(META)GENOMIC CHARACTERIZATION OF A BIOREACTOR WITH POLYHYDROXYALKANOATES (PHA) AND BIOCHAR AS BIOMATERIALS TO PROMPT REDUCTIVE DECHLORINATION

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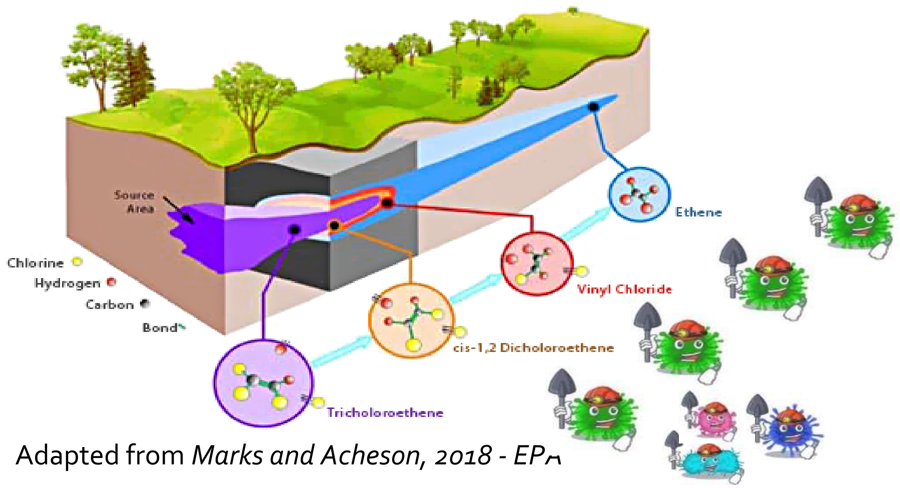
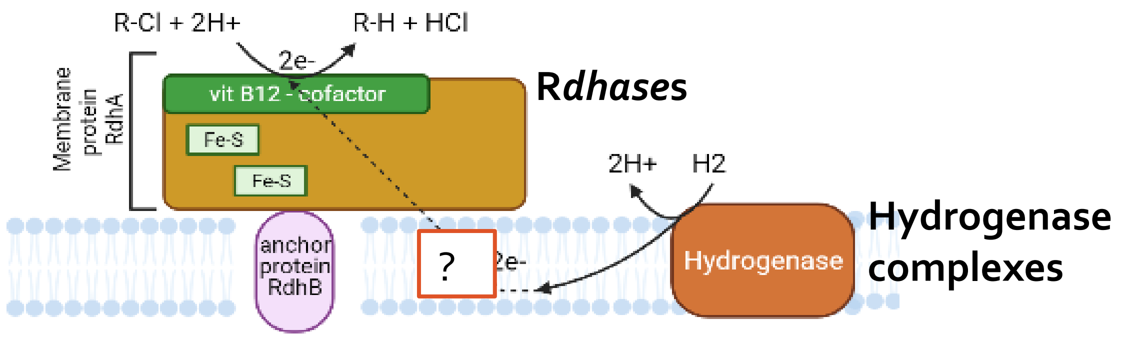
Biodegradation of Chlorinated Solvents



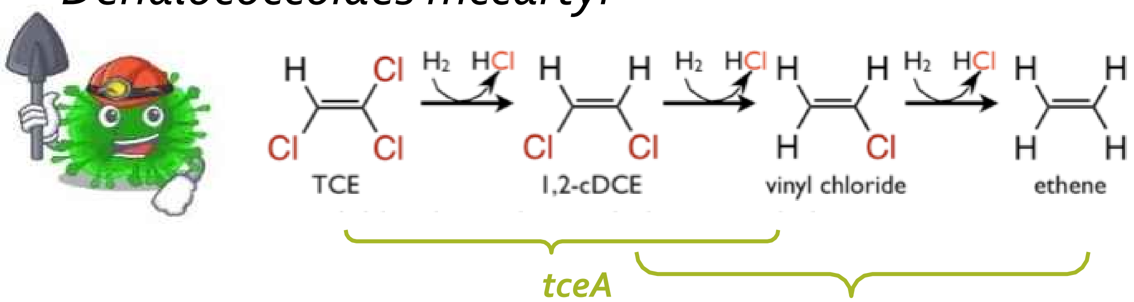
Adapted from *Marks and Acheson, 2018* - EPA

