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Use of microbiology in forensics

Case of a vinyl chloride pollution

*Cédric Malandain
Senior Project Manager*



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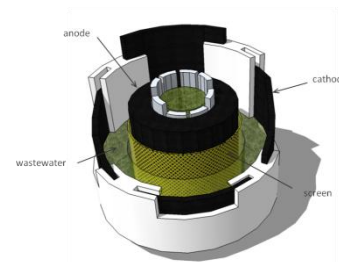
- Company created in May 2008
- 4 partners:
 - *Post-doc, Molecular microbiology*
 - *PhD, Environmental Microbiology*
 - *Chemist*
 - *Professor, Environmental Microbiology and Bio-Engineering*
- 3 employees :
 - *Msc, Project Engineer*
 - *Msc, Sales Engineer*
 - *PhD, Senior Project Manager*
- Development and application of innovative technologies for diverse environmental problems.
- 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





- **Forensics:**
 - Relating to the use of science or technology in the investigation and establishment of facts or evidence in a court of law.
- **Environmental forensics:**
 - scientific methodology developed for identifying potentially hazardous environmental contaminants and for determining their sources and time of release.
- **Microbial forensics:**
 - Means by which a microbial signature is used to trace a contaminant source, similar to the use of DNA in criminal forensic (Budowle *et al*, 2003).



Microbiology applied to forensics (classical uses)

- Tracking of the source of fecal contamination:
 - Human/animal origins
 - Bacterial species present
 - Ratio between different specific populations (ex: fecal coliforms/fecal streptococque)
 - Phenotypic analysis
 - Immunology
 - Pattern of antibiotic resistance
- Toxicology:
 - Basal respiration (metabolic ratio qCO_2) in response to toxic compounds
 - CLPP analysis (community level physiological profiling): profiling using biolog plates. Profiles are altered in response to heavy metal toxicity
- Isotopic fractionation $^{13}C/^{12}C$ and $^{37}Cl/^{35}Cl$:
 - Show biodegradation
 - Show volatilization

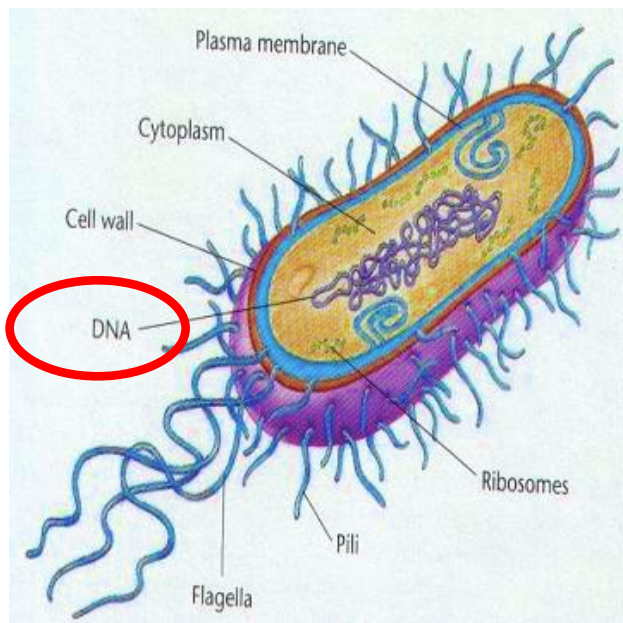


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New tools for forensics: innovative tools of molecular biology

- Community profiling
- Search for specific genes and analyses of their activity



DNA: Genetic material responsible for
bacterial degradation capabilities

—————→ Codes for enzymes

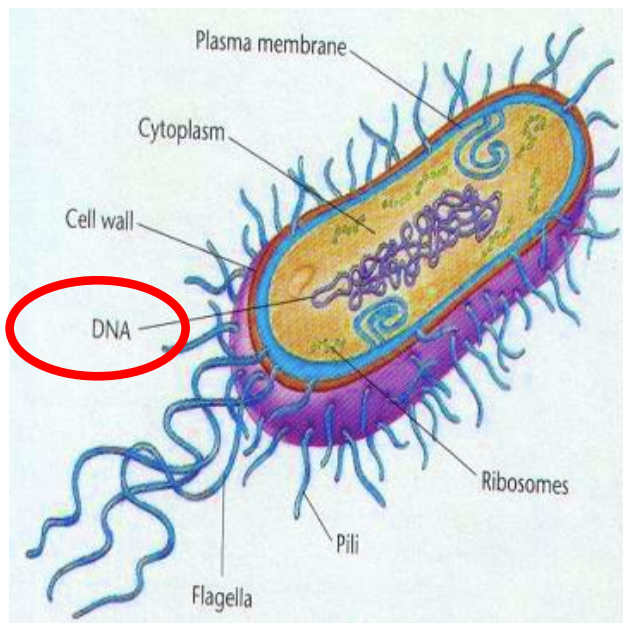


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New tools for forensics: innovative tools of molecular biology

- Community profiling
- **Search for specific genes and analyses of their activity**



DNA: Genetic material responsible for
bacterial degradation capabilities

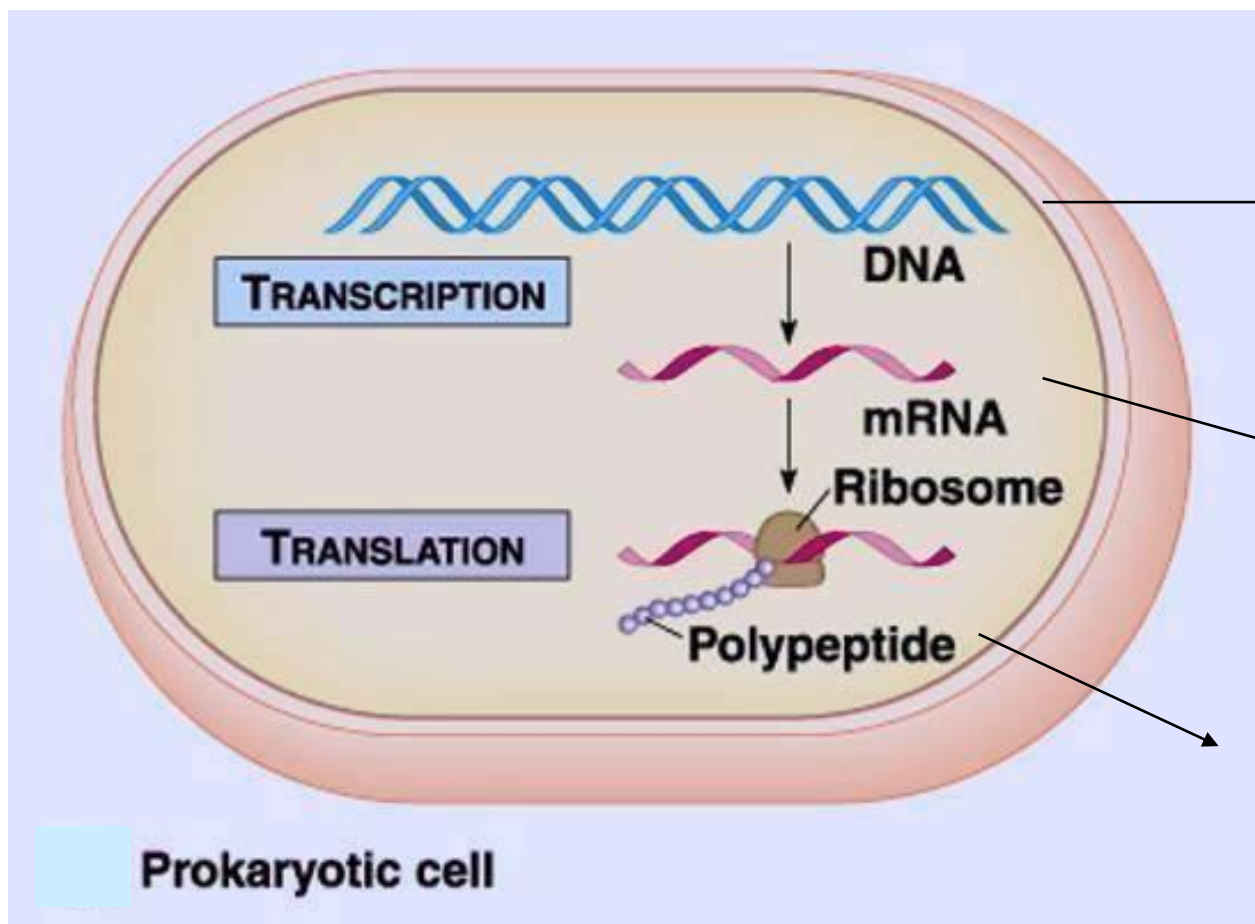
—————→ Codes for enzymes



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Process



DNA : long half-life in soil

RNA : short half-life in the soil

Enzymes responsible for pollutant degradation



- 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: degradation
+	-	→ Genes are present but not active: no degradation but potential
-	-	→ Genes are not present: no degradation

