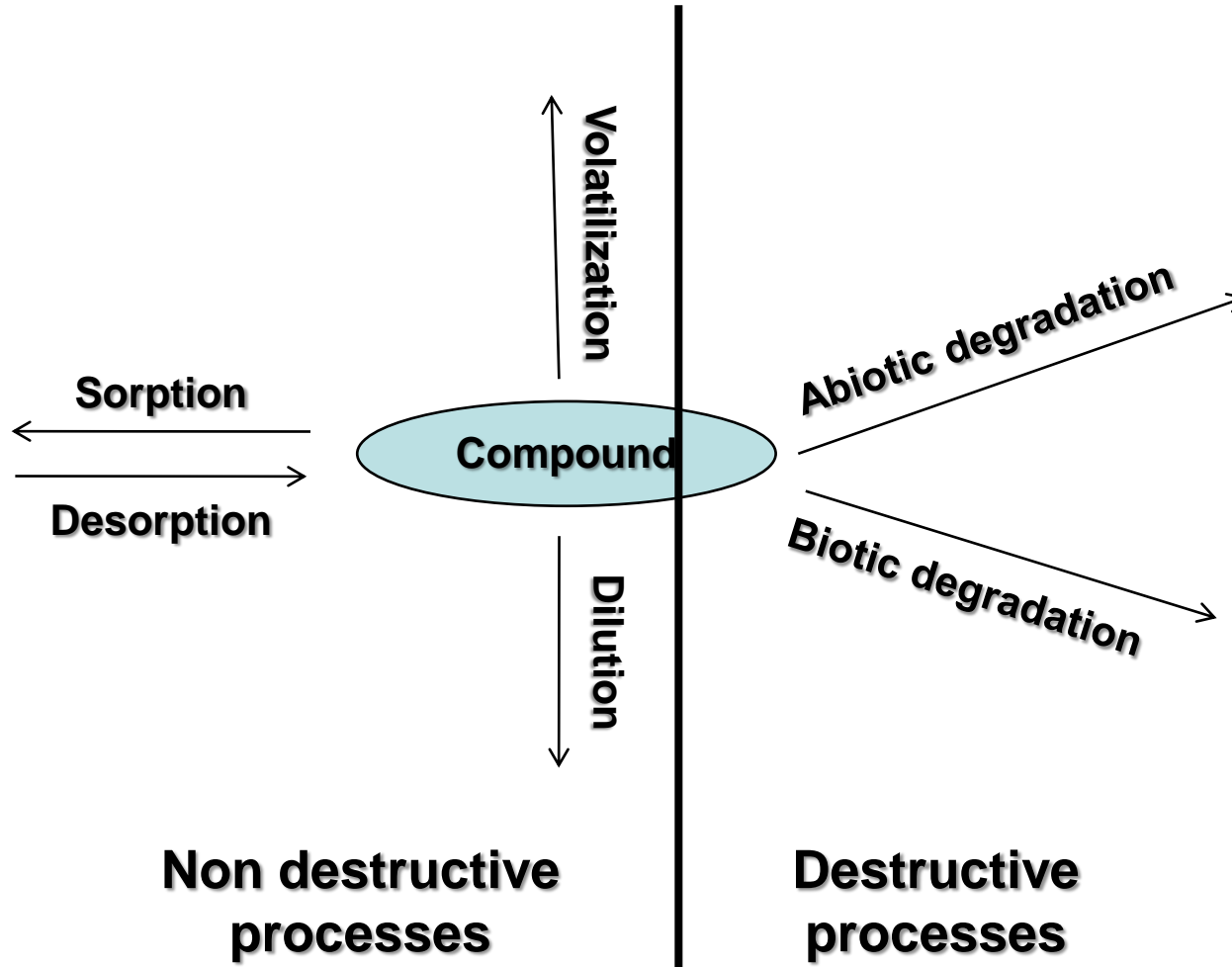




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# Natural Attenuation processes

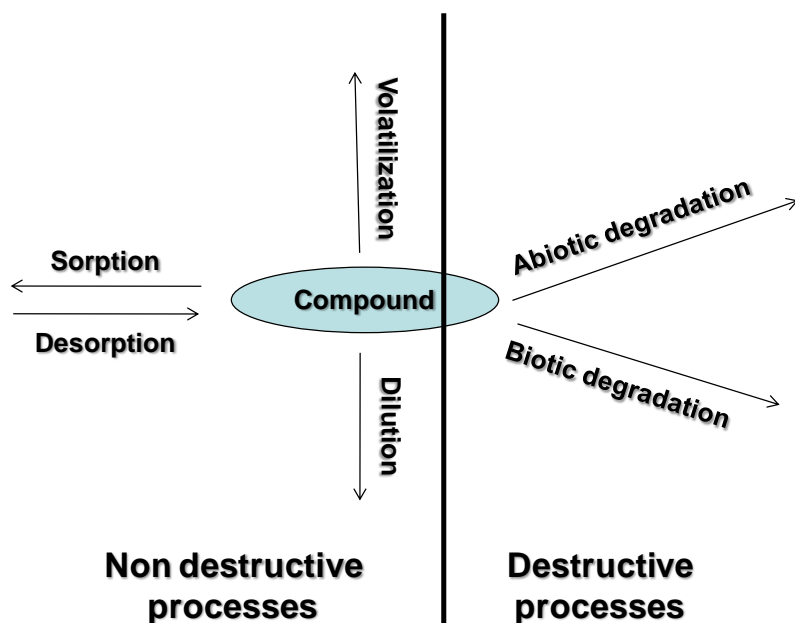




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# Natural Attenuation processes



**Currently monitored by:**

- **chemical analysis**
- **isotopic fractionation**

## Limits:

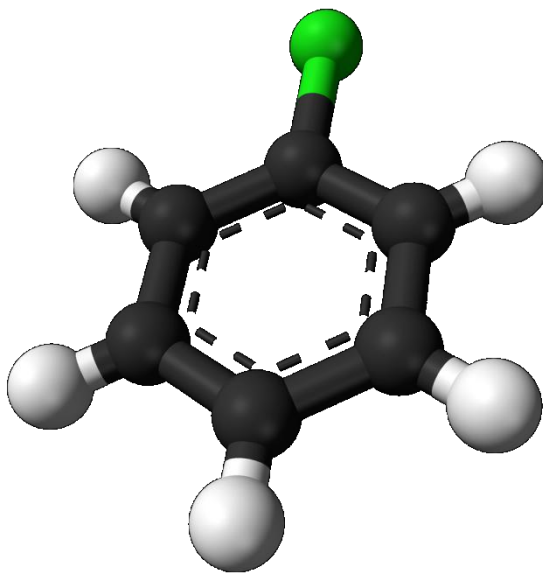
- **sometimes abiotic and biotic processes have the same daughter-products**
- **half-life of daughter-products**
- **when the biodegradation happened? Is it still ongoing?**



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# Monochlorobenzene (MCB) case study







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# Monochlorobenzene (MCB)

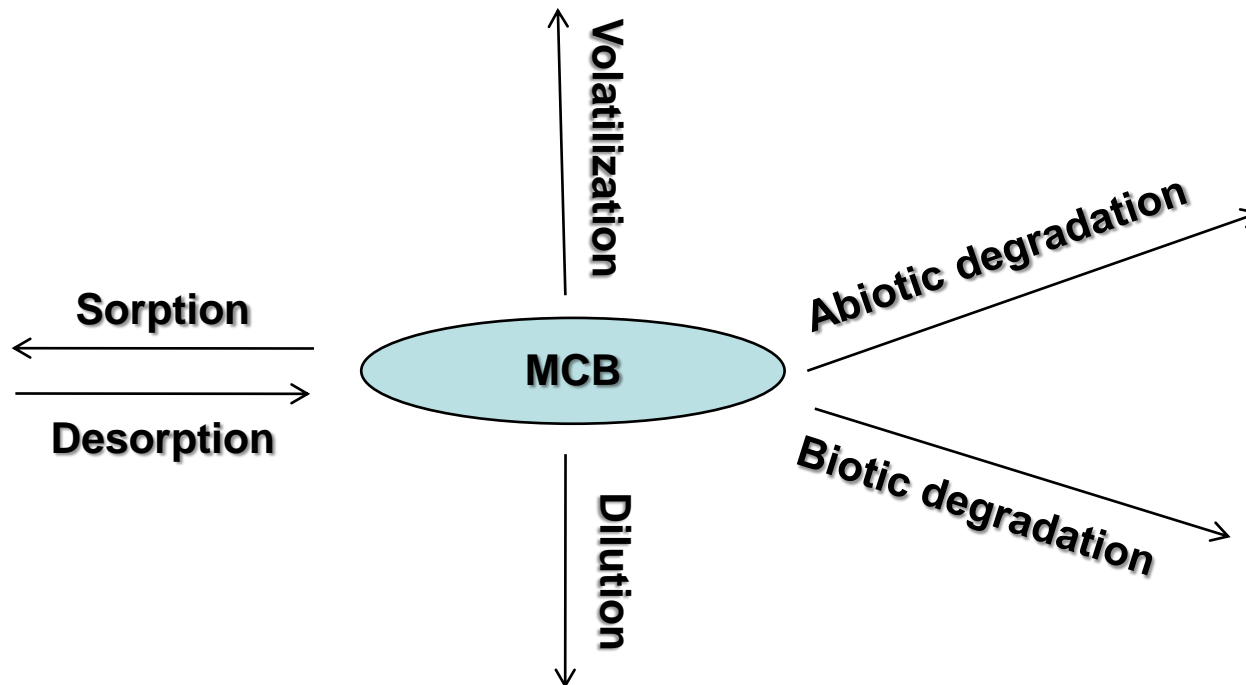
- Biodegradable under aerobic conditions
- Poorly biodegradable under anaerobic conditions
- Induction of aerobic degradation: injection of oxygen in the aquifer
- Highly volatile