Reductive dehalogenases→ Organohalide Respiration Complex
at the cell membrane involved in the respiratory electron transfer for RD



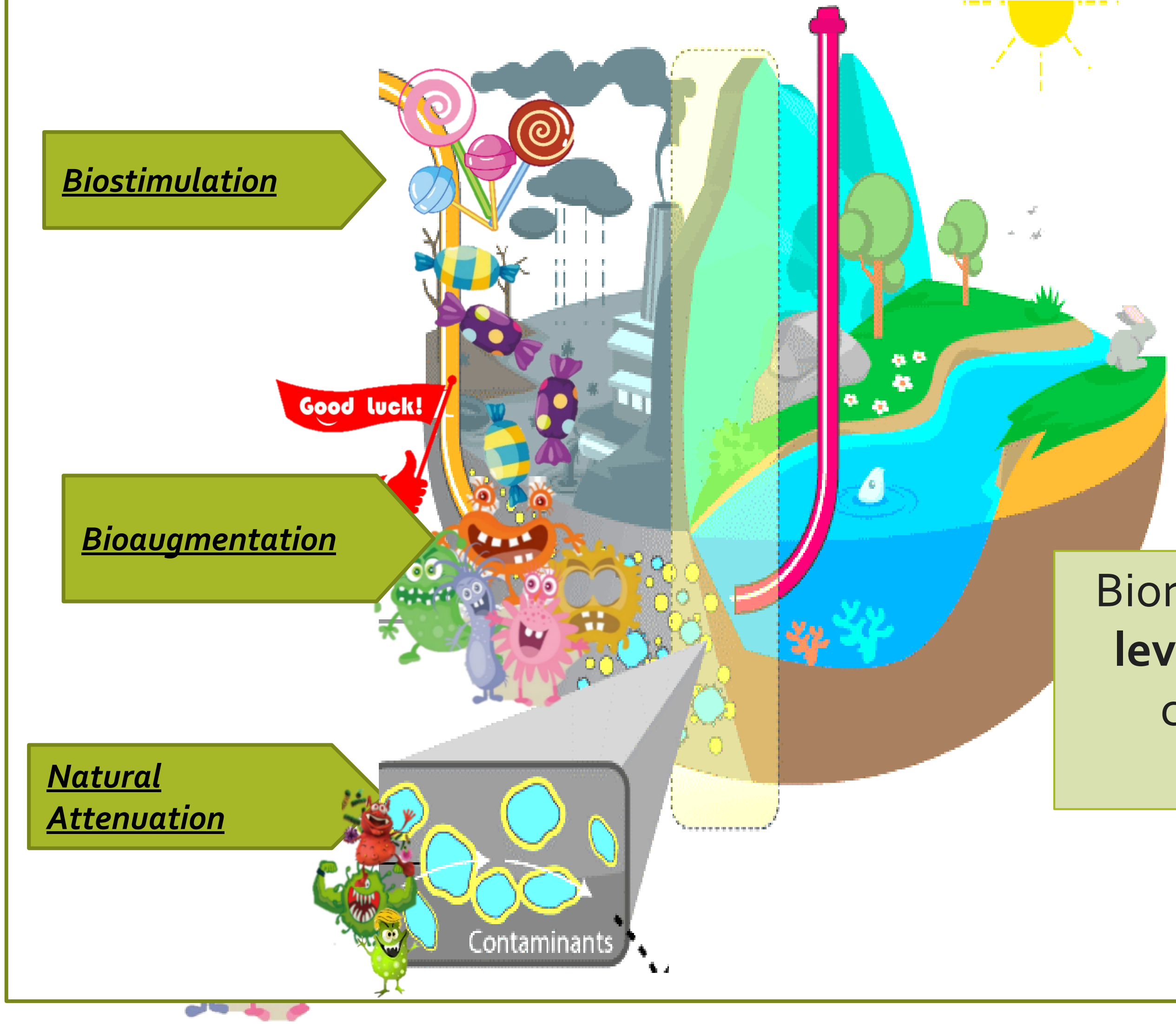
- TCE biodegradation to harmless ethene via **anaerobic reductive dechlorination** (RD)
- **H2 or fermentable organic substrates** as electron donor
- **Organohalide respiring bacteria (OHRB)**
- **Reductive dehalogenases (Rdases)** are distributed in various phylogentic group of OHRB and are central for RD