Improve our understanding of site microbiology

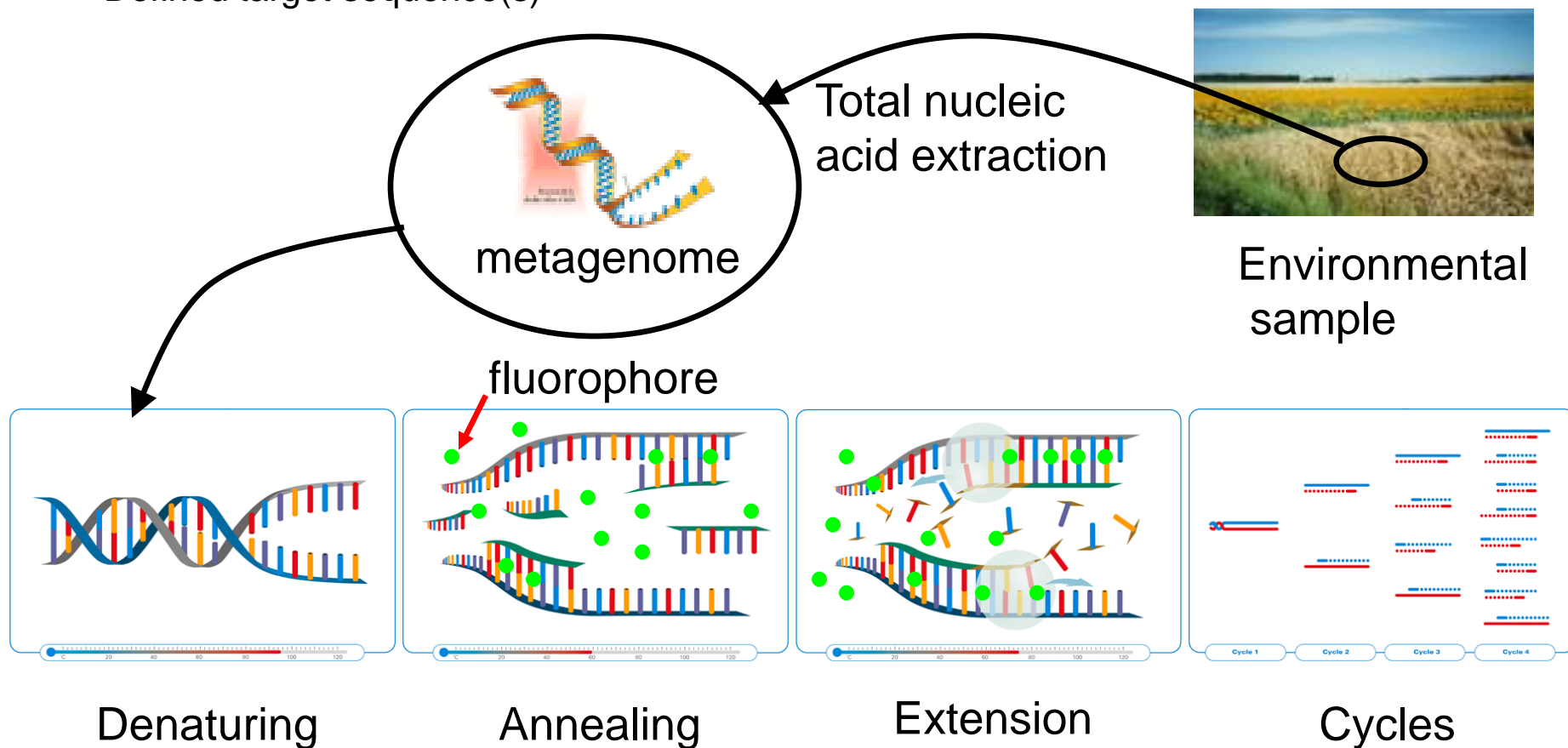


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DNA/RNA quantification

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



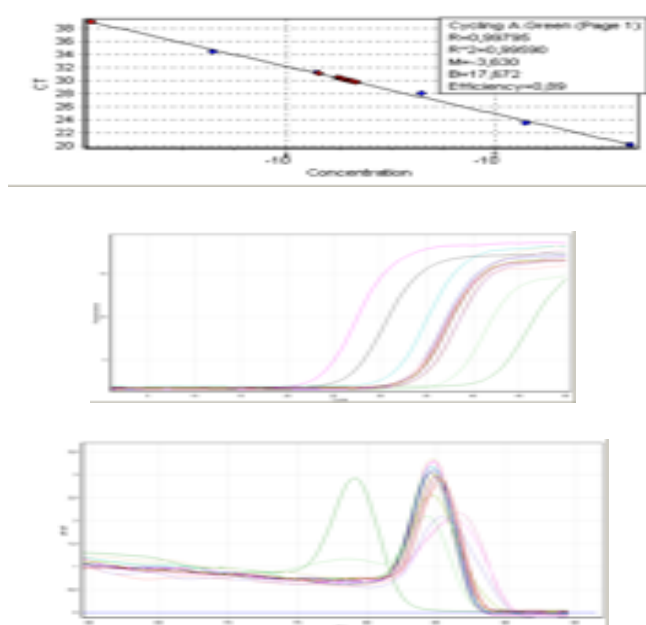


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qPCR
thermocycler



Statistical
analyses
Reaction
specificity

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



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Environmental forensics

Source ?



Pollutant



Description of the site:

- Spills of TCA by the former owner in the 50's
- Chlorinated solvents are still in use but no accident known
- Cross-contamination by several chlorinated solvents
 - PCE contamination
 - TCA contamination
 - VC contamination
 - Anaerobic environment

Origin of the VC :

=> **Present pollution or past pollution?**

➡ **Determine whether biodegradation mechanism could be responsible for the VC contamination**

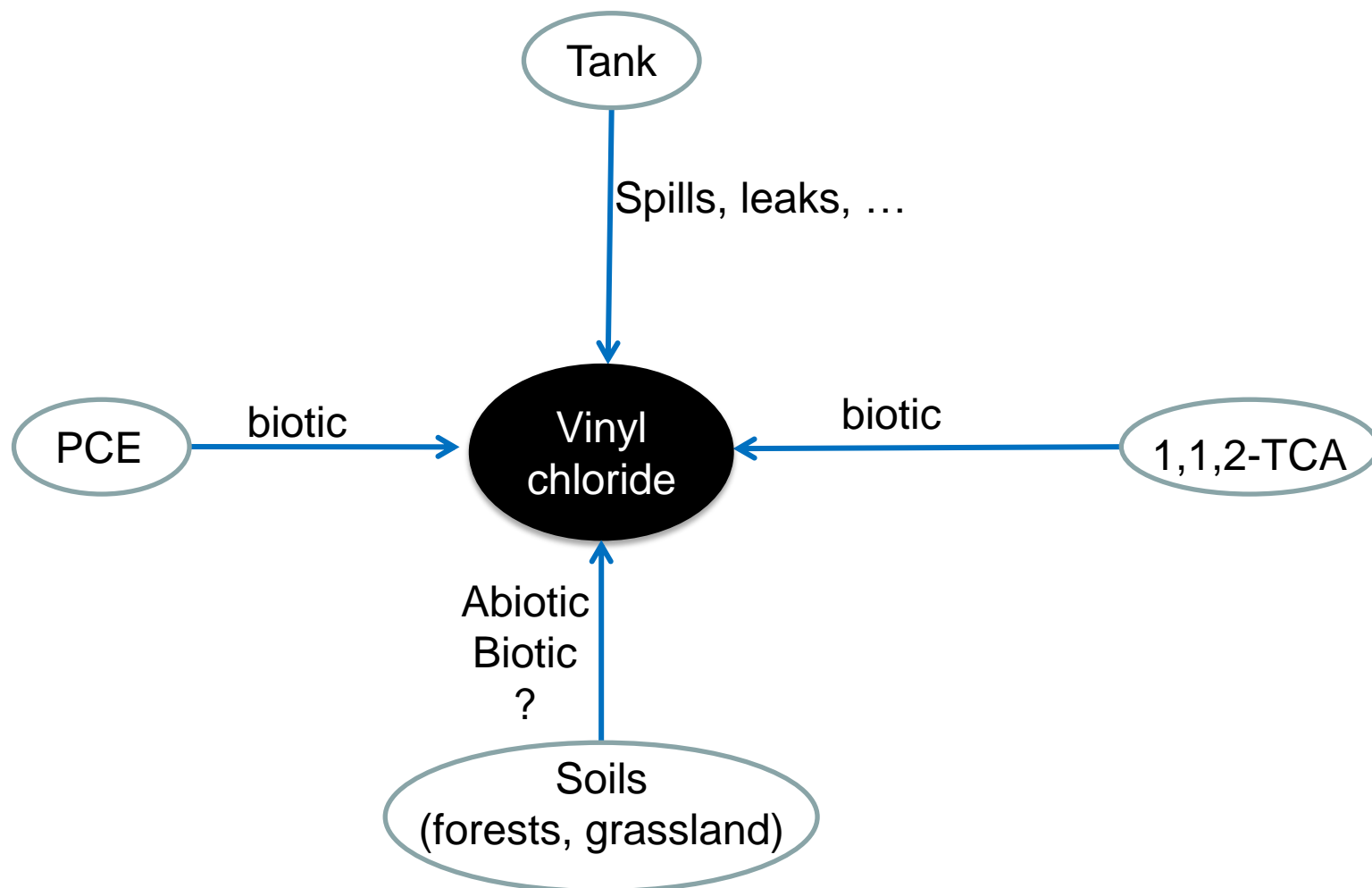
➡ Actual owner isn't responsible of the VC contamination



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Possible origins of VC in the environnement