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## MCB natural attenuation

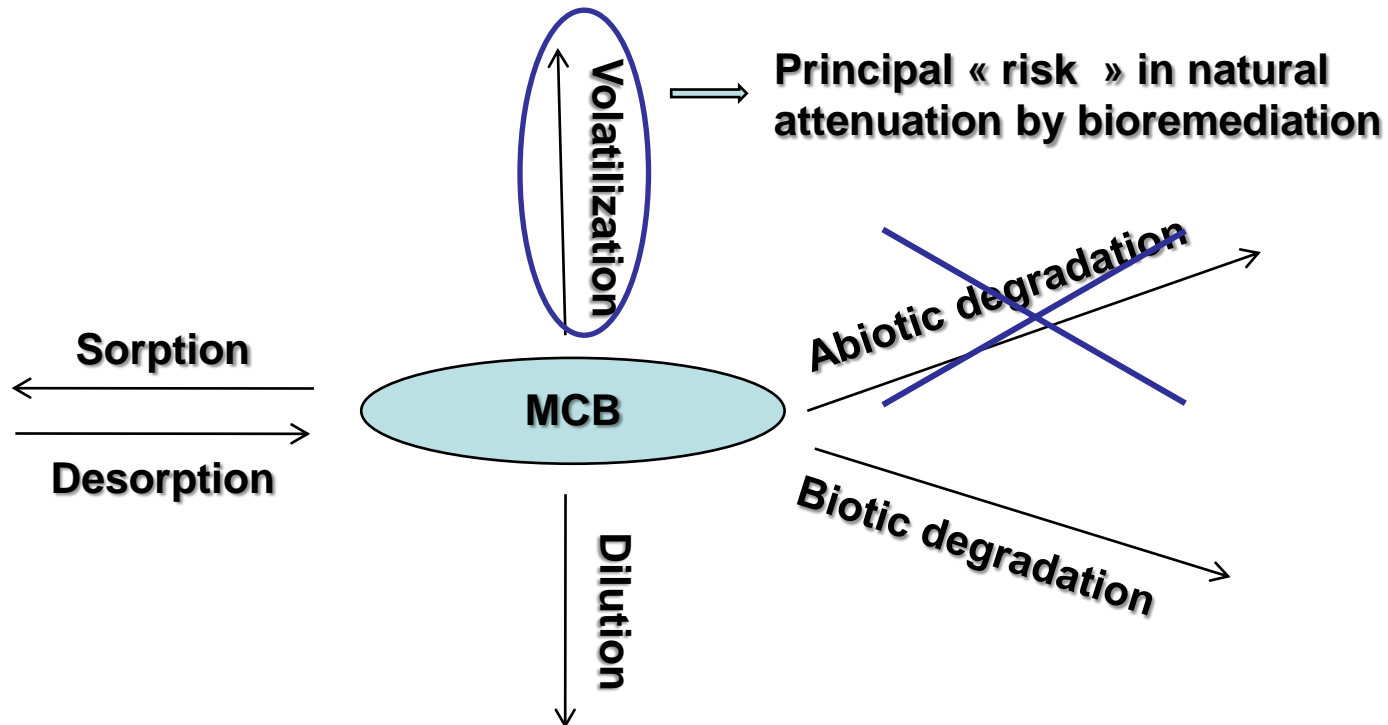




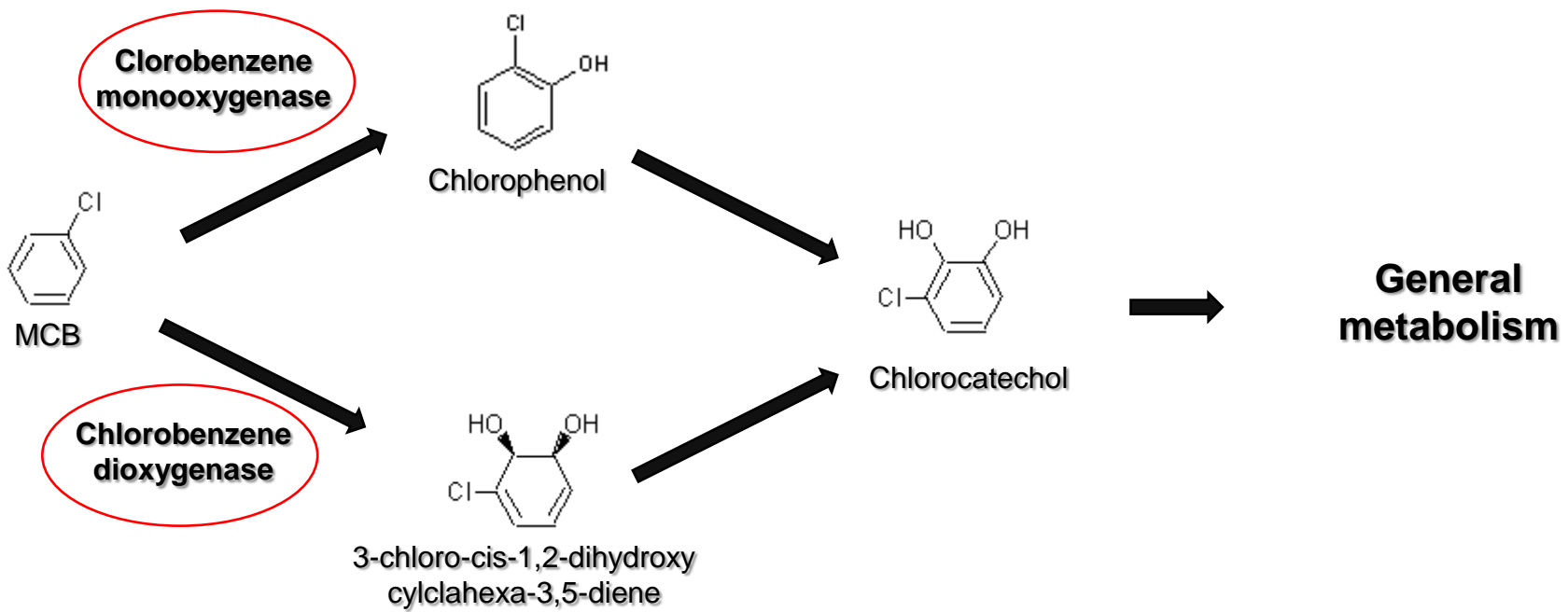
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## MCB natural attenuation



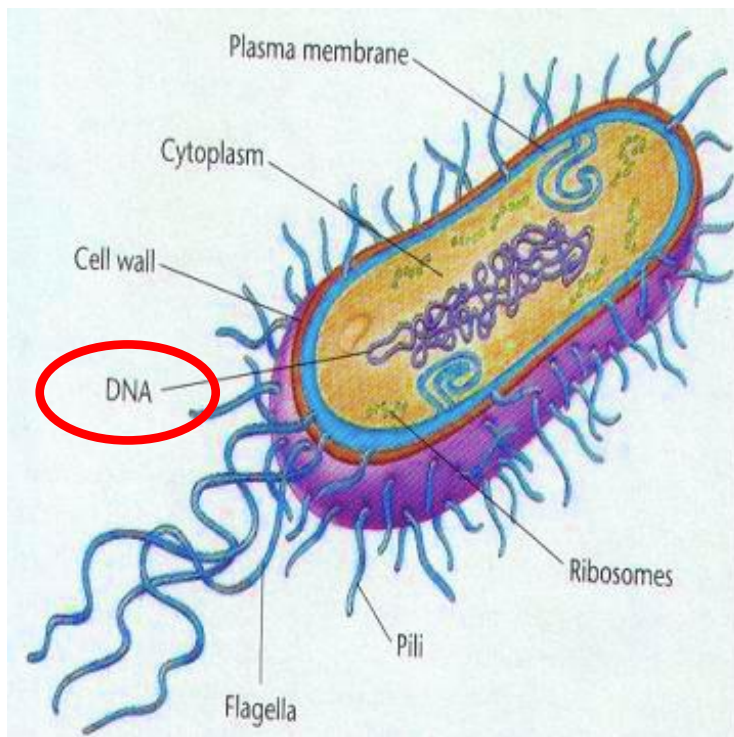
# MCB biodegradation pathway





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**Genetic material :**  
**Responsible for bacterial degradation capabilities**

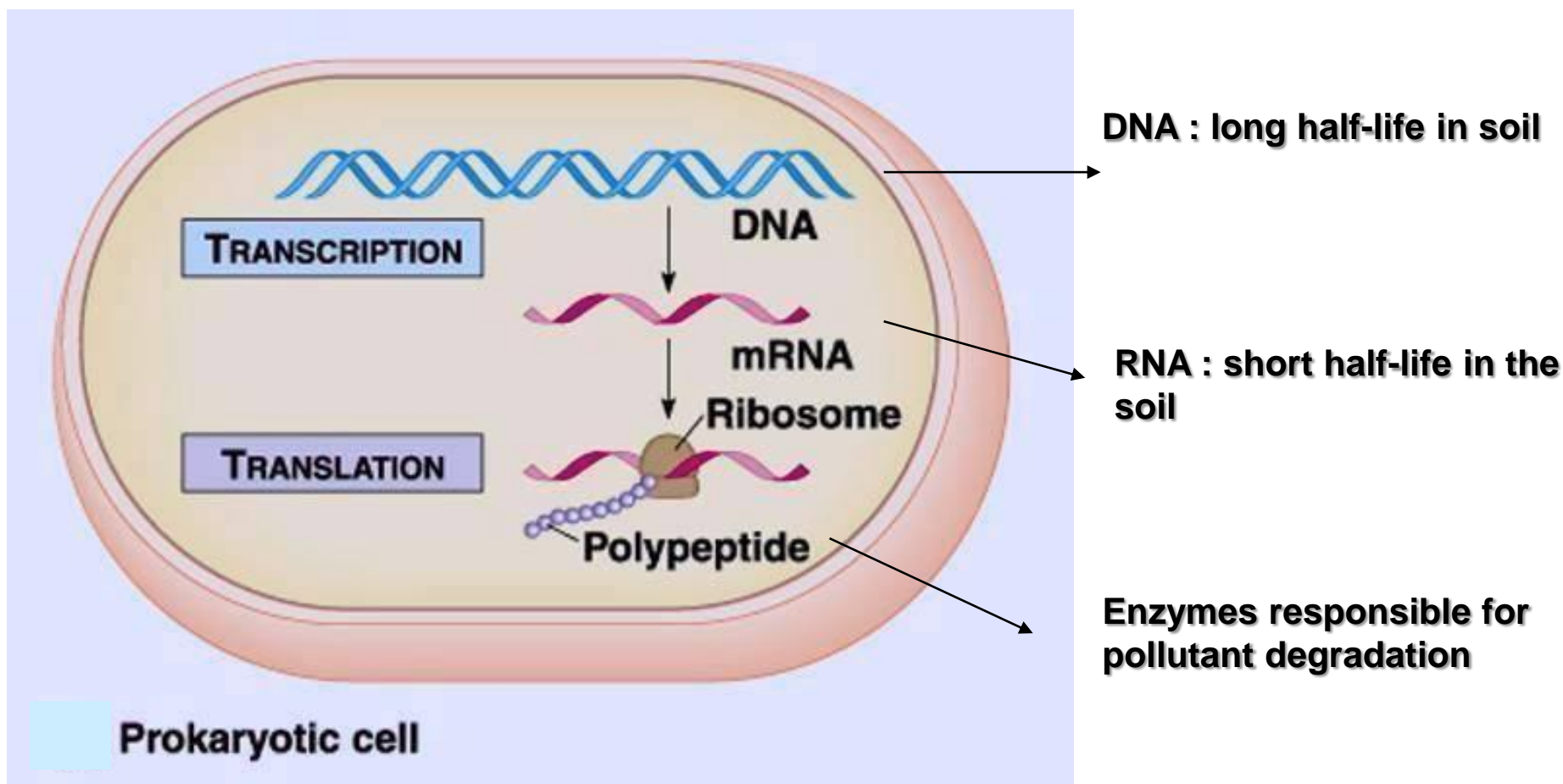
—————→ **Codes for enzymes**



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# Process





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- Quantification of target sequences : gene of interest
- DNA Quantification : potential of soil bacteria to degrade a pollutant
- RNA quantification : measure degradation activity

**Improve our understanding  
of site microbiology**

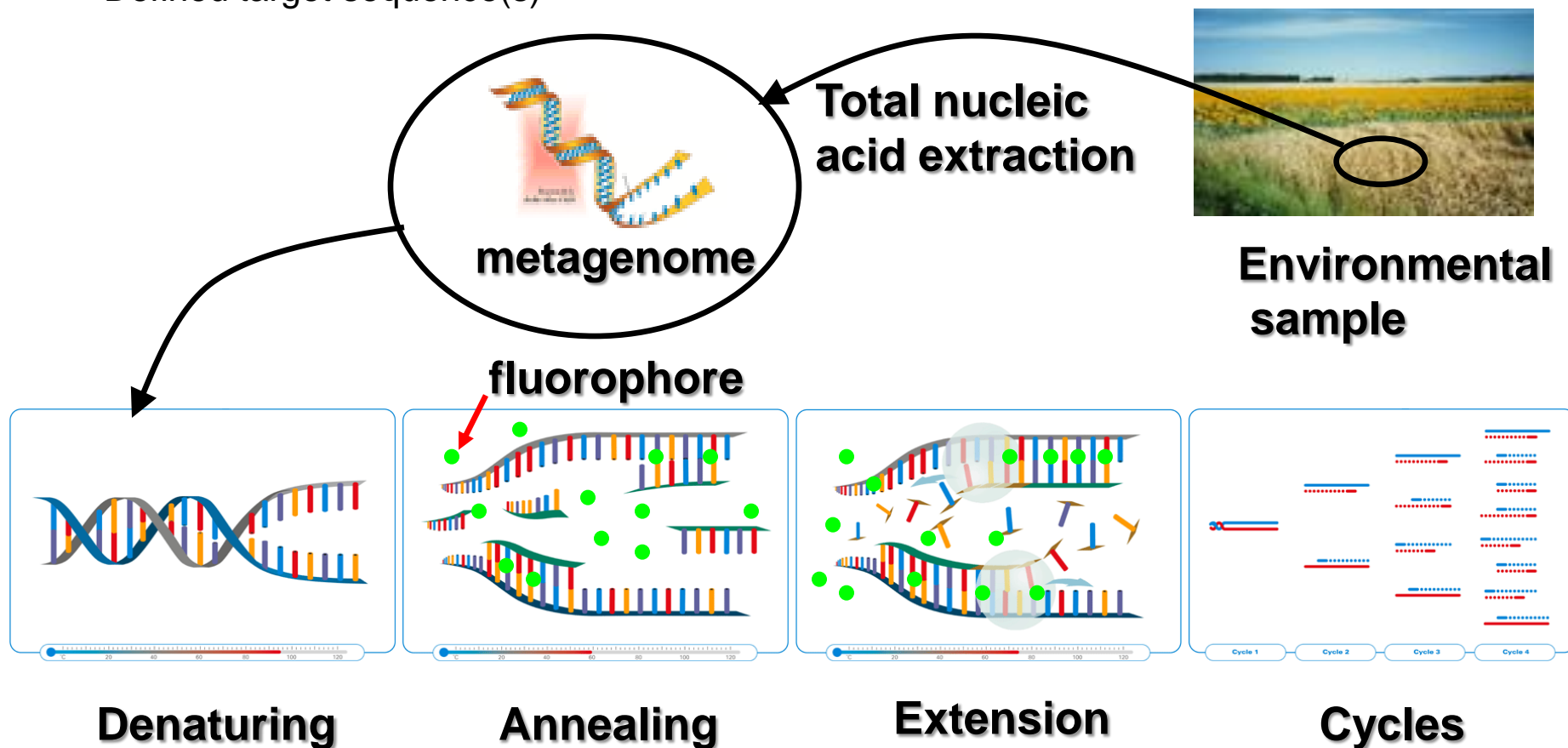


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# DNA/RNA quantification

- Well known technique: quantitative Polymerase Chain Reaction (qPCR)
- Defined target sequence(s)