Dehalococcoides mccartyi



- **H2ases and *Rdhases* are fundamental** for *Dhc* energy metabolism

Dehalococcoides mccartyi (Dhc) and RD

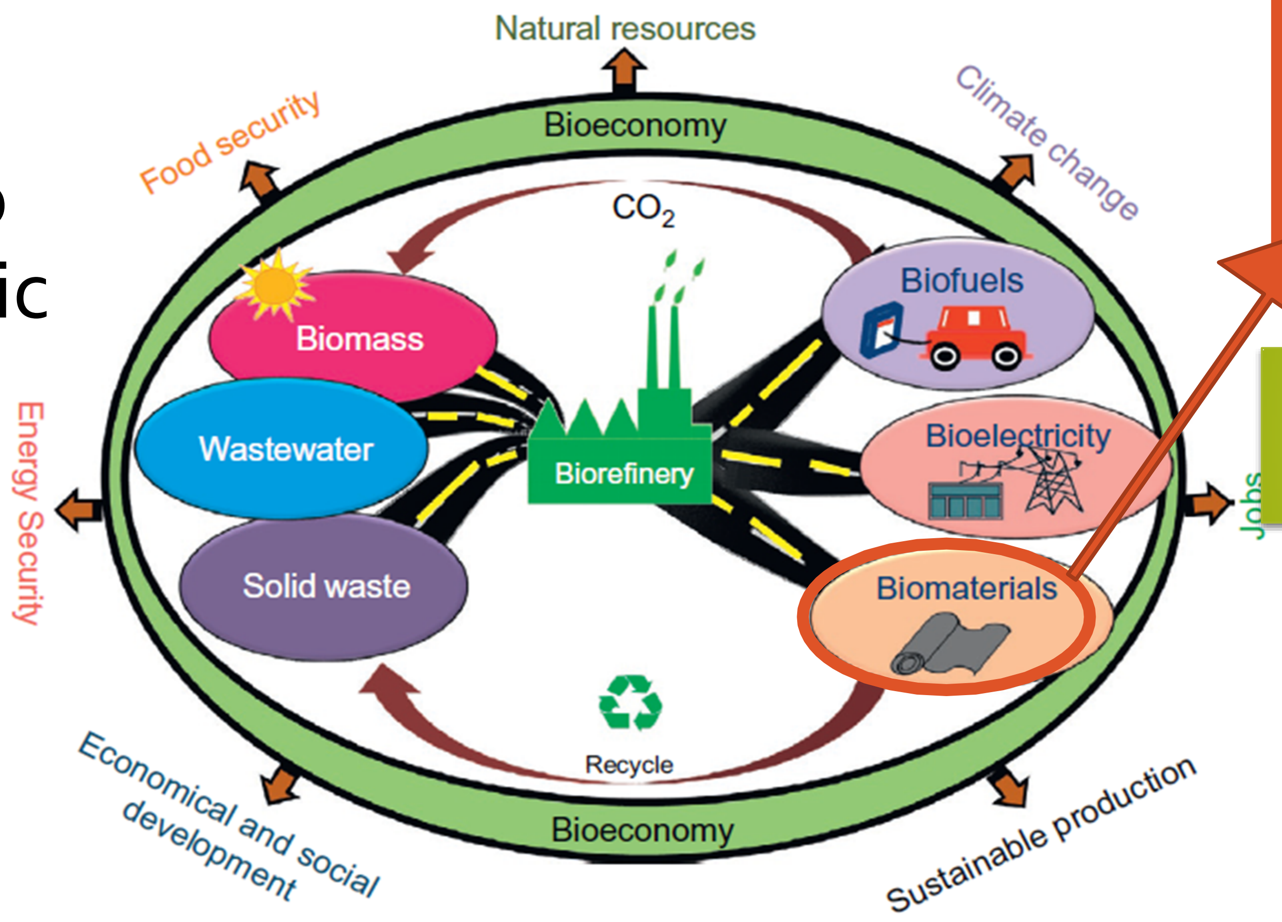


Network of **interactions between *Dhc*, its syntrophic partners and other coexisting community members** needs to be deciphered

Bioremediation technologies benefit by a **system-level understanding** of structural and functional characterization of dechlorinating microbial communities

Bioremediation technologies in a circular bio-based economy perspective

- Exploit the capacity to turn organic waste into valuable products