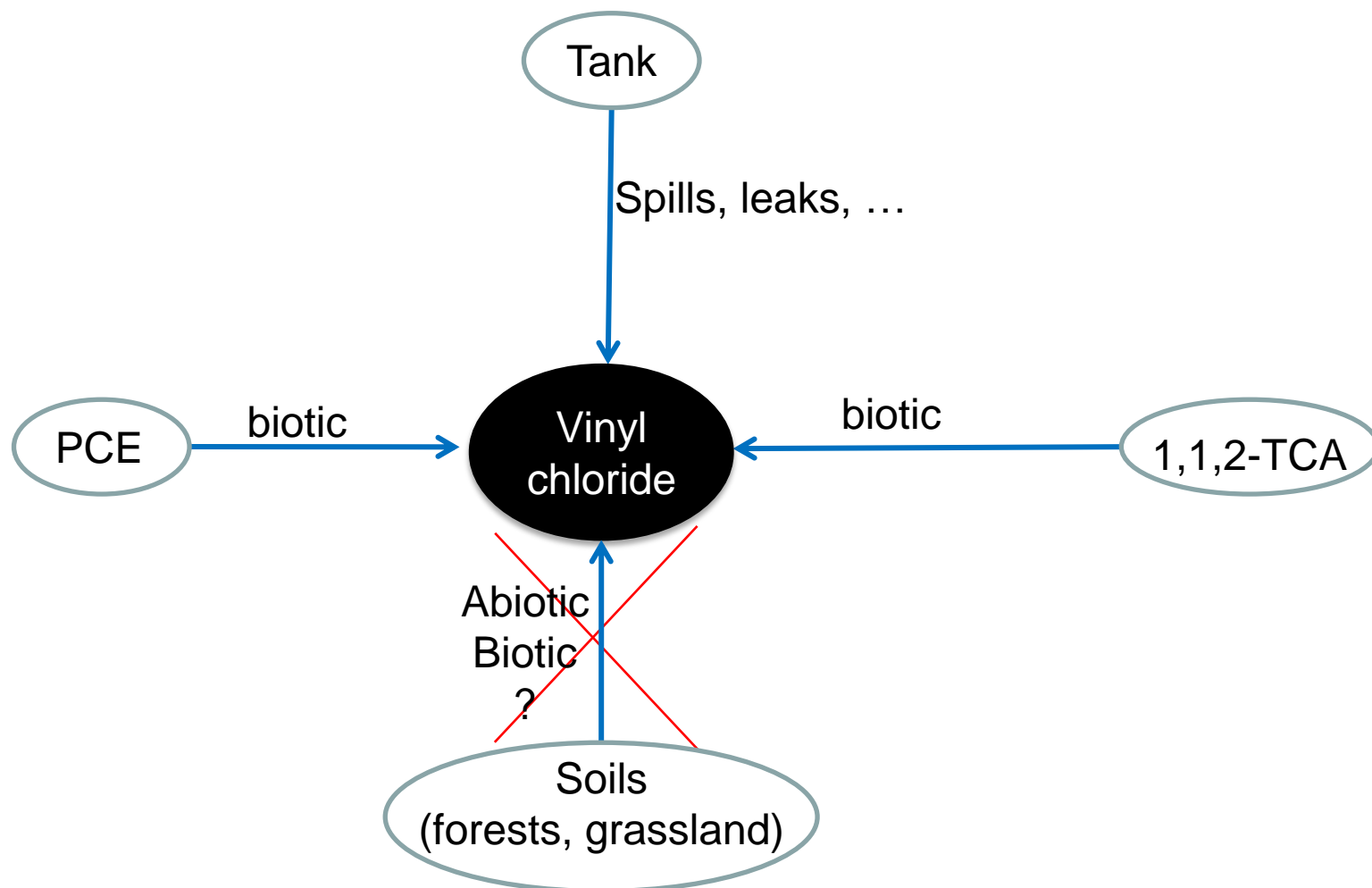




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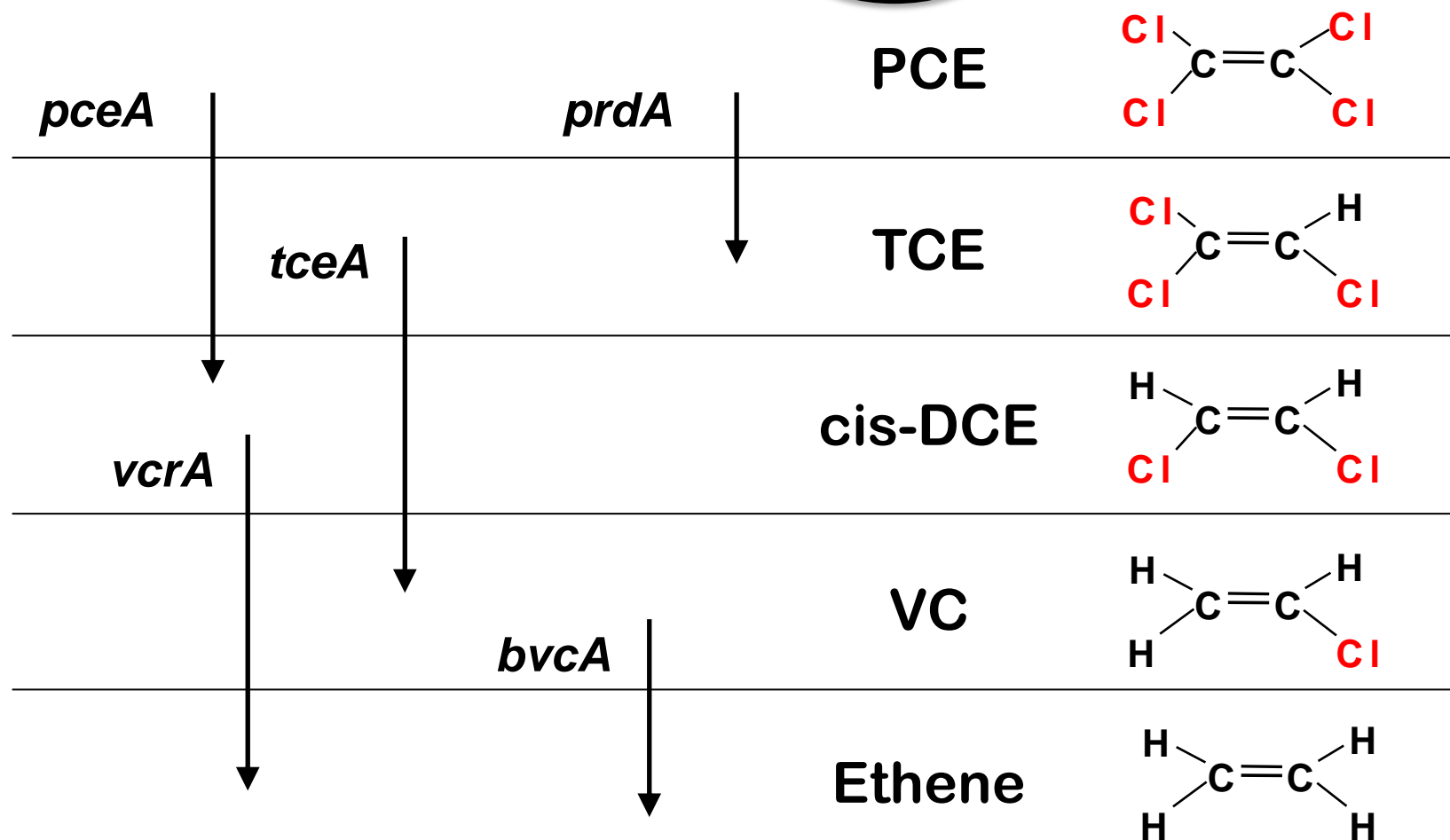
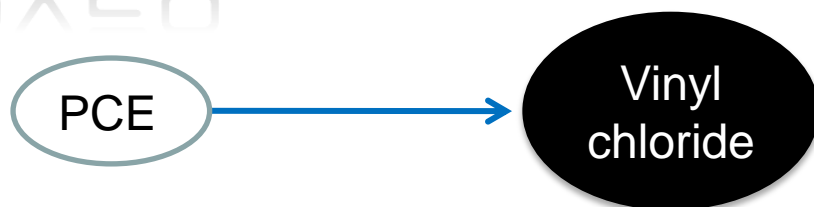
Possible origins of VC in the environnement