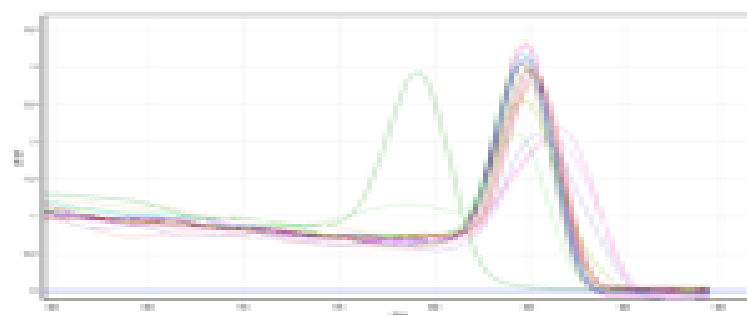
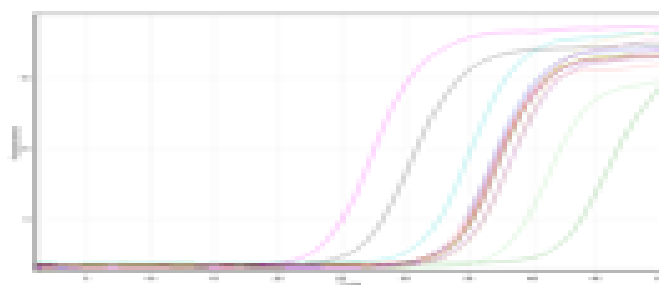
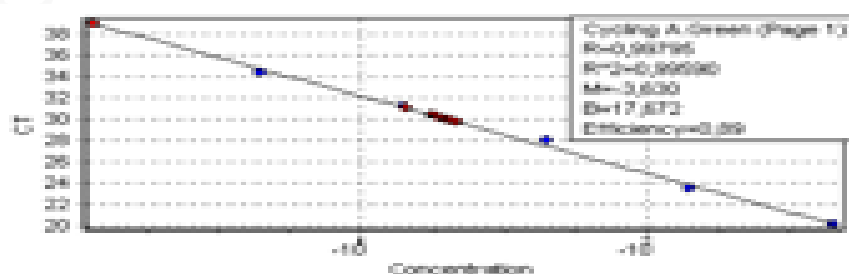


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qPCR  
thermocycler



Statistical  
analyses

Reaction  
specificity

→ Identification and quantification of general and specific activity  
within the total microbial community

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- Quantification of target sequences : gene of interest
- DNA Quantification : potential of soil bacteria to degrade a pollutant
- RNA quantification : measure degradation activity

DNA	RNA
+	+
+	-
-	-

Genes are present and active =  
**ongoing degradation**

Genes are present but not active =  
**past degradation**

Genes are not present =  
**no biological degradation = other process**

## Improve our understanding of site microbiology



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## Preliminary tests: Pilot studies

- Abiotic control ( $\text{HgCl}_2$  addition)
- Biotic control
- Amended nutriment 1
- Amended nutriment 2



Incubation  
- Aerated  
- Temp controlled

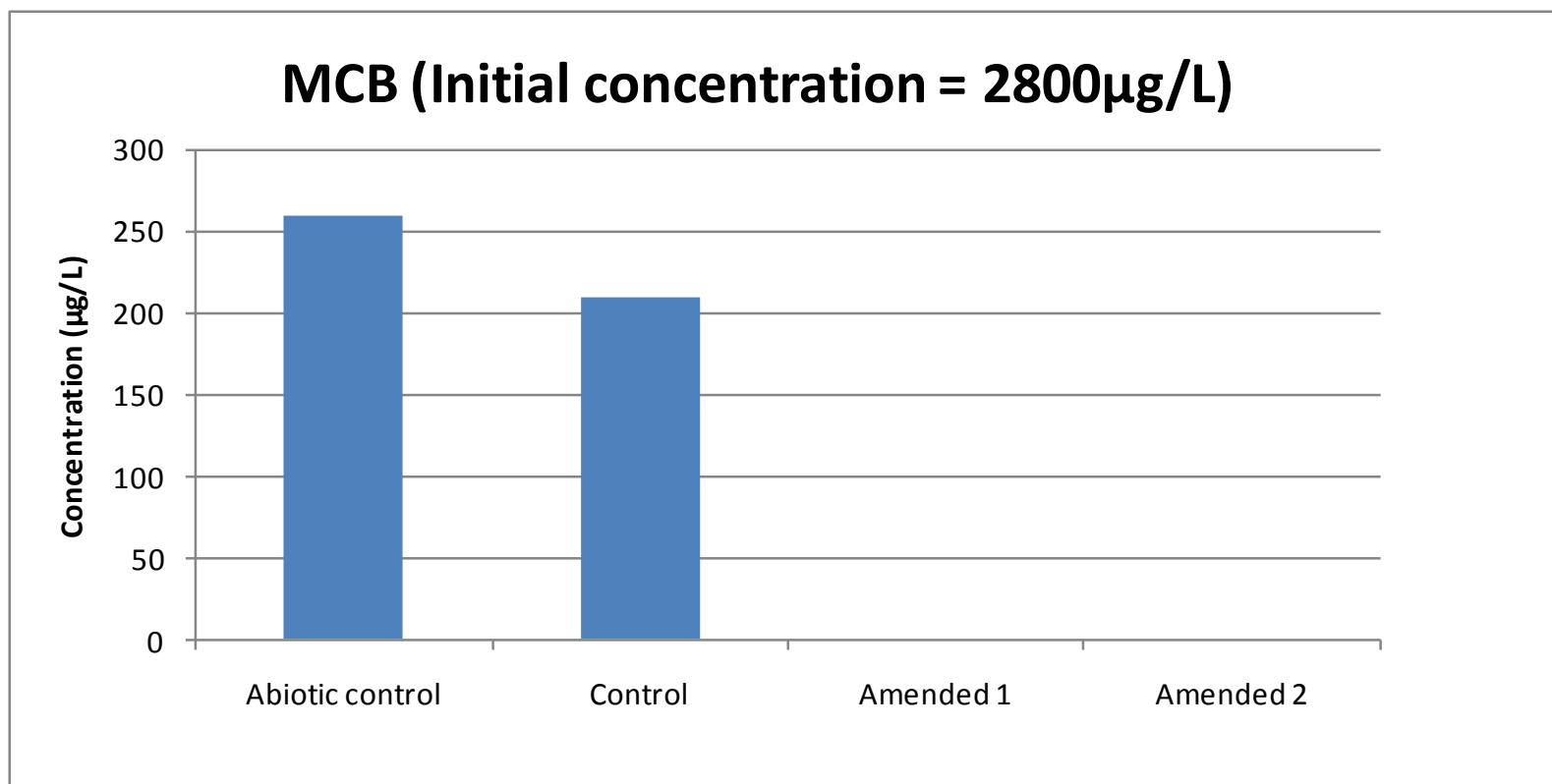
- T0 and T30 days
- Chemical analysis
- Biological analysis



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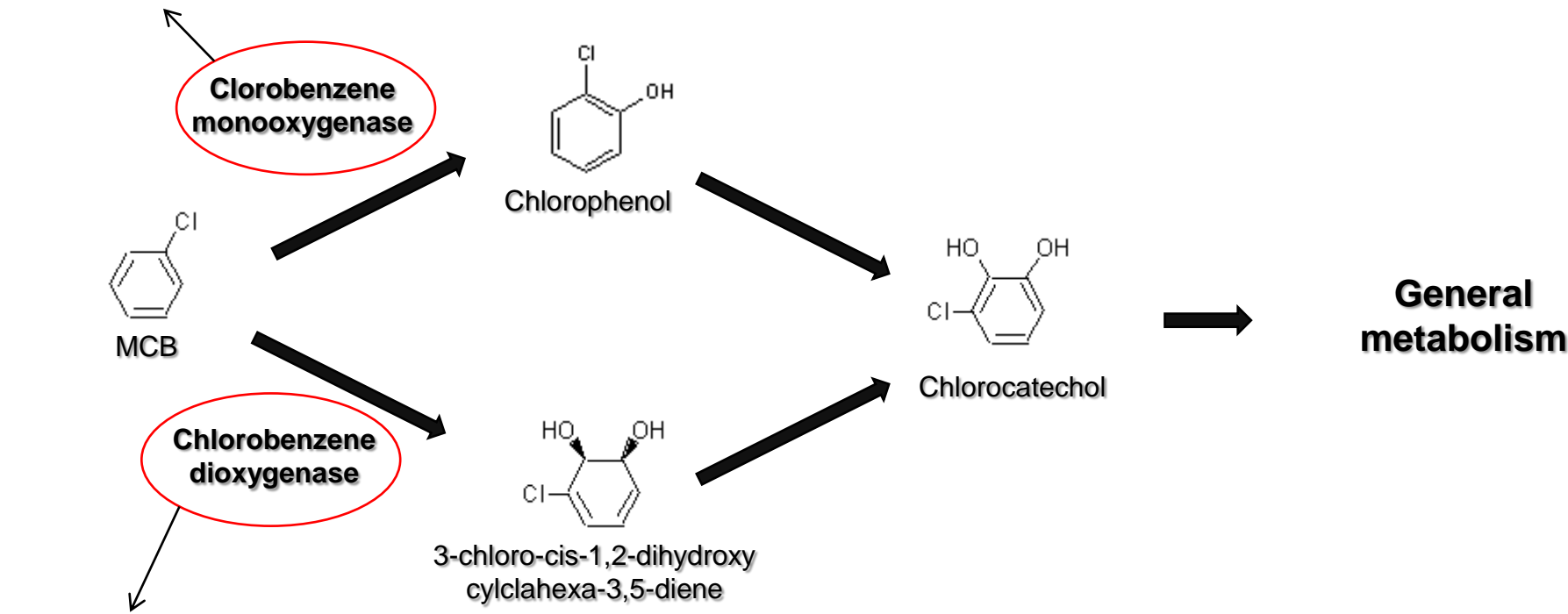
# Chemical analysis