BIO-BASED MATERIALS FOR BIOREMEDIATION TECHNOLOGIES

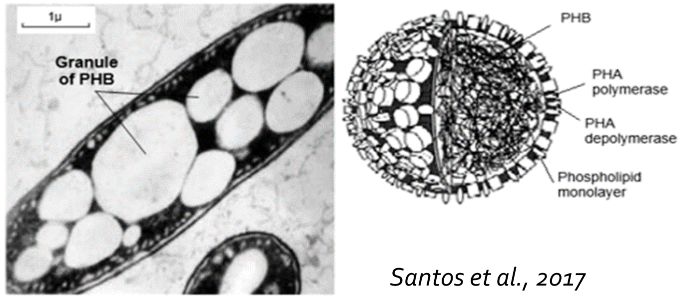
Poly(hydroxyalkanoates) PHA

Biochar

Adapted from Prasad, 2016 Bioremediation and bioeconomy

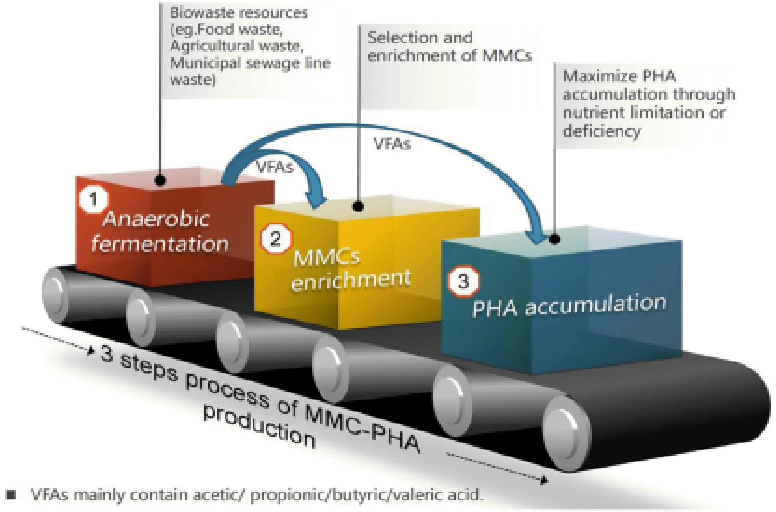
Bio-based materials for bioremediation technologies: **Poly-Hydroxybutyrate (PHB)**

- Polyhydroxyalkanoates (PHA) are biodegradable and biocompatible aliphatic polyester with linear polymer chain **accumulated intracellularly** by many **microorganisms under unfavorable growth conditions** and used **as carbon and energy reserves**