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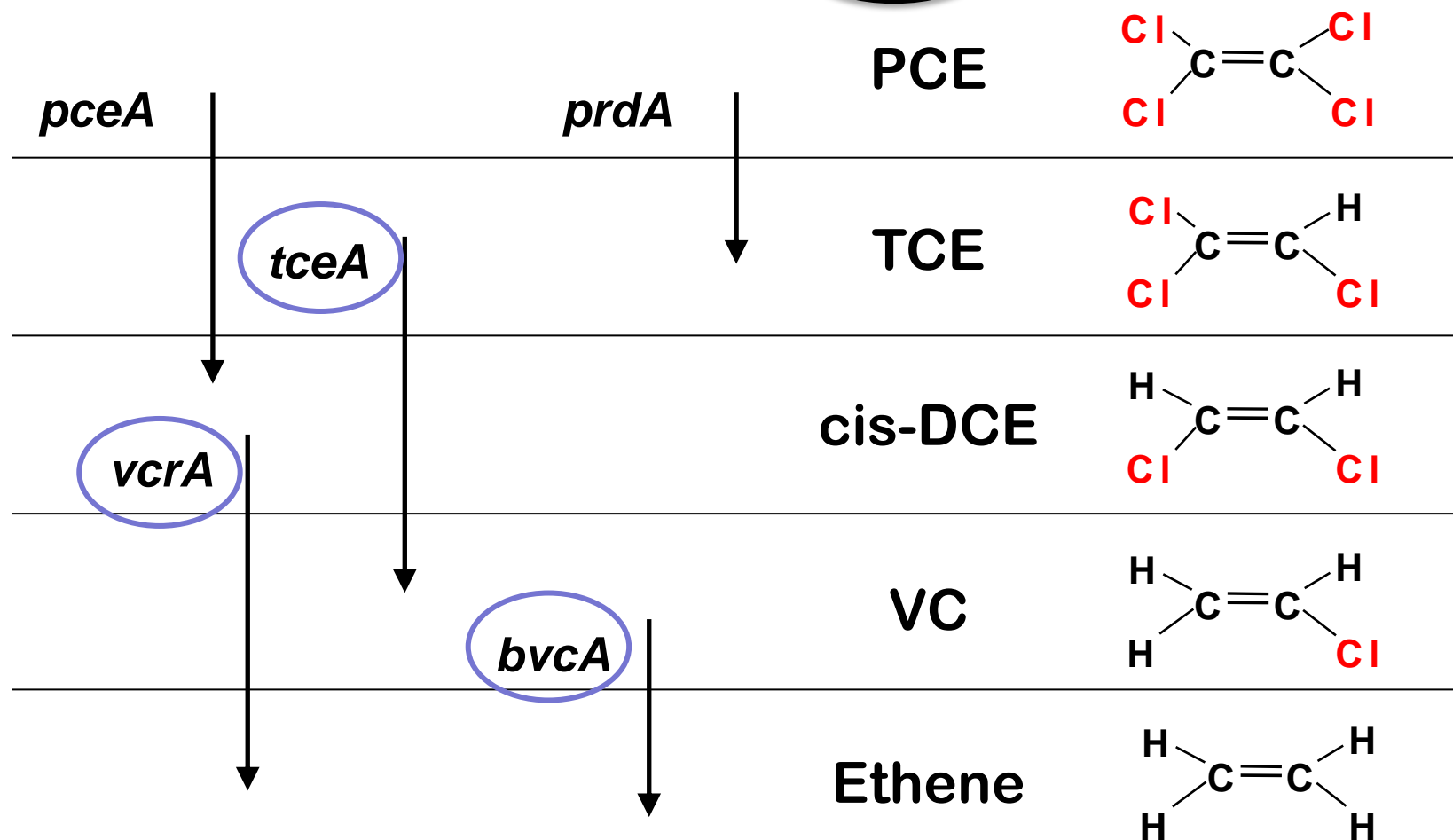
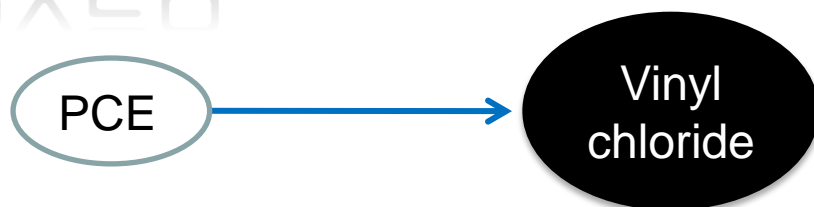
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On site analysis

Genes specific to
PCE degradation
are missing

<u>Gène recherché et analysé</u>	Puits 1	
	ADN (copies/L d'eau)	ARN (copies/L d'eau)
bvcA	n.d.	n.d.
vcrA	l.q.	l.q.
tceA	n.d.	n.d.

n.d.: non détecté

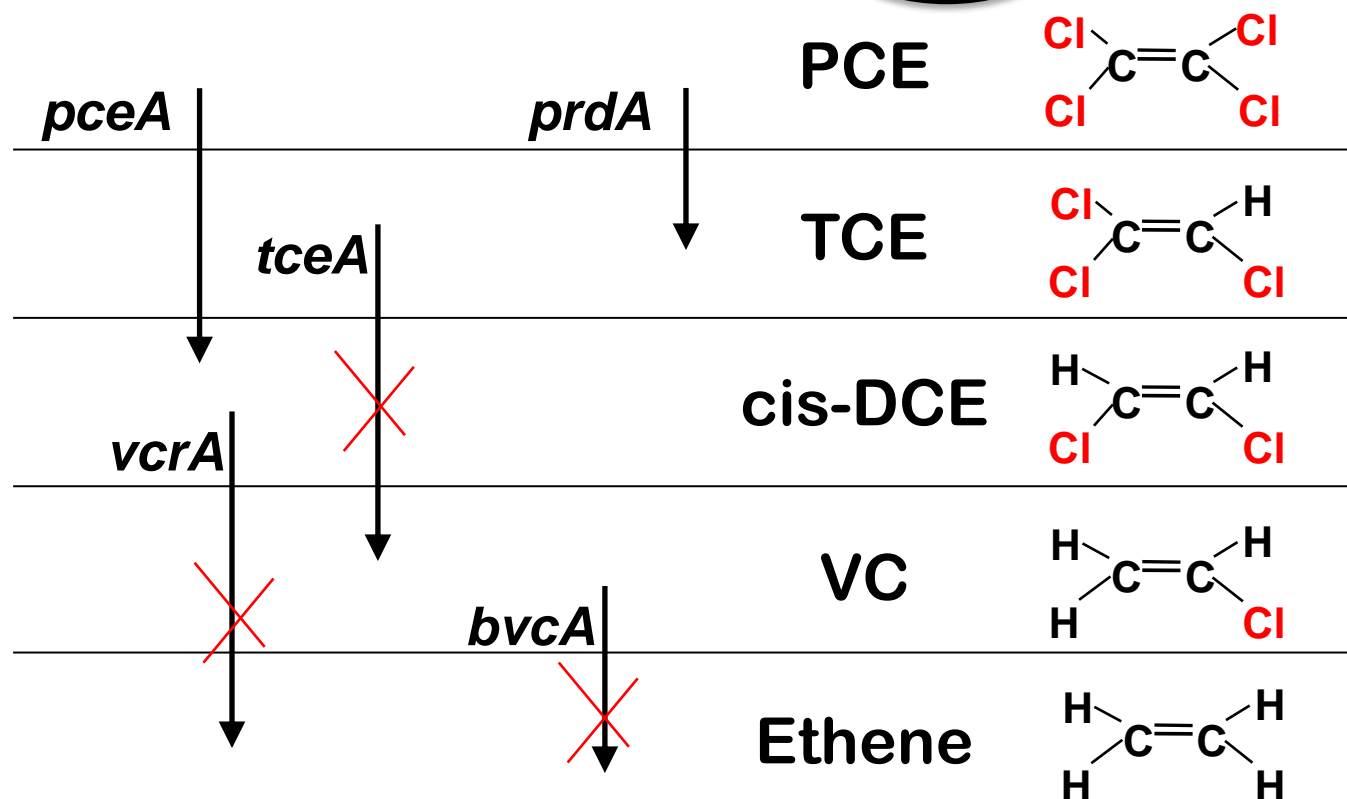
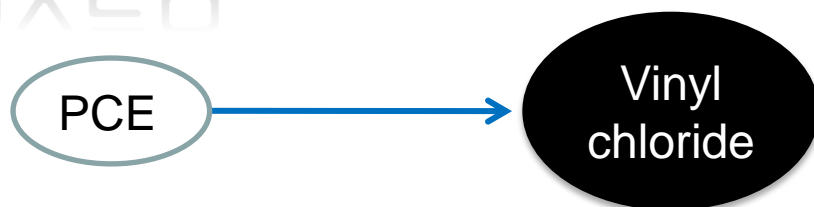
l.q.: sous la limite de quantification de 500 copies/L