 **90 to 100% MCB removal**

# MCB biodegradation pathway

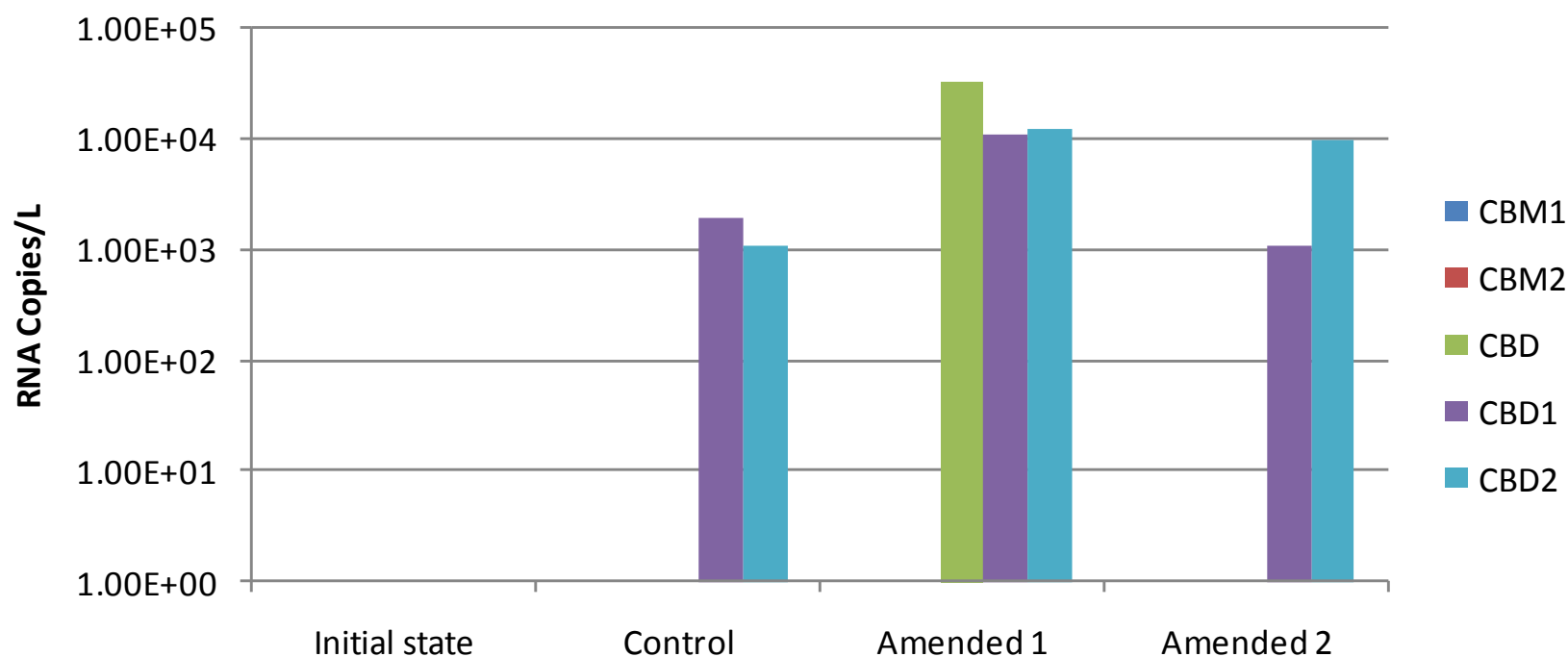
2 monooxygenases



3 dioxygenases



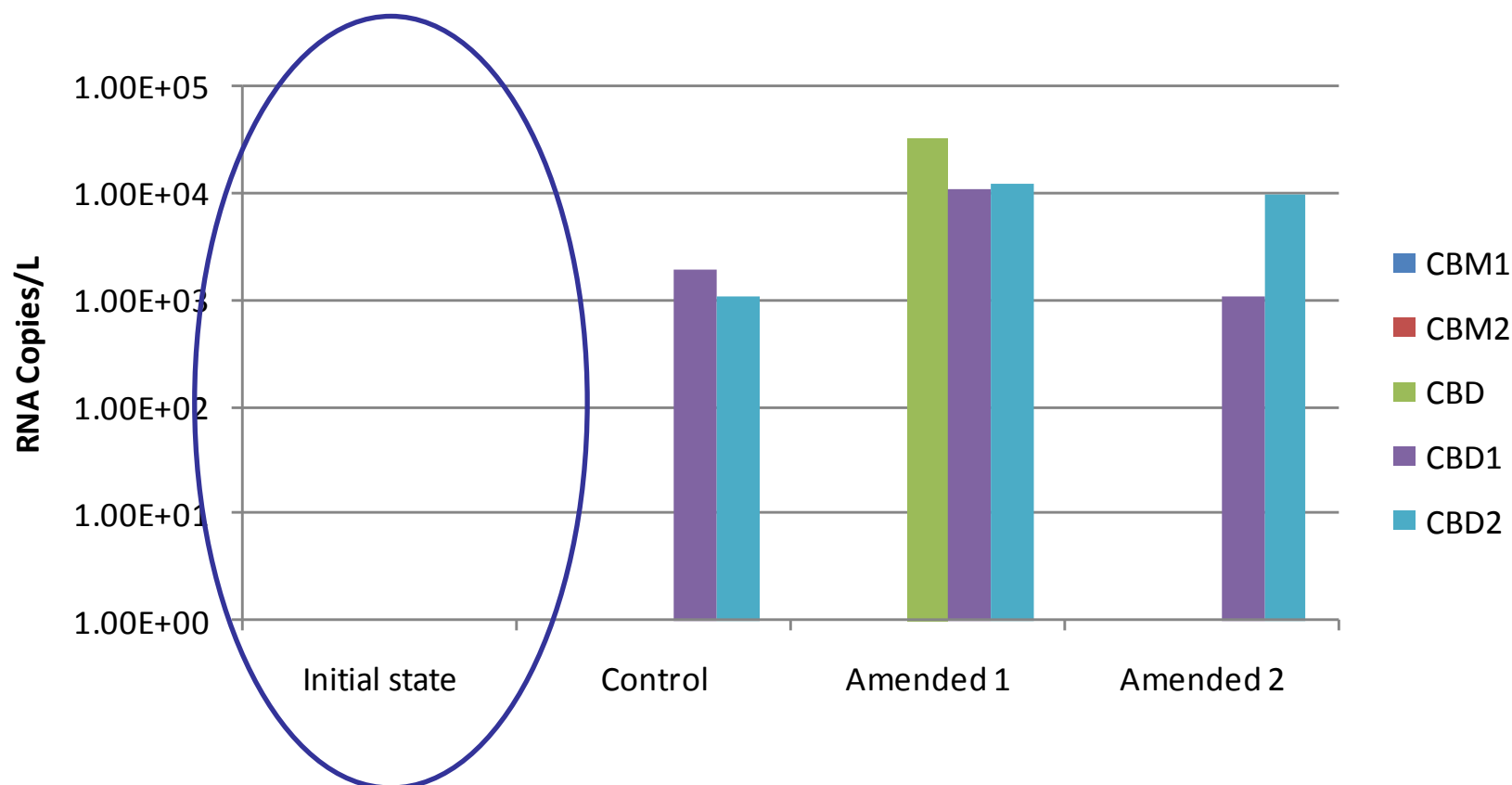
## Biomarker activities (Copies of RNA/L)



**Amended pilots show enhanced activity compared to the Control**



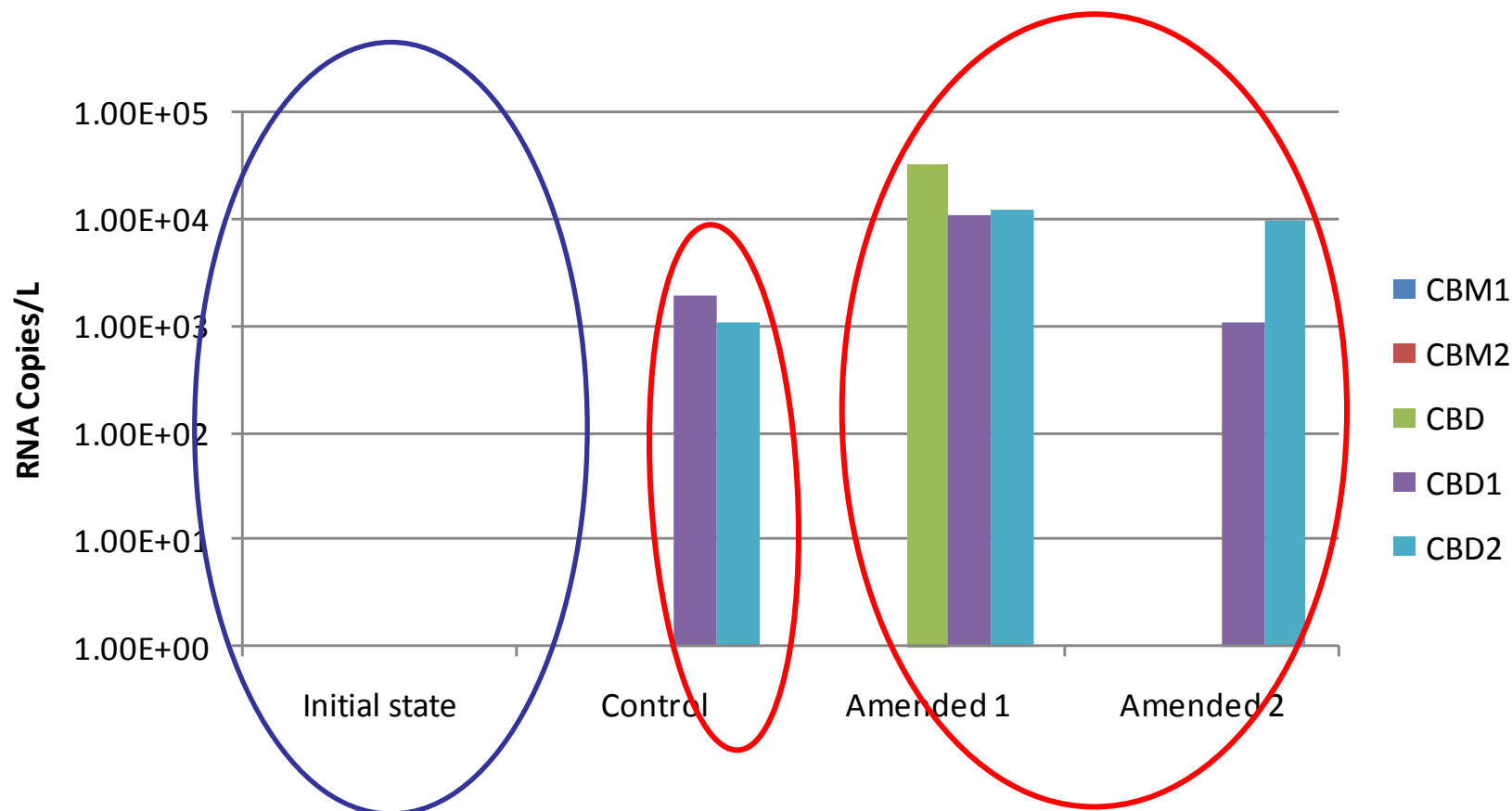
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## Biomarker activities (Copies of RNA/L)



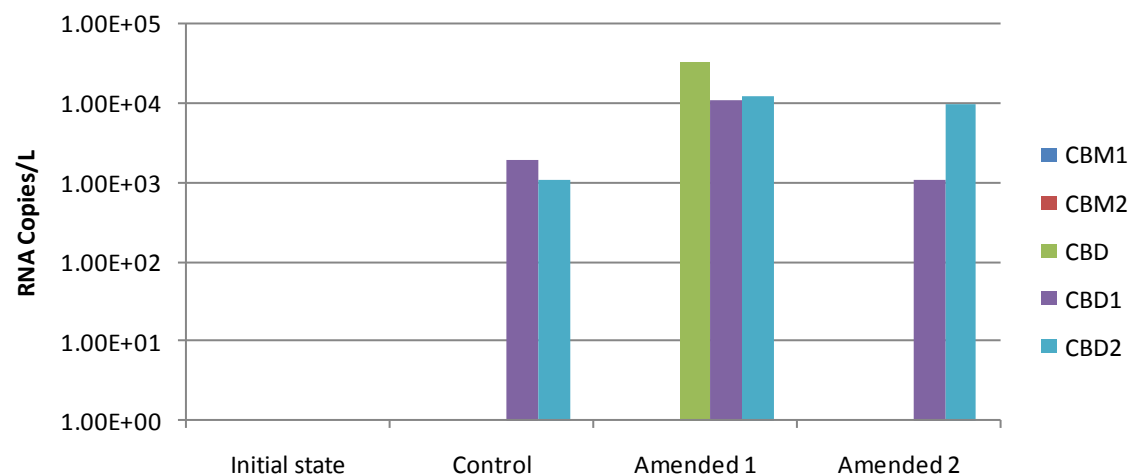
**Amended pilots show enhanced activity compared to the Control**