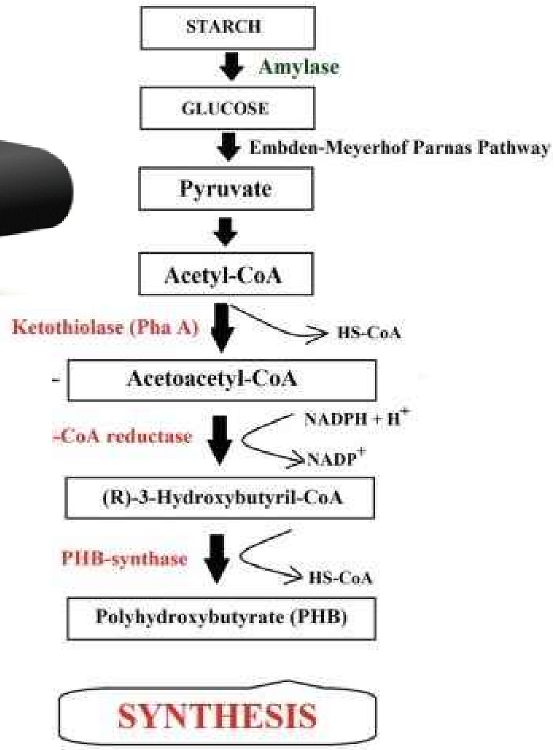


Santos et al., 2017

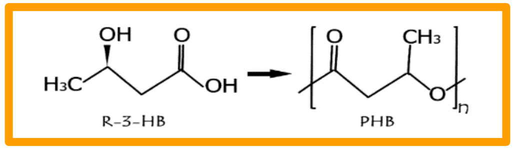
- **Produced as Pure or Mixed Microbial Cultures (MCC)**



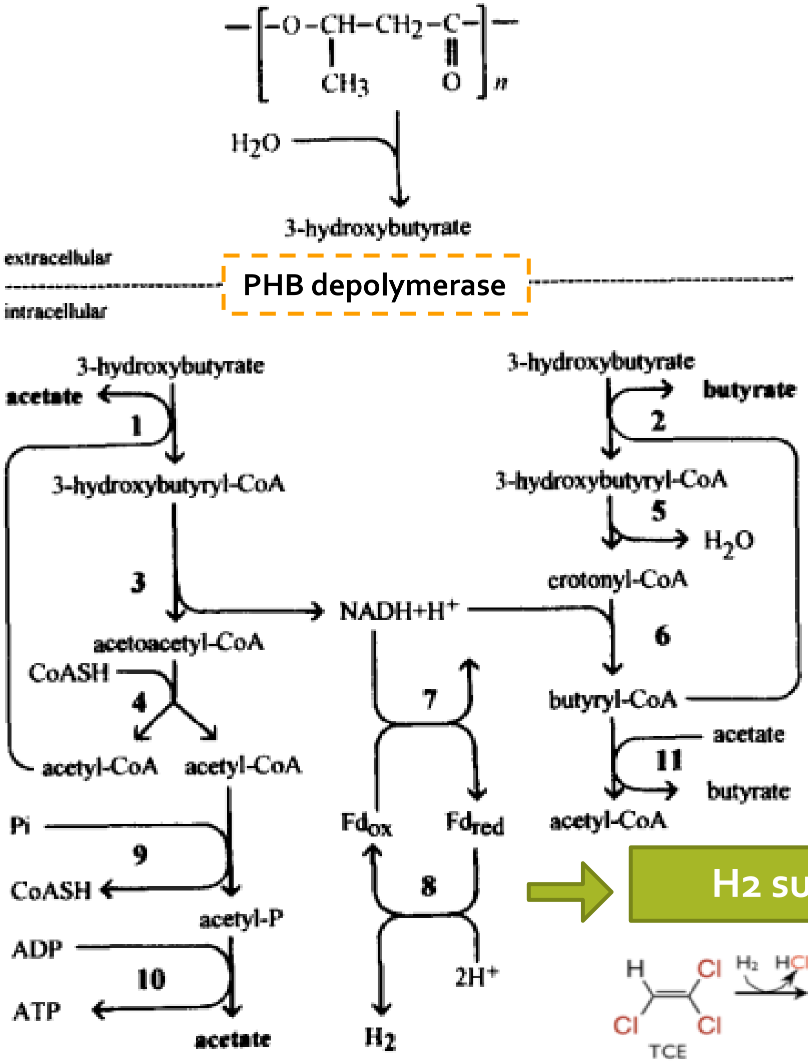
Wei and Fang, 2022



- **Poly-β-hydroxybutyrate (PHB): member of PHA family with a methyl functional group (CH3) and an ester linkage group (–COOR). It is** composed of **3-hydroxybutyrate monomers** strung together by β-bonds.