➡ **Confirmed by the absence of metabolites (TCE and cisDCE)**



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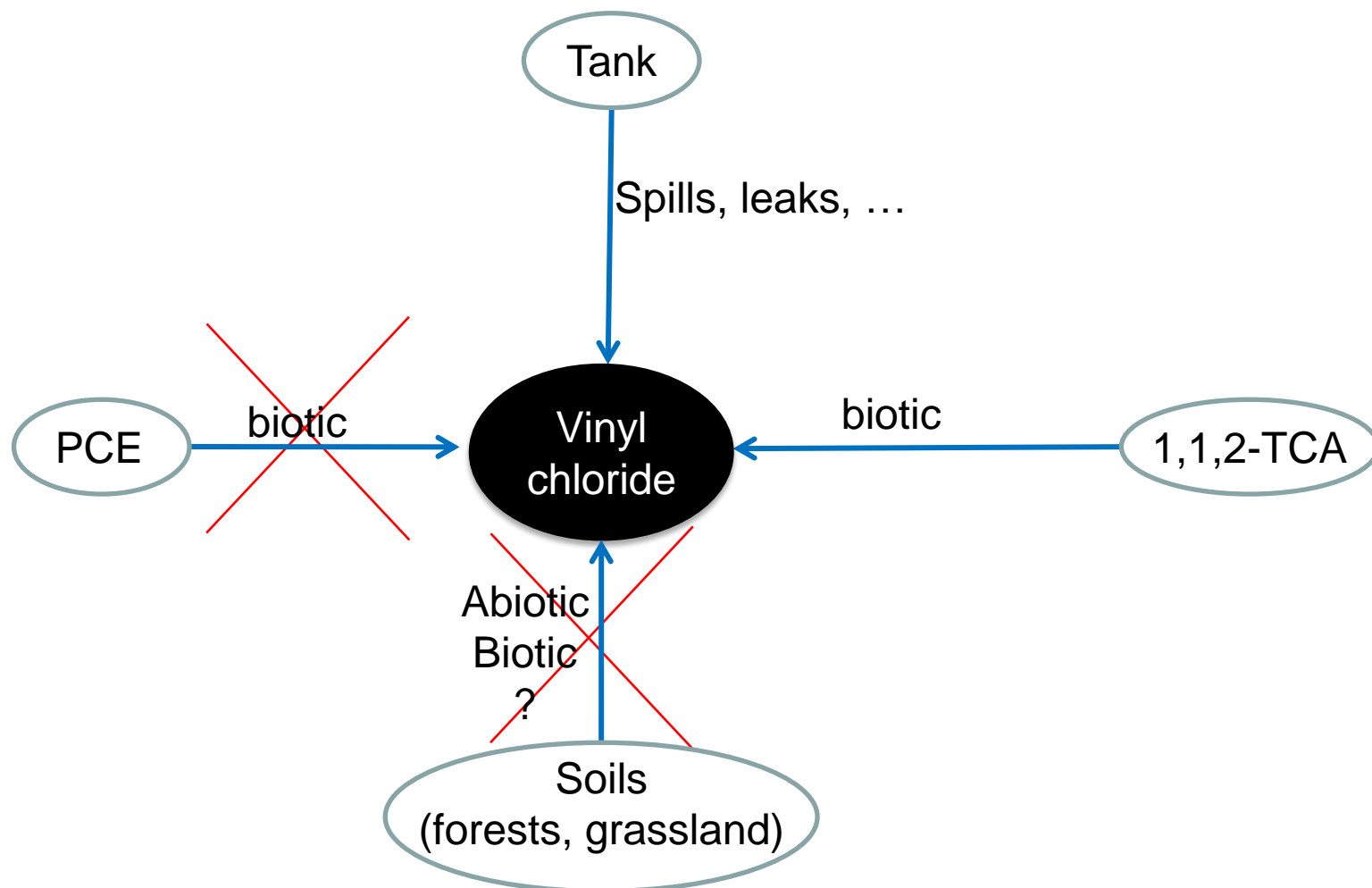
PCE degradation pathway is inactive



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Possible origins of VC in the environnement

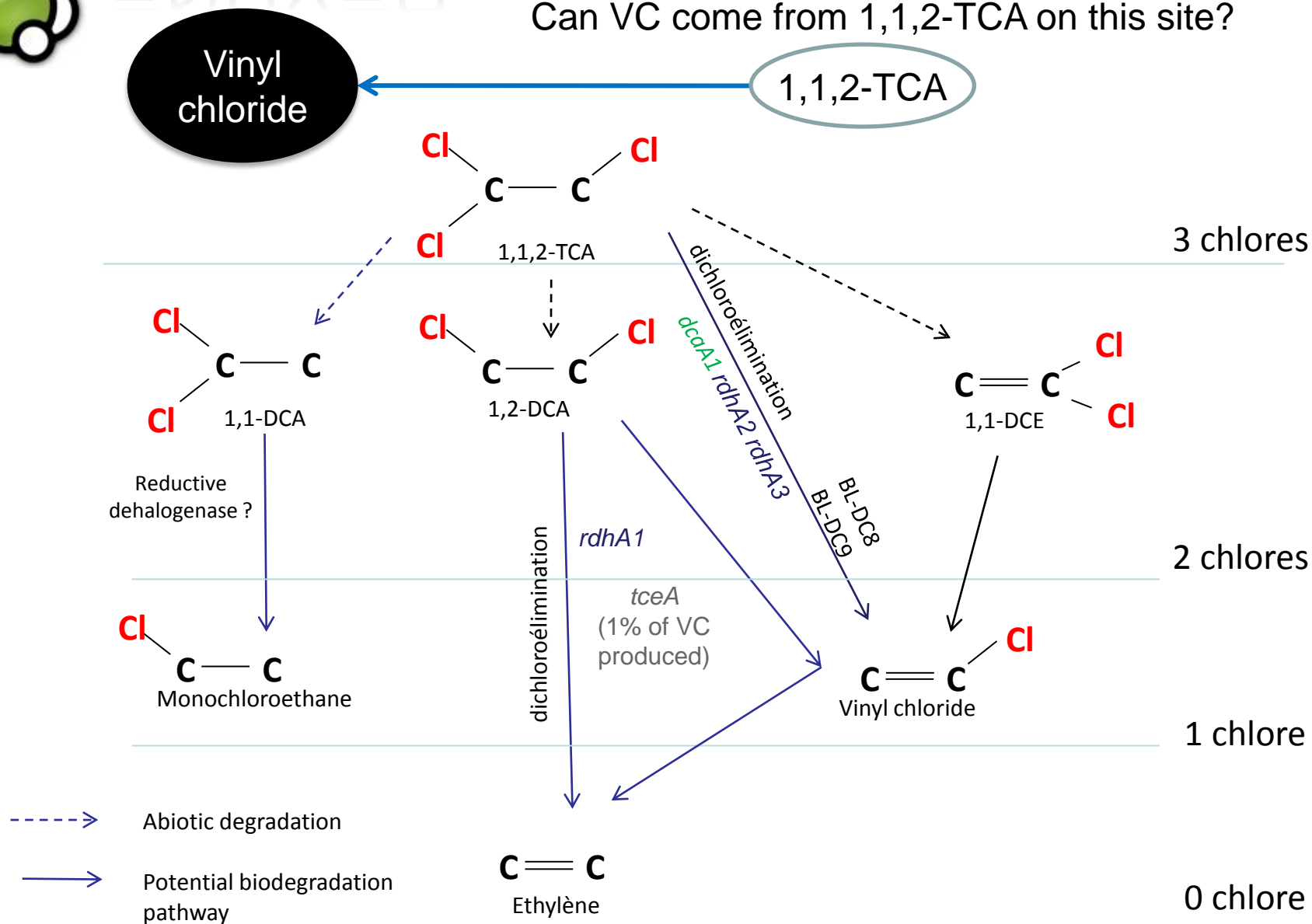




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Can VC come from 1,1,2-TCA on this site?





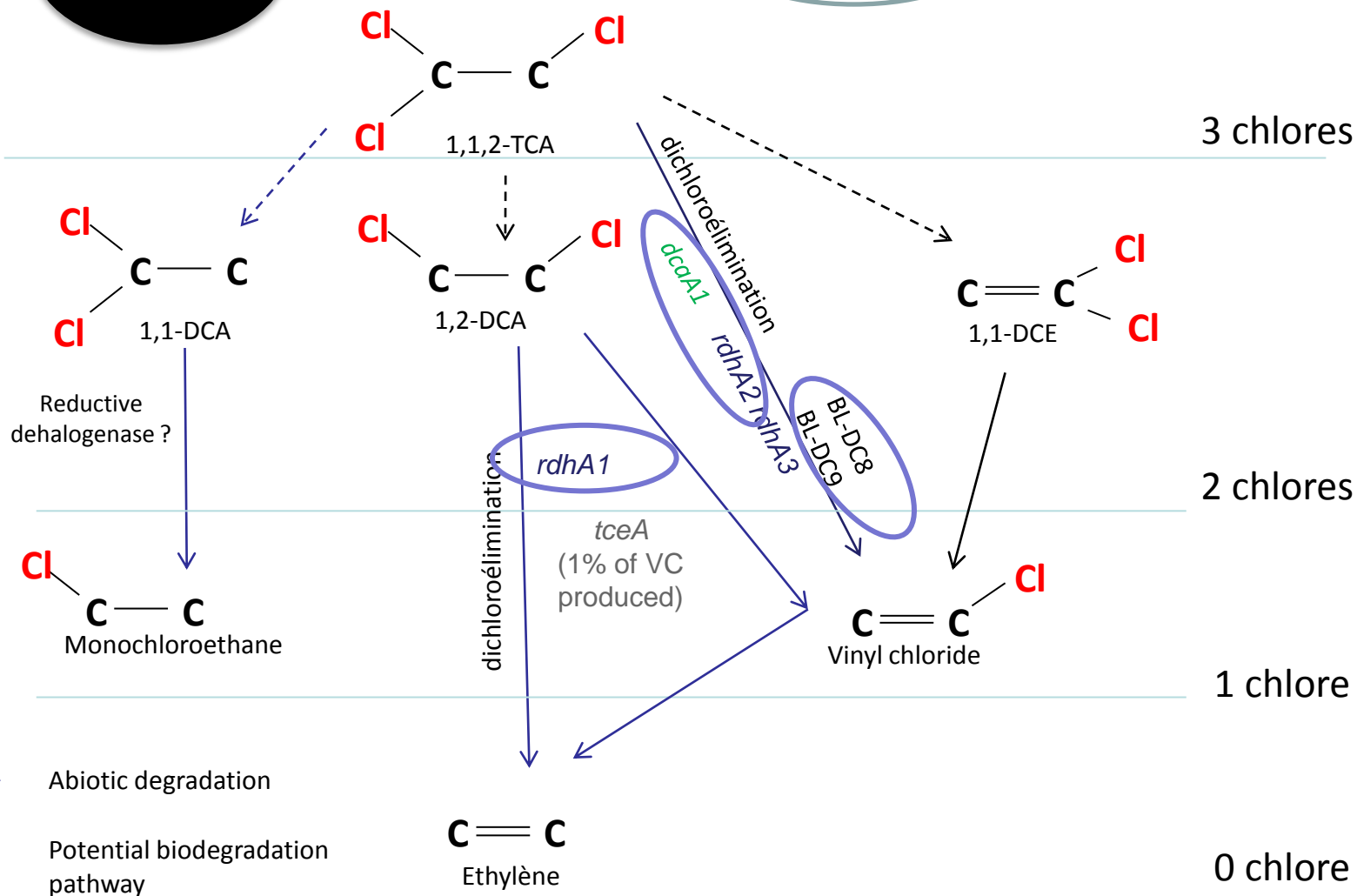
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Can VC come from 1,1,2-TCA on this site?