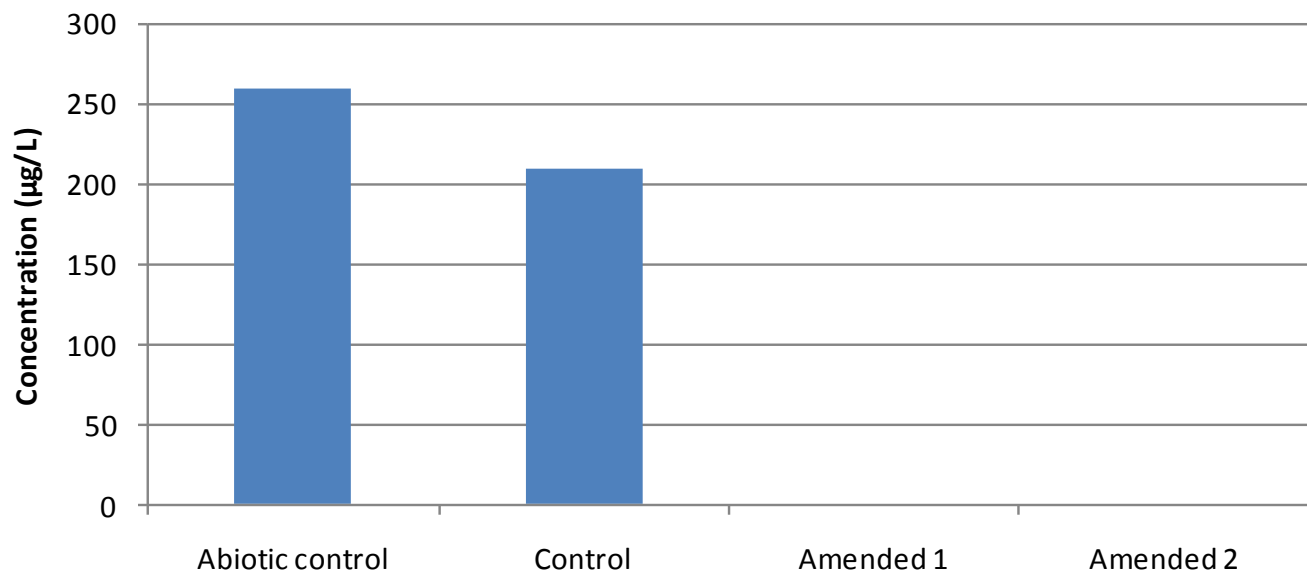


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## MCB (Initial con



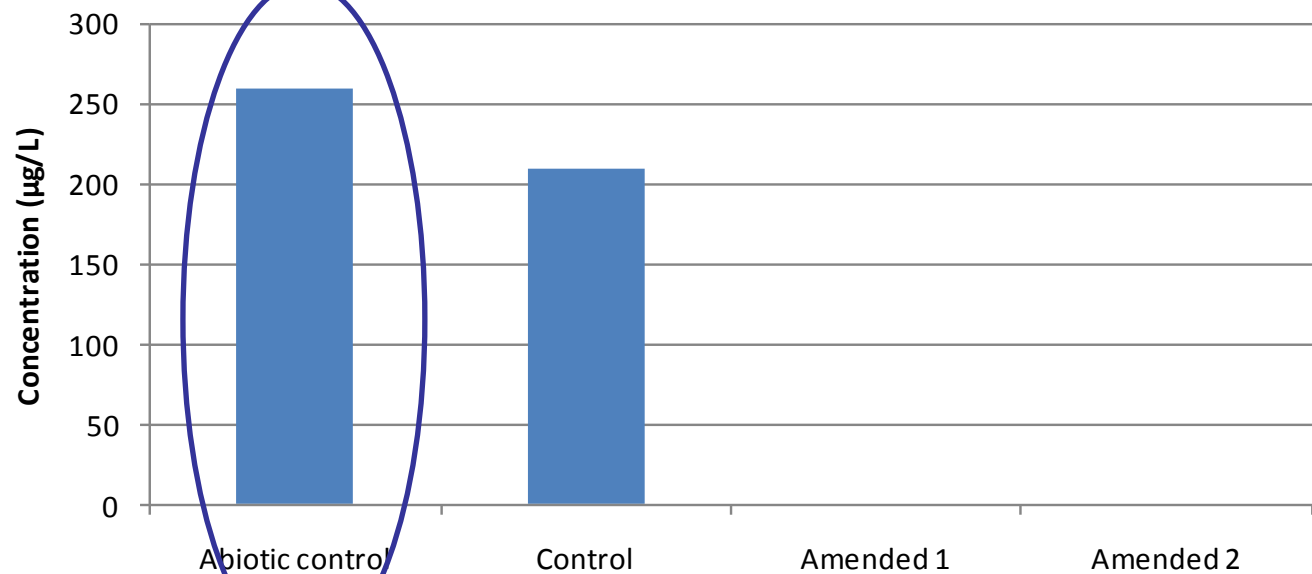
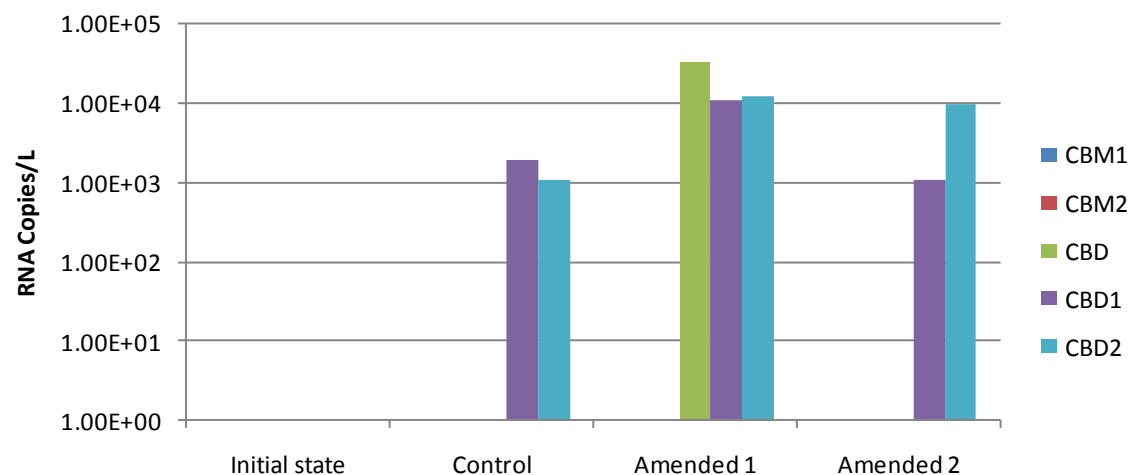


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**Volatilization**

**MCB (Initial con**



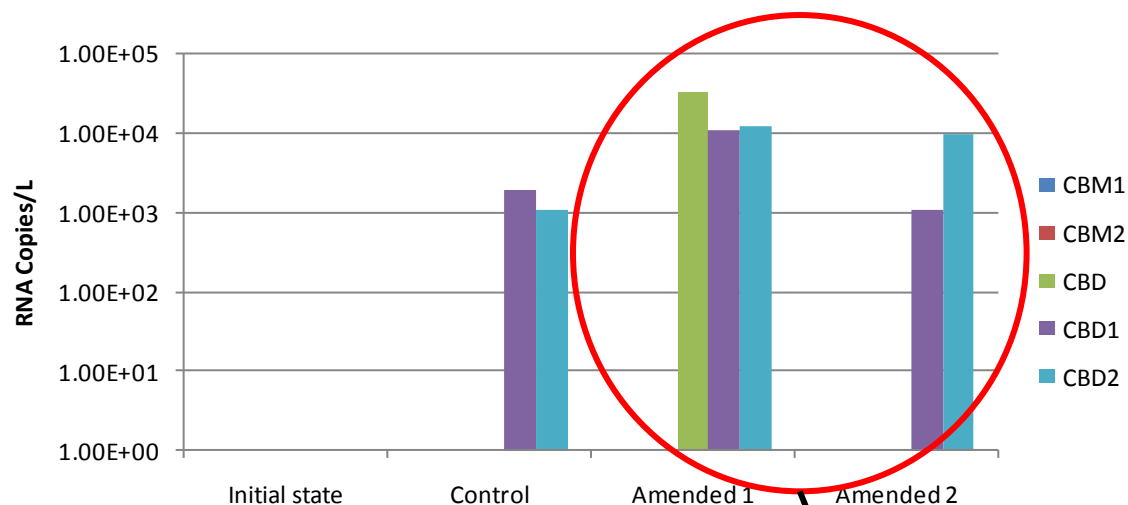
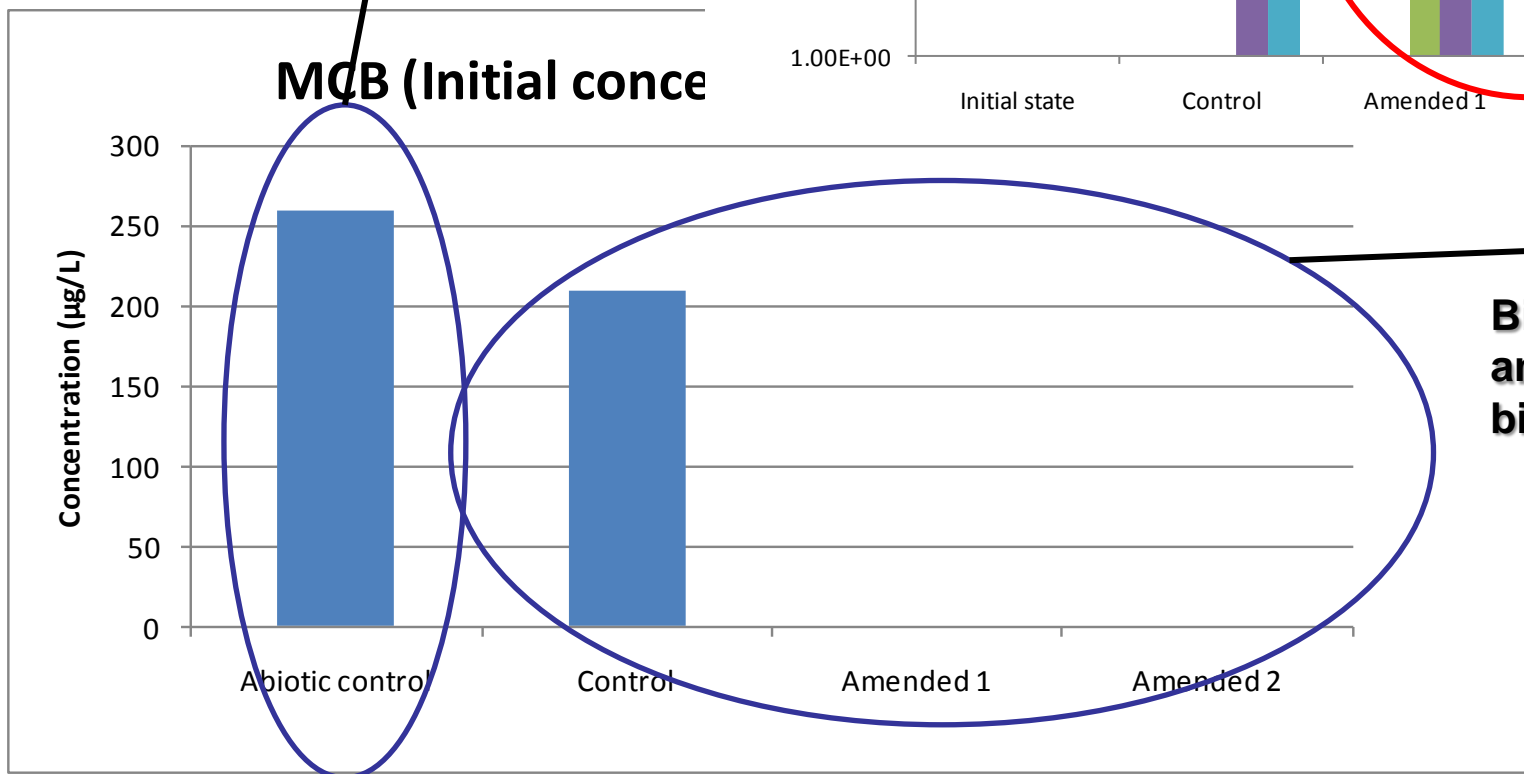