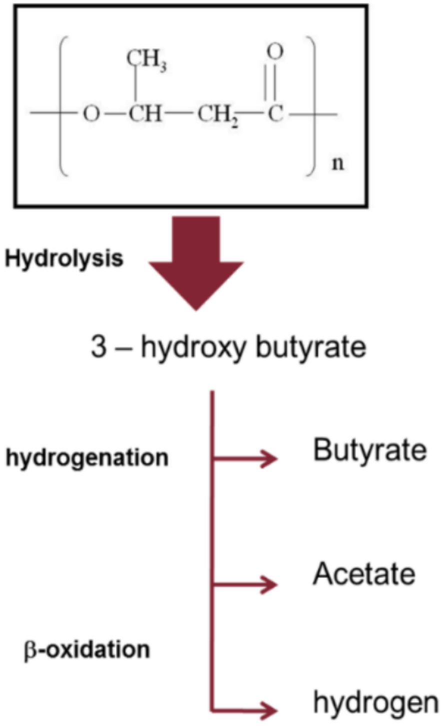


Bio-based materials for bioremediation technologies: **Poly-Hydroxybutyrate (PHB)**

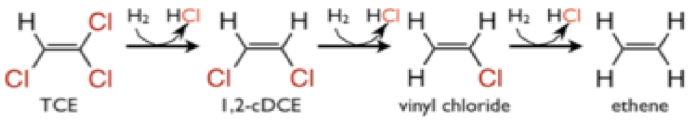


1. Acetyl-CoA 3-hydroxybutyrate CoA-transferase
2. Butyryl-CoA 3-hydroxybutyrate CoA-transferase
3. 3-Hydroxybutyryl-CoA dehydrogenase (NAD)
   3-hydroxybutyrate dehydrogenase
   3-hydroxybutyrate CoA ligase
4. Acetyl-CoA acetyltransferase [thiolase]
5. 3-Enoyl-CoA hydratase [crotonase]
6. Butyryl-CoA dehydrogenase (DCPIP)
7. Ferredoxin:NAD oxidoreductase
   ferredoxin:NADP oxidoreductase
8. Hydrogenase (MV)
9. Phosphate acetyltransferase
10. Acetate kinase
11. Butyryl-CoA acetate CoA transferase