Vinyl chloride

1,1,2-TCA





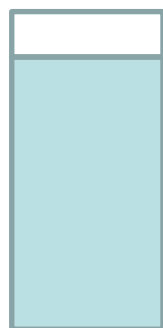
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How to show the biological origin of the VC production?

- Show biological activity
- Show metabolites or final product apparition/accumulation

- 3 microcosms
- 3-month incubation



Aerated

= inhibition of anaerobic
biological reaction



Control



Acetate

= enhancement of
the biological processes

- **Monitoring and maintenance of biological activity**
- **Monitoring of chemical products**



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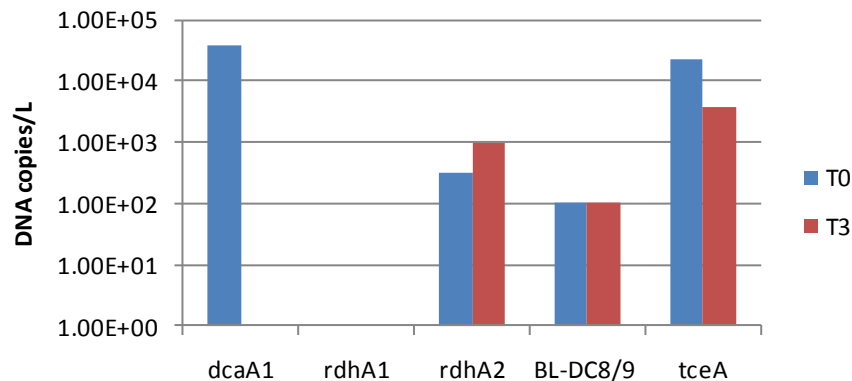
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Aerated microcosm

DNA quantification



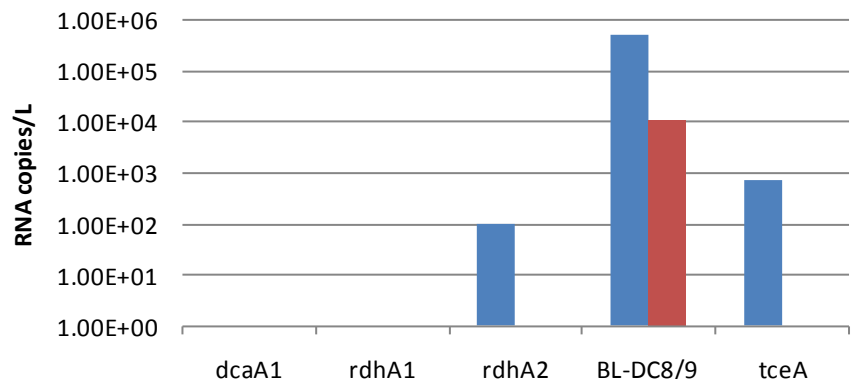
Genes are present



RNA quantification



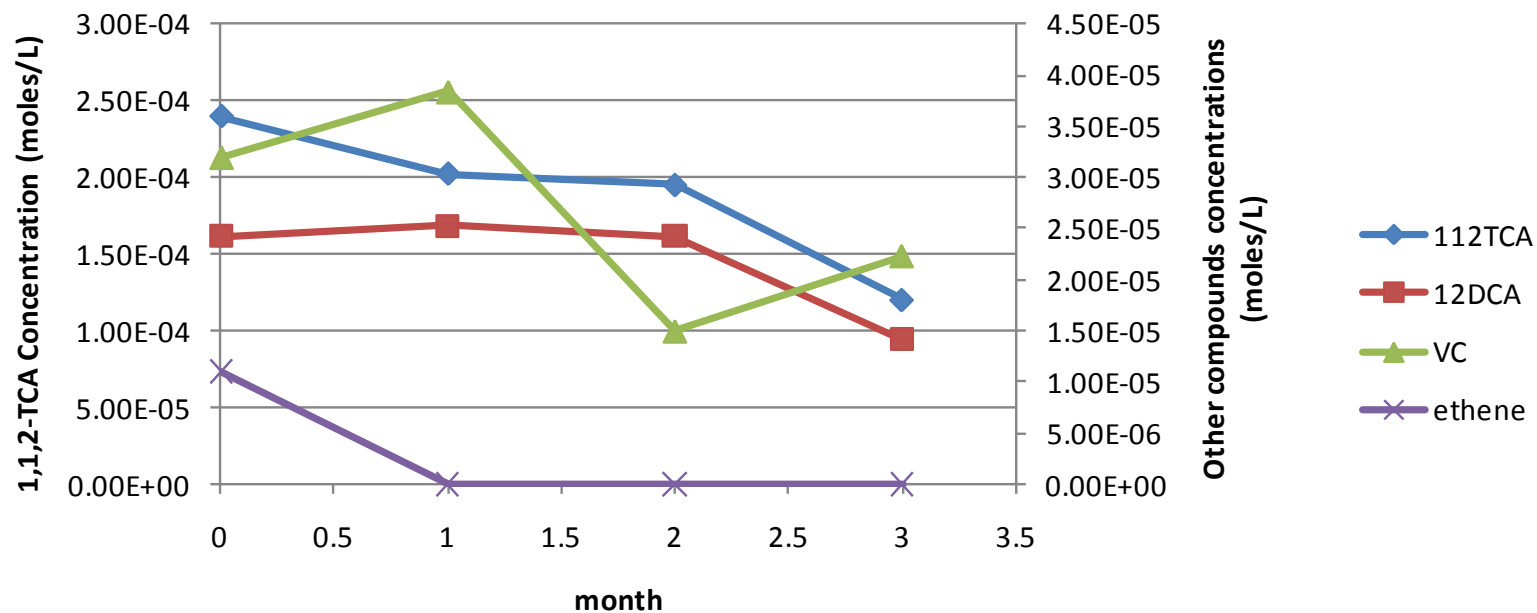
Genes are not active



No gene activity



Aerated microcosm



No VC production



No genes activity
No VC production



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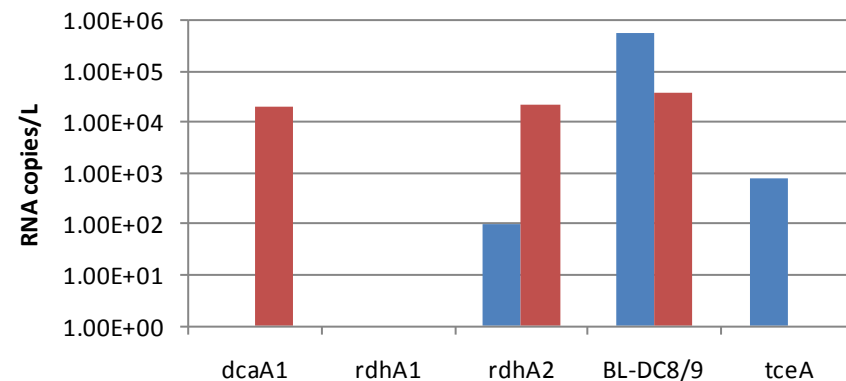
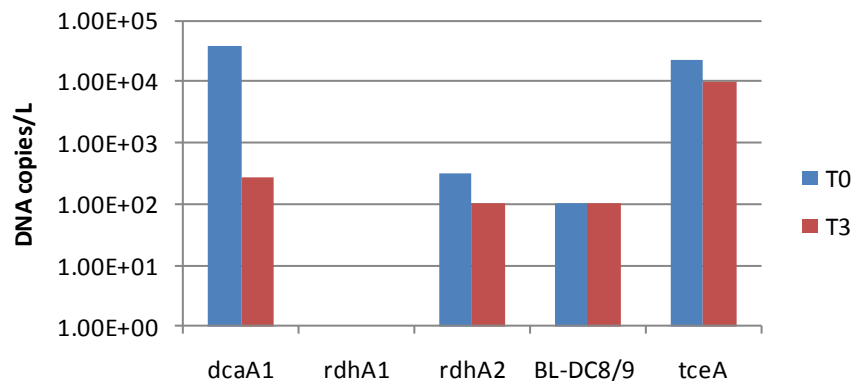
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Control and acetate microcosms