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**Volatilization**

**MCB (Initial conce**



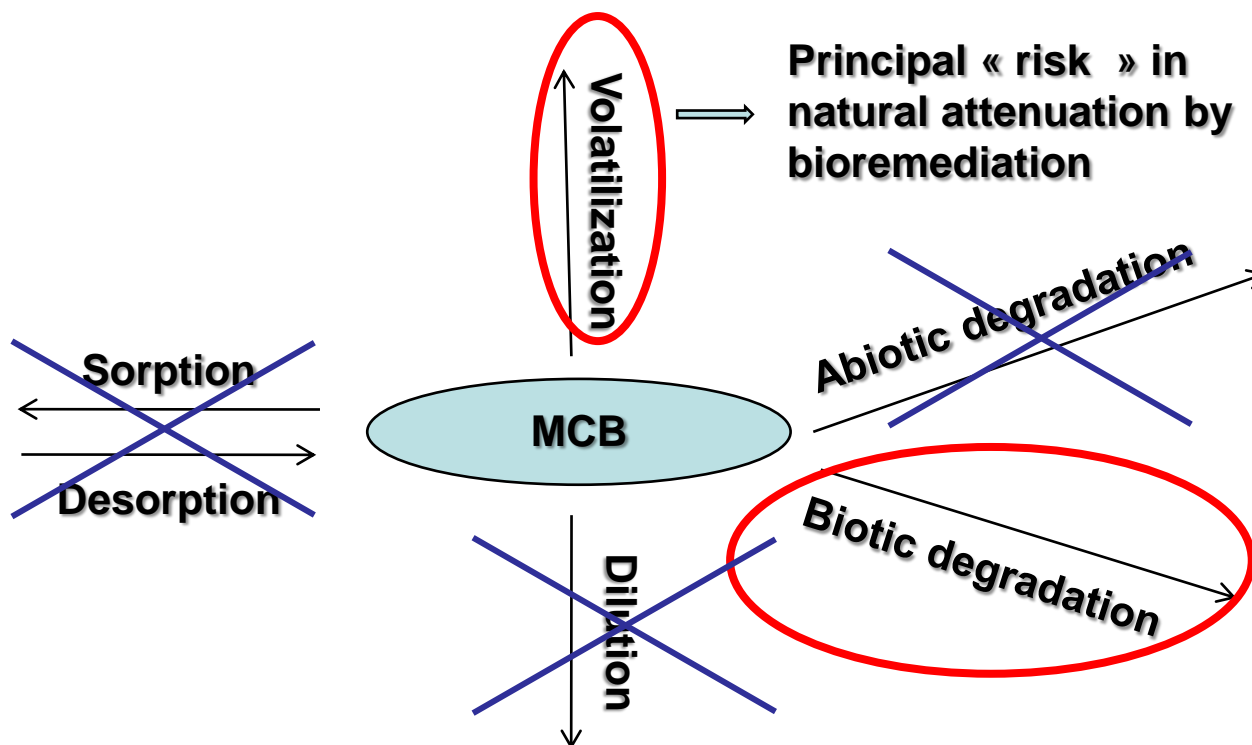
**Biodegradation and Enhanced biodegradation**



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## MCB natural attenuation



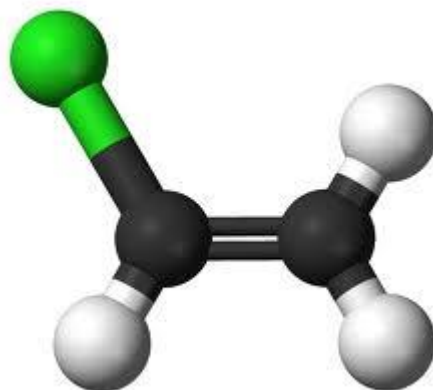
**Careful attention to volatilization risks**  
**Monitoring of the biological activity on-site**



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## Chlorinated solvent: VC

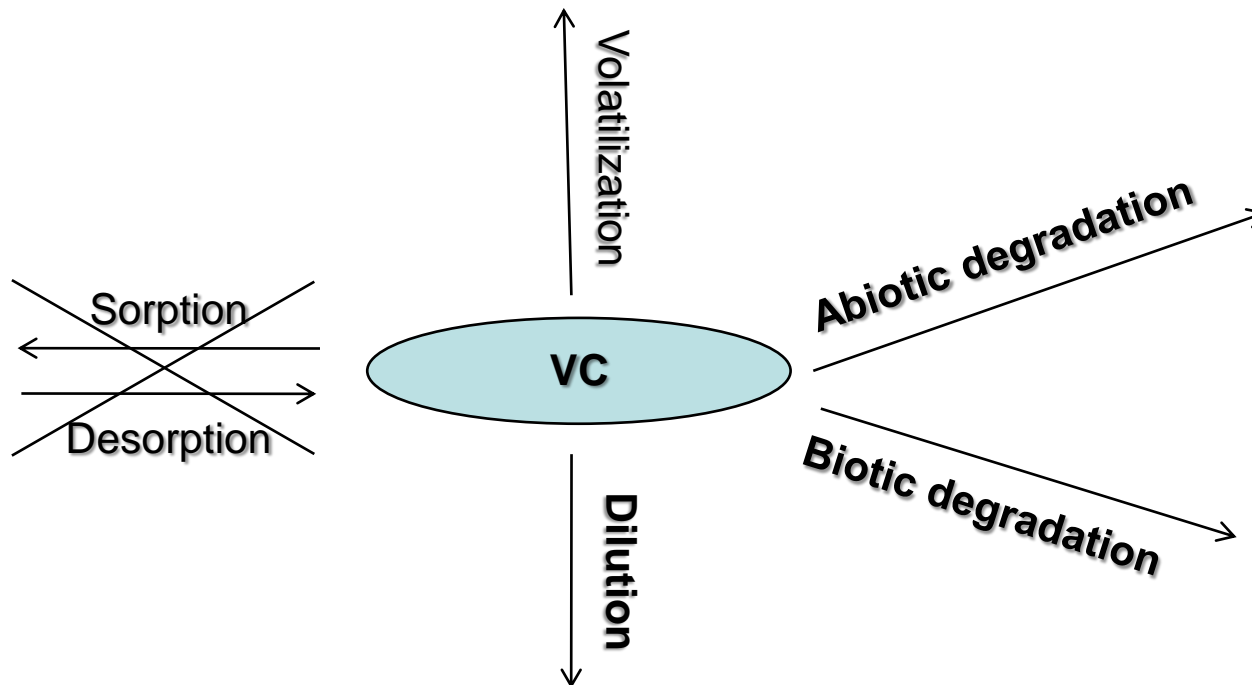




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## Chlorinated solvents

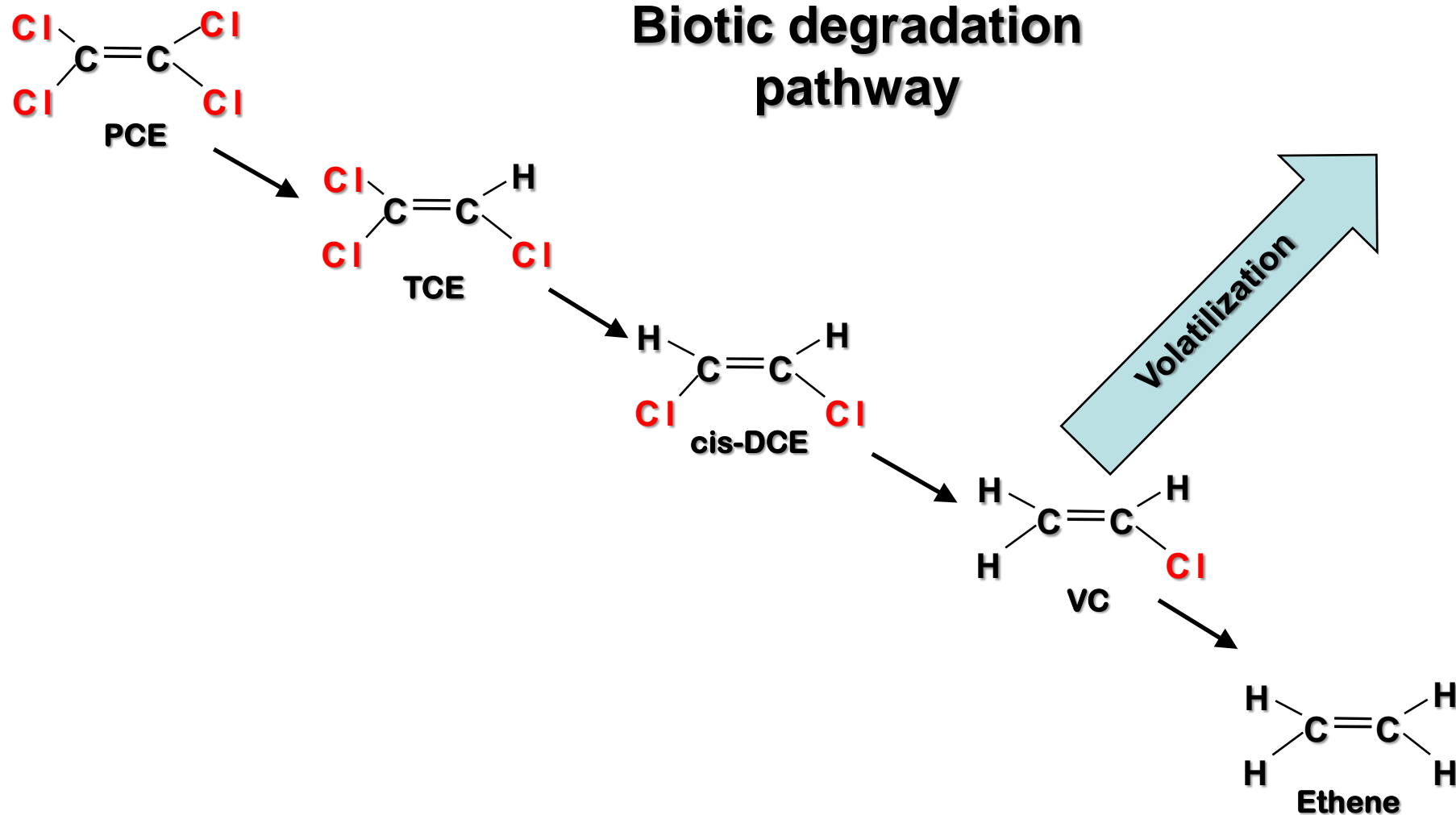




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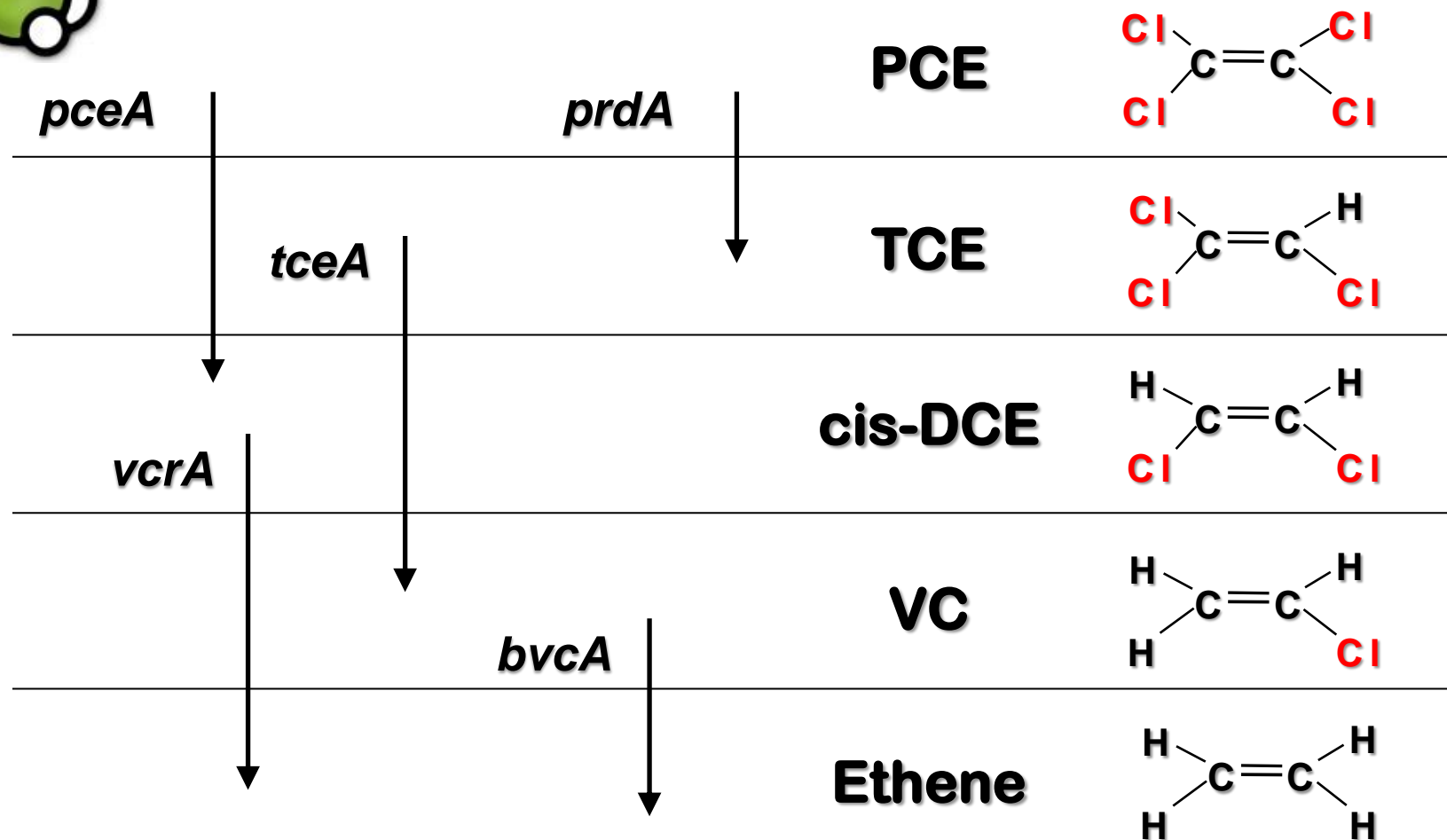
## Biotic degradation pathway