Others…



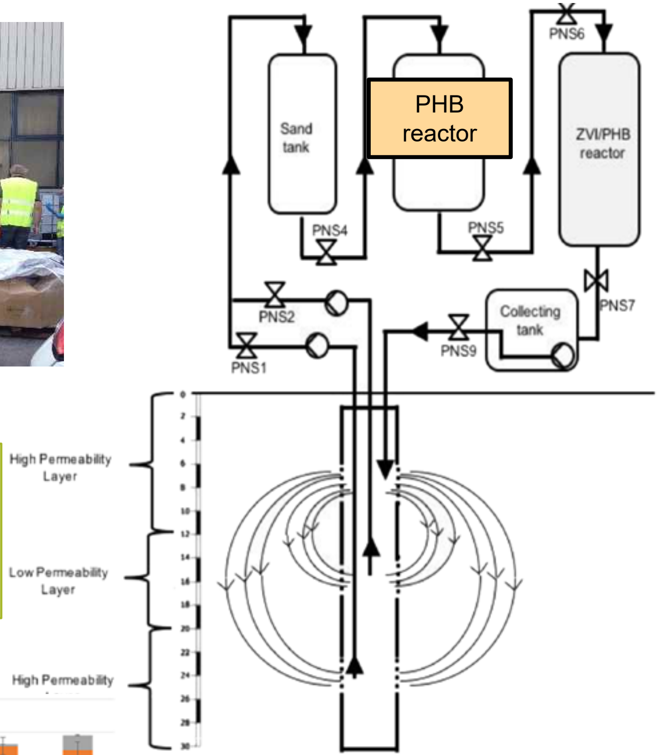
H2 supply for RD processes



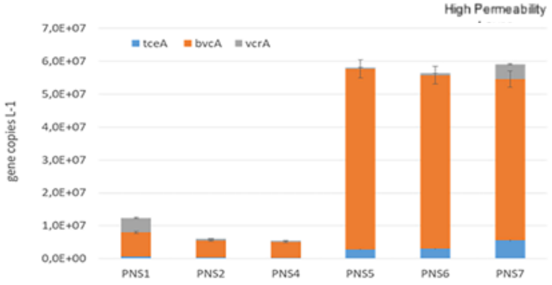
Fermentation (H₂-producing)	$CH_3(CH_2)_2COO^- + 2\ H_2O \rightarrow 2\ CH_3COO^- + 2\ H_2 + H^+$ (Butyrate → Acetate)
Reductive dechlorination	$CCl_2CHCl + 3\ H_2 \rightarrow CH_2CH_2 + 3\ H^+ + 3\ Cl^-$ (TCE → Ethene)

PHB as slow release carbon source for RD: previous experiences

Groundwater Ricirculation Well system at field scale

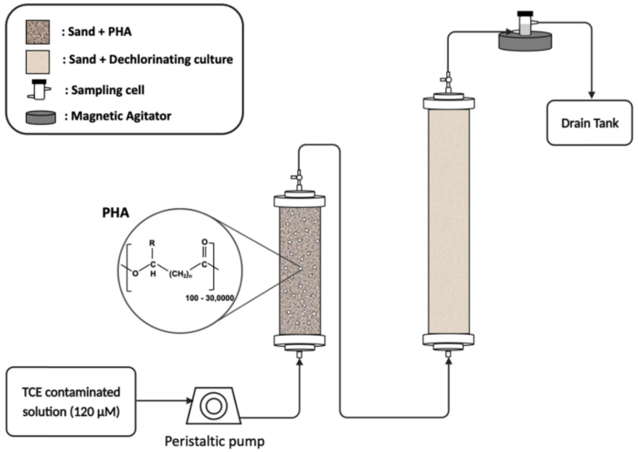


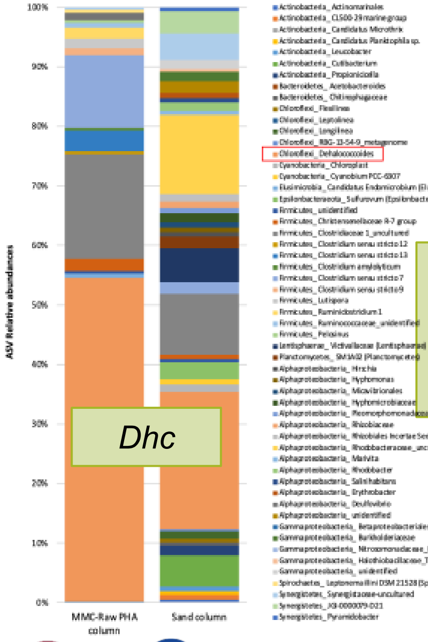
- *Dhc growth after PHB reactor unit;*
- *Increment of chlorinated solvents biodegradation*



Pierro et al., 2017; Matturro et al., 2018

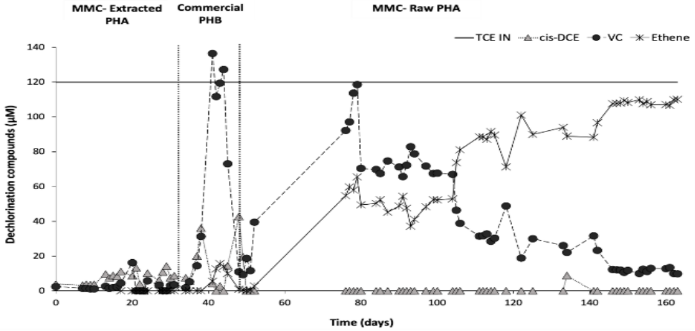
Column system at lab scale with PHA from pure and MMC cultures