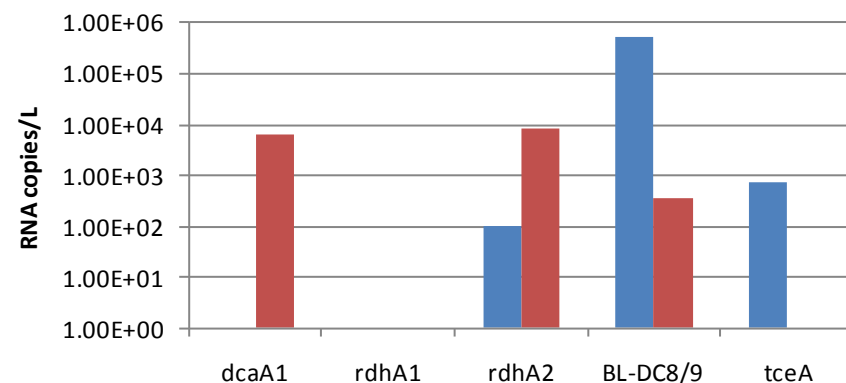
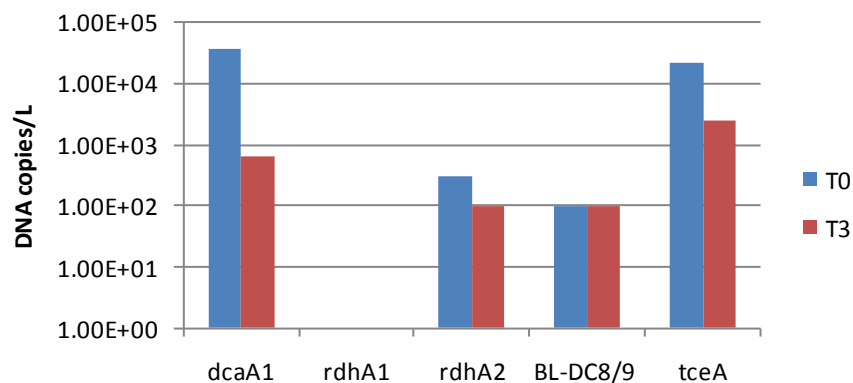
DNA quantification

RNA quantification

control



acetate



⇒ Genes are present

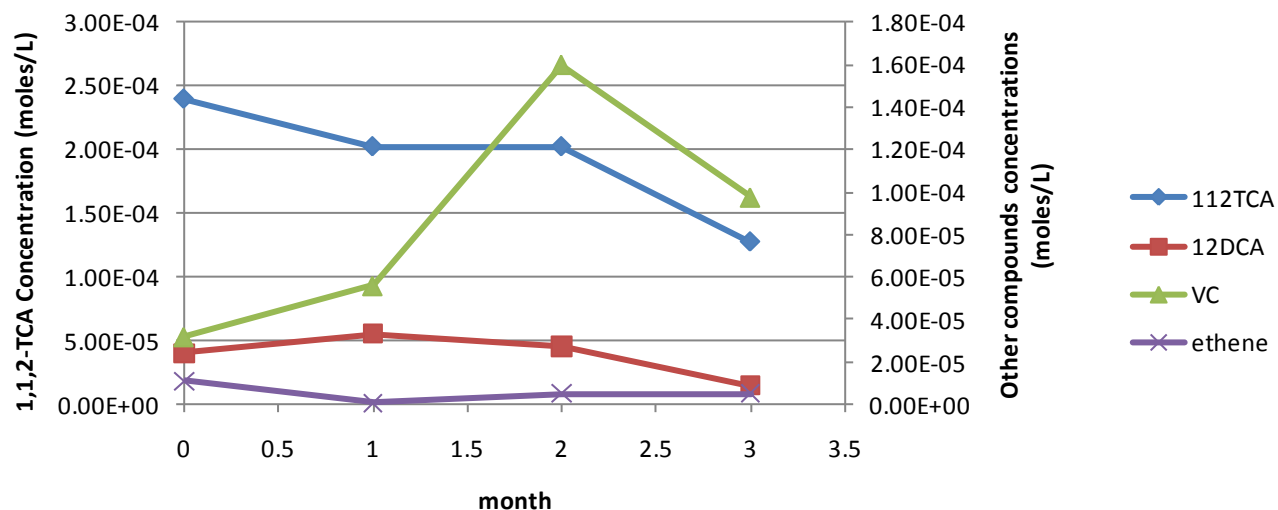
⇒ Genes are active



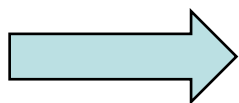
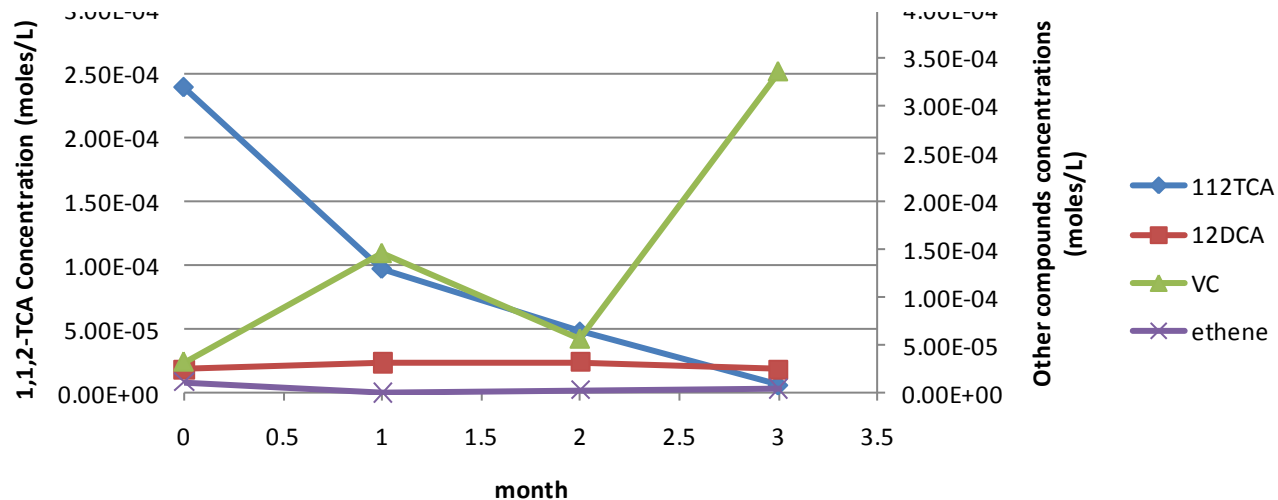
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Control

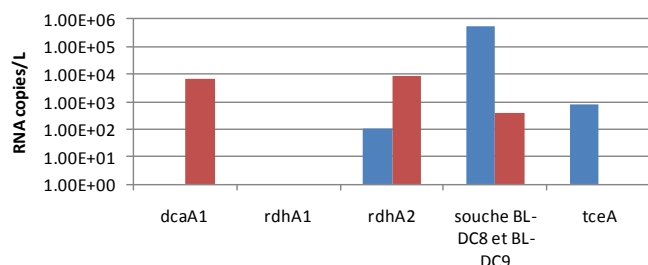
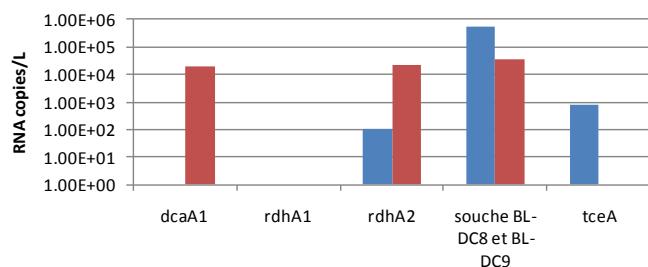
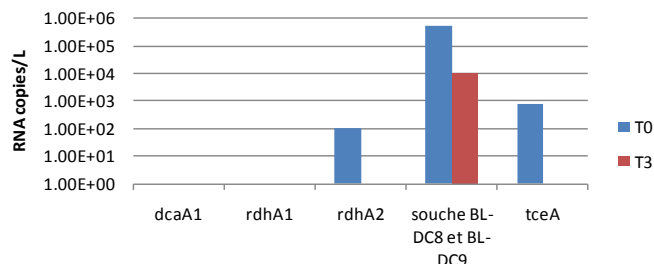


Acetate



1,1,2-TCA degradation
VC production

Biological activity

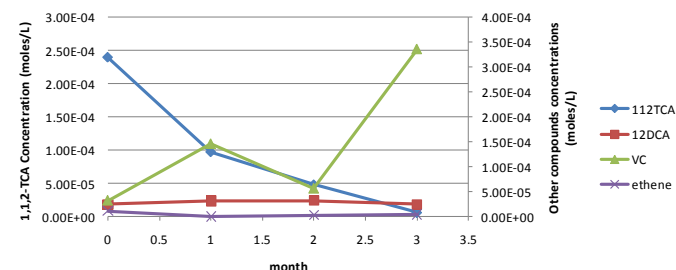
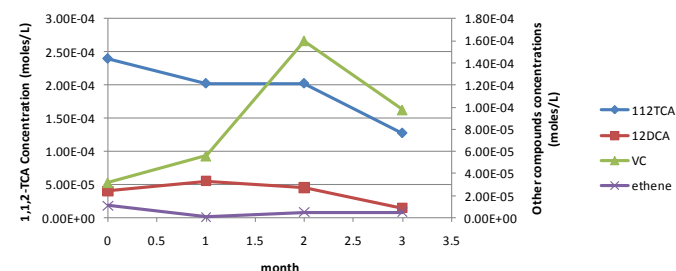
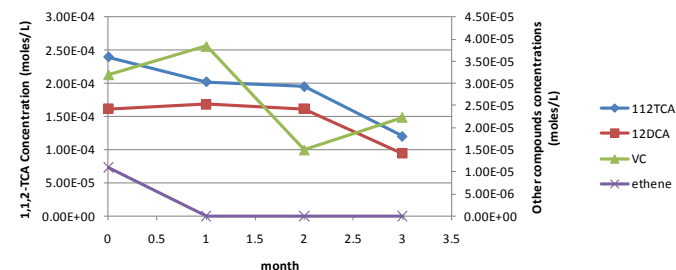


Aerated

Control

Acetate

Chemistry



VC is massively produced only when specific genes are activated
Favorable conditions to bacteria (i.e. acetate addition) lead to more VC produced.