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**Monitoring of the genes**





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## **Site:**

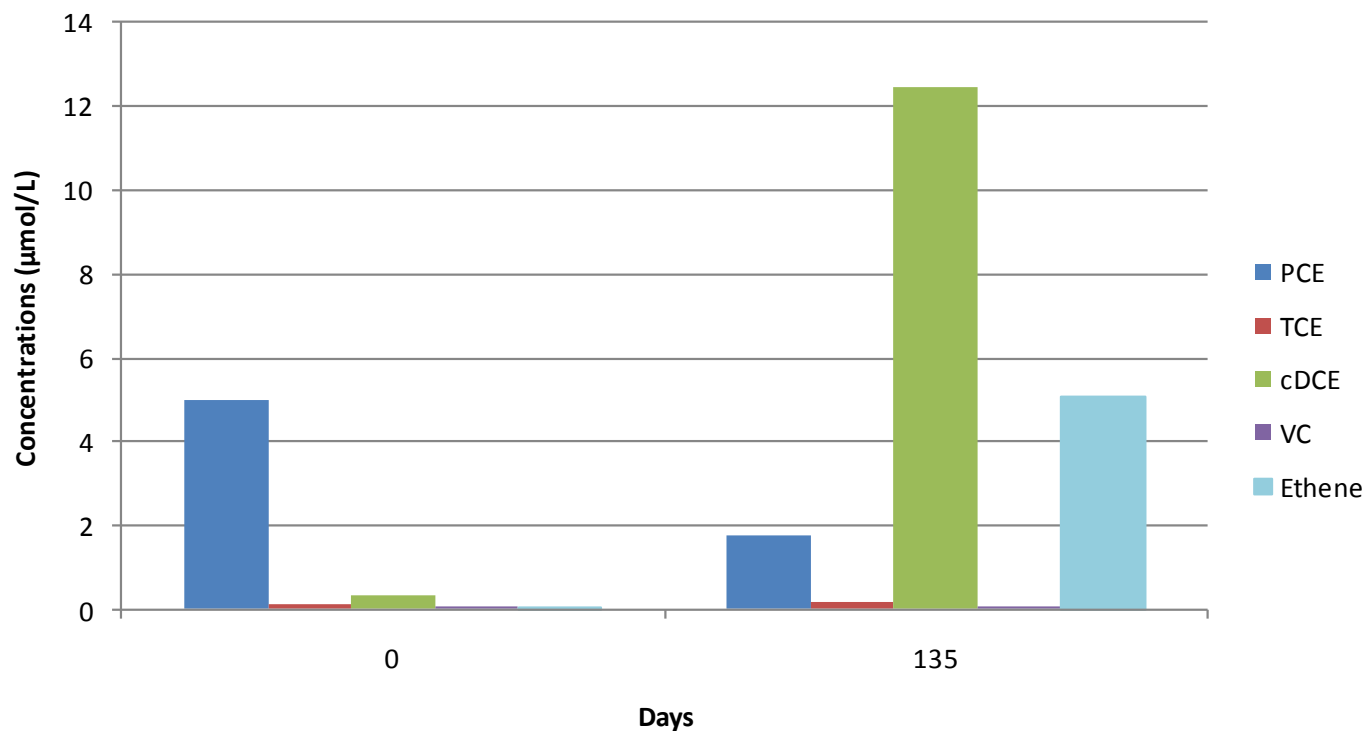
- **PCE contamination**
- **Continuous source of PCE**
- **Injection of HRC on Day 0**
- **Accumulation of cDCE and ethene production**
- **Monitoring:**
  - **Chemistry**
  - **Molecular Biology**



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## Chemical results

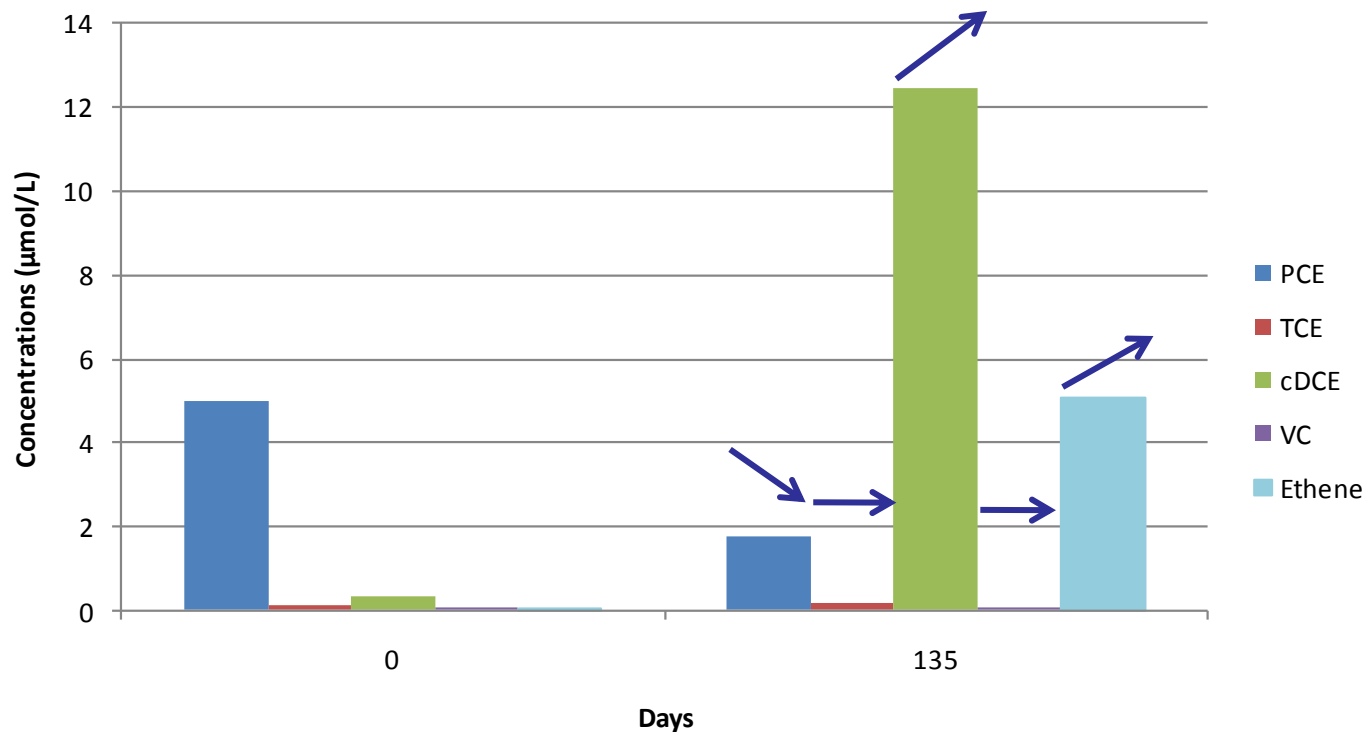




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## Chemical results



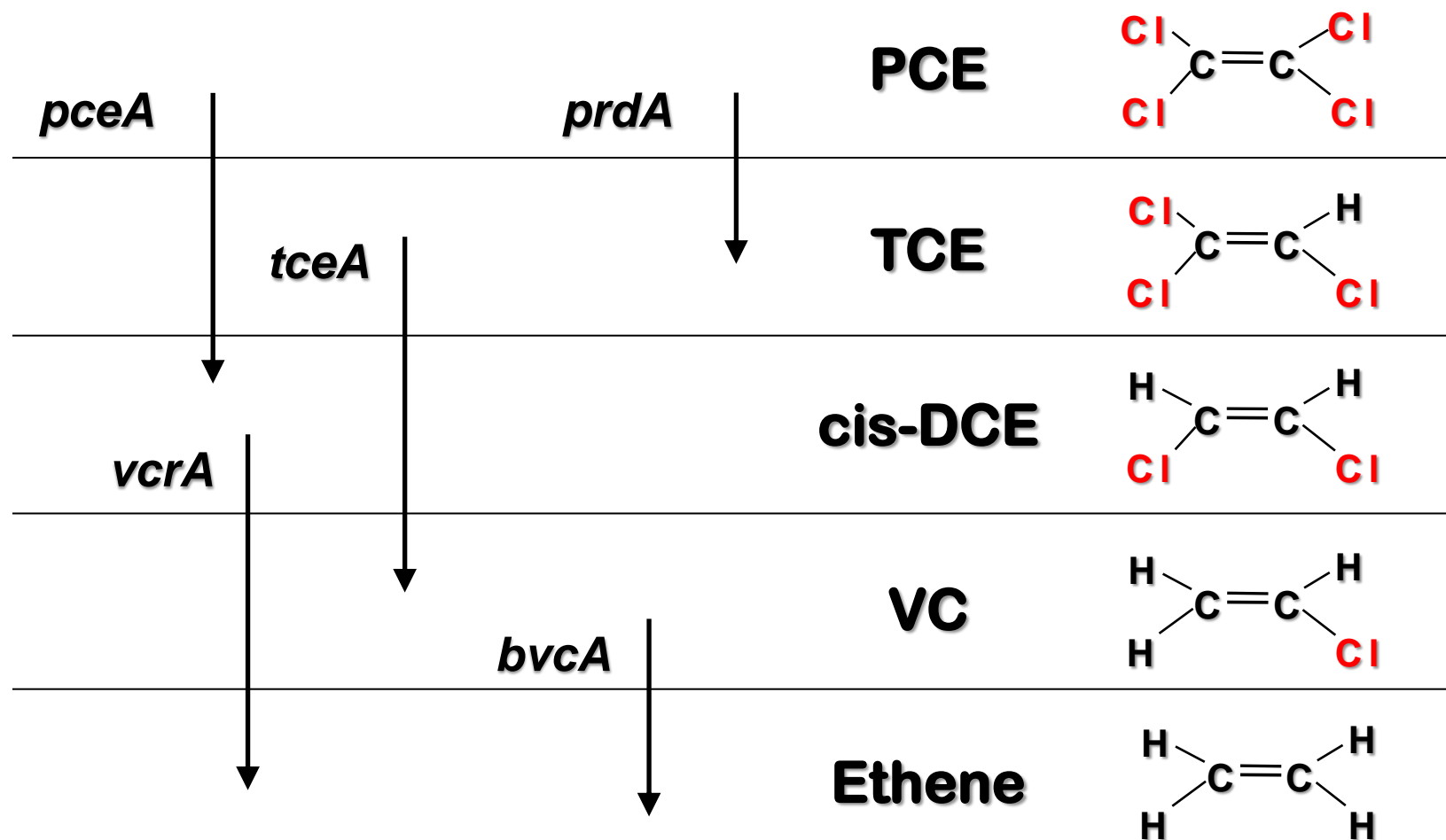
**TCE ?**  
**VC ?**



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## Biodegradation pathway



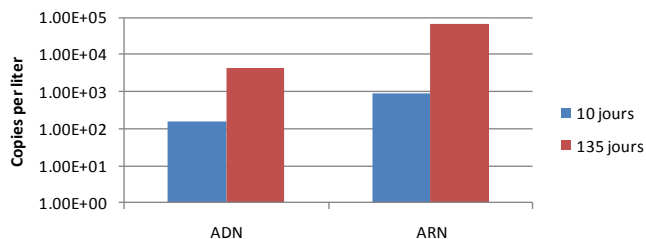


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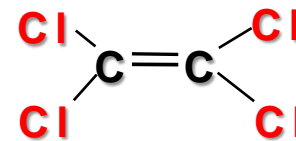
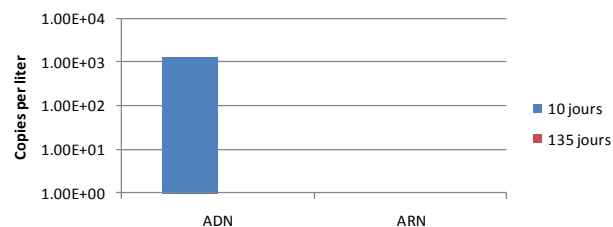
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## Biological results

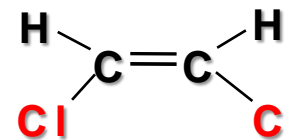
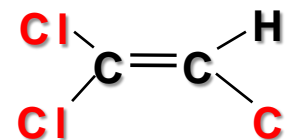
*pceA*



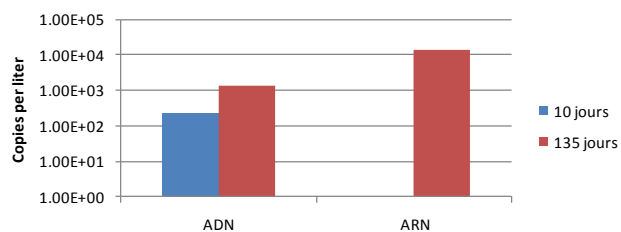
*prdA*



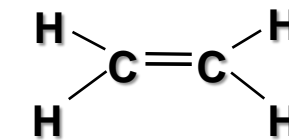
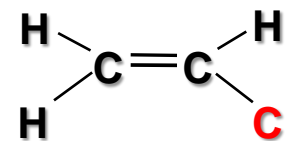
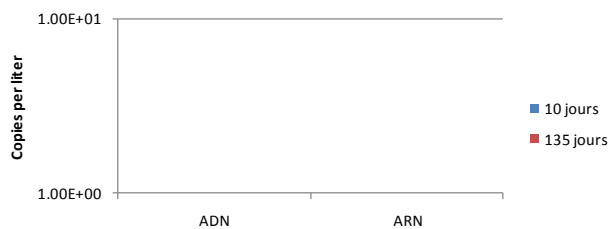
*tceA*



*vcrA*



*bvcA*

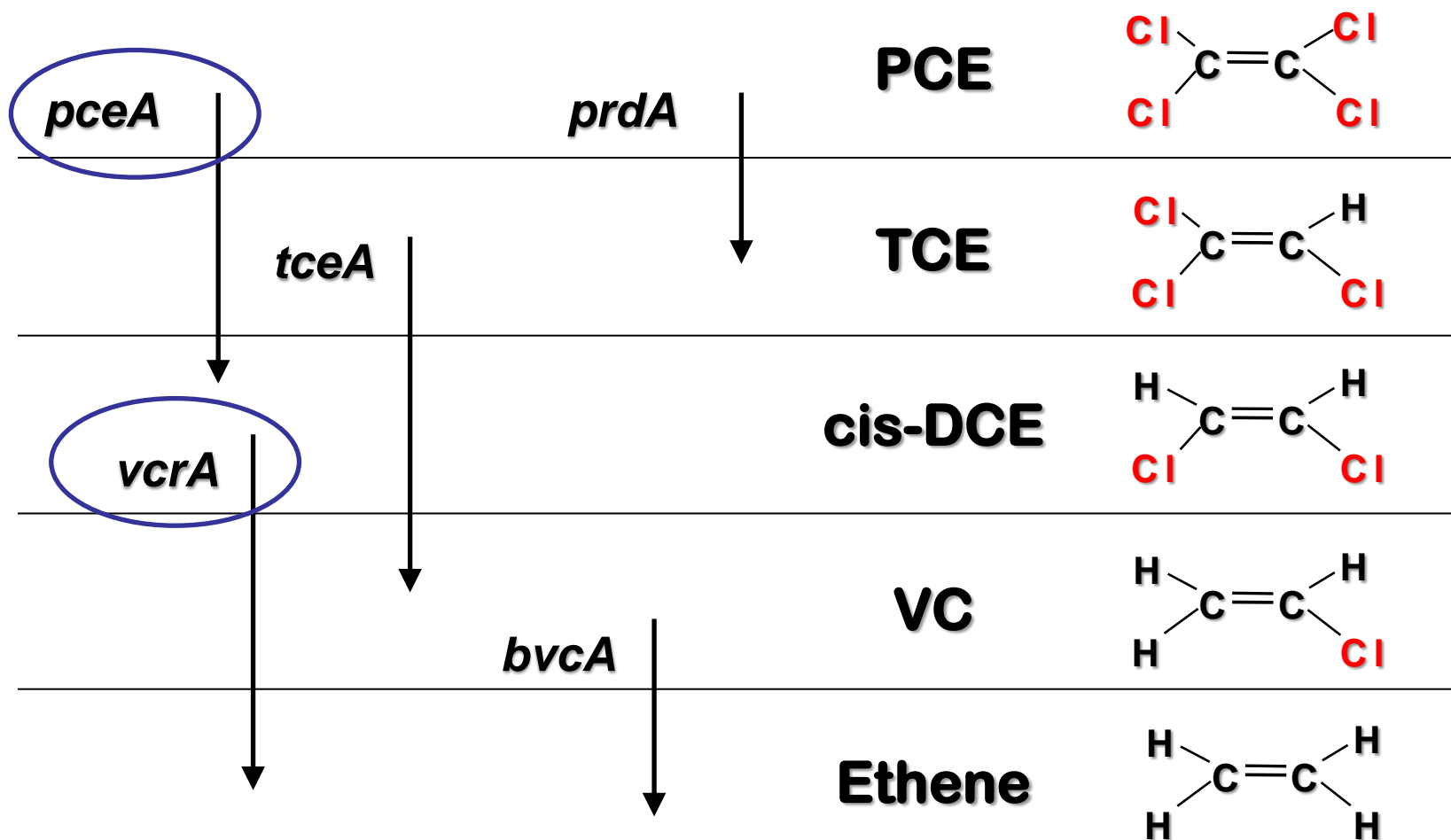




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## Biodegradation pathway



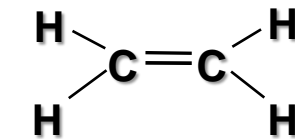
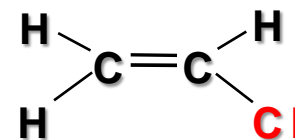
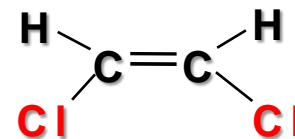
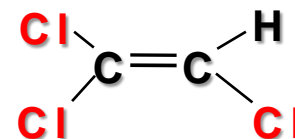
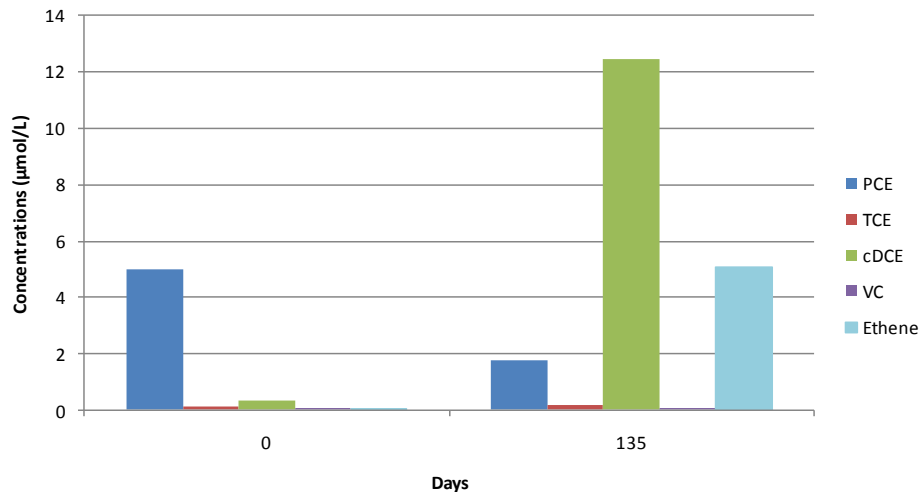
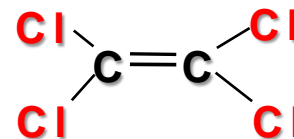
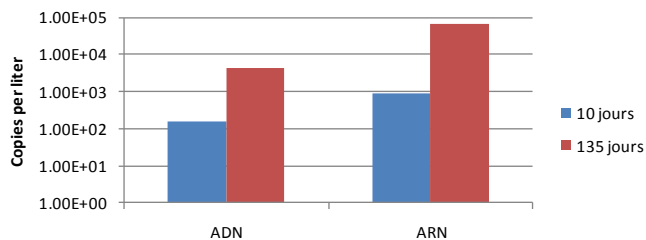


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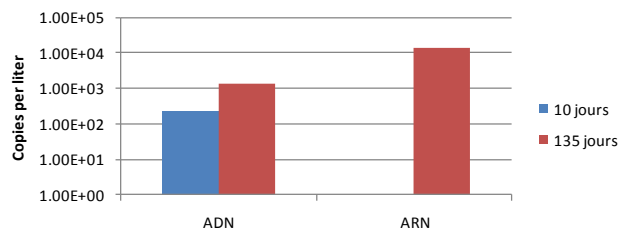
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## Biological results

*pceA*



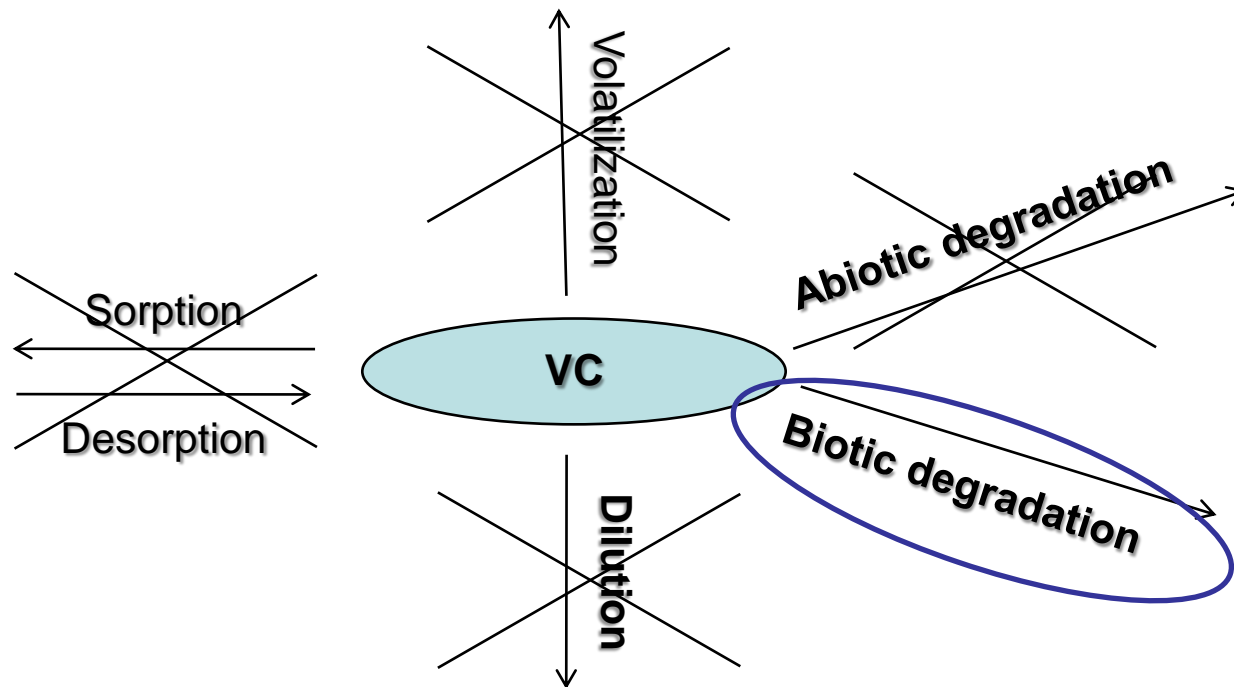
*vcrA*





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- **Realisation of Pilots : Assess the potential for biodegradation**
- **Show the ongoing process of biodegradation and estimation of biodegradation kinetics**
- **Show biodegradation despite other processes occurring**
- **Show past biodegradation – Age dating**



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# Thanks for your attention

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