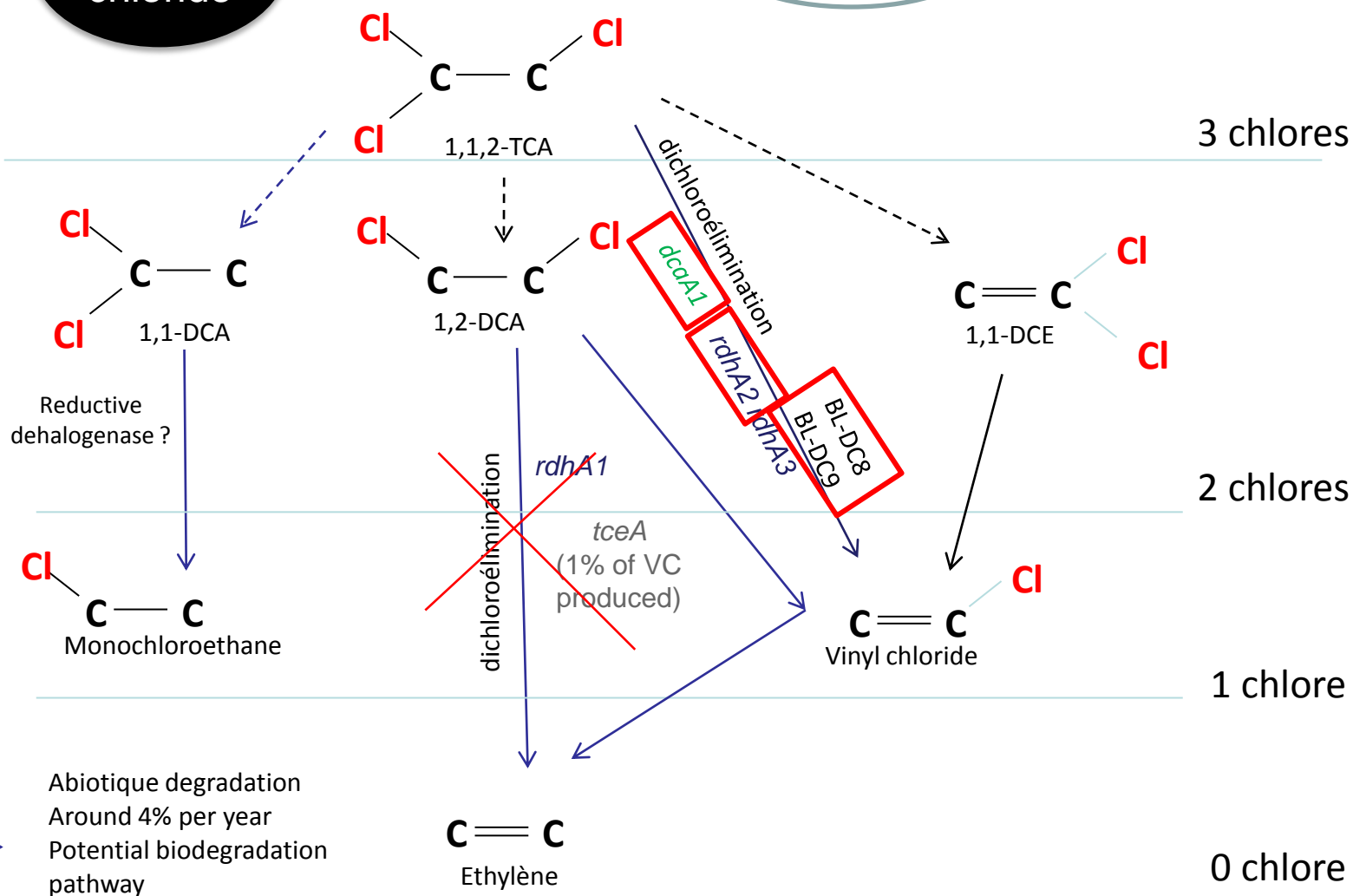
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Can VC come from 1,1,2-TCA on this site?

Vinyl chloride

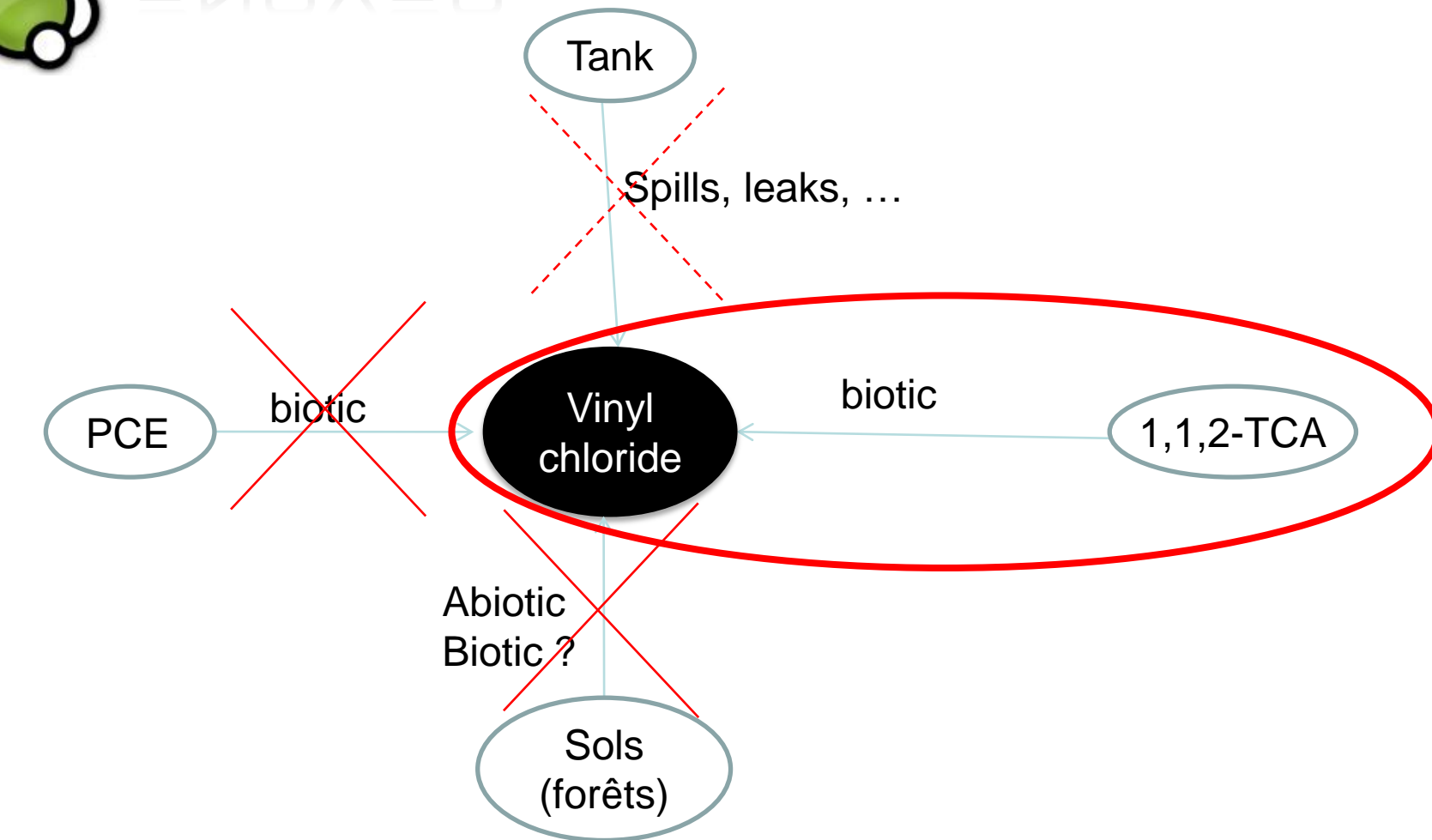
1,1,2-TCA





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Using combination of chemical and microbiological analyses
Identification of vinyl chloride origin



Microbial forensics:

1. determine the contamination origin and its history (in some cases).
2. discriminate the origin of soil or water sample.

To be done:

3. reveal past pollution
4. Determine the age of the pollution



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