- PHA from a municipal foodwaste treatment plant
- Enhanced TCE biodegradation

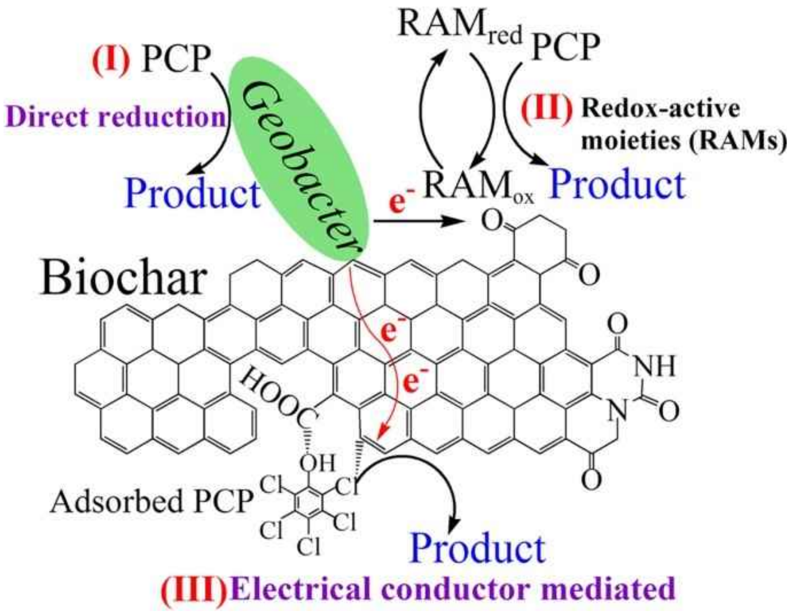
→ Microbial interactions during the fermentation need to be clarified



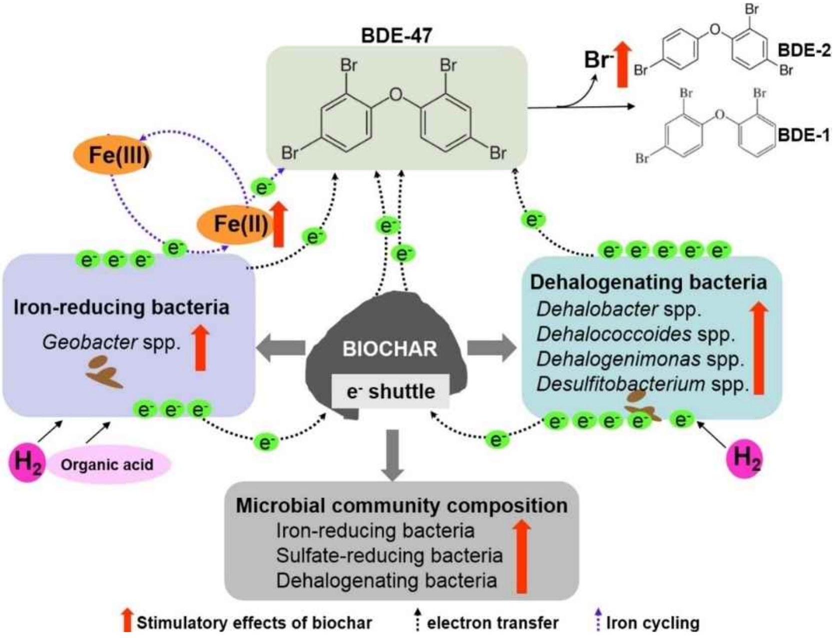
Amanat et al., 2021; Amanat et al., 2022

Bio-based materials for bioremediation technologies: **BIOCHAR**

- **Biochar selectively enriched microbial communities involved** in halogenated pollutant **degradation**, mostly aromatic compounds
- **Few studies** report for **chlorinated solvents biodegradation**



Yu et al., 2015



Chen et al., 2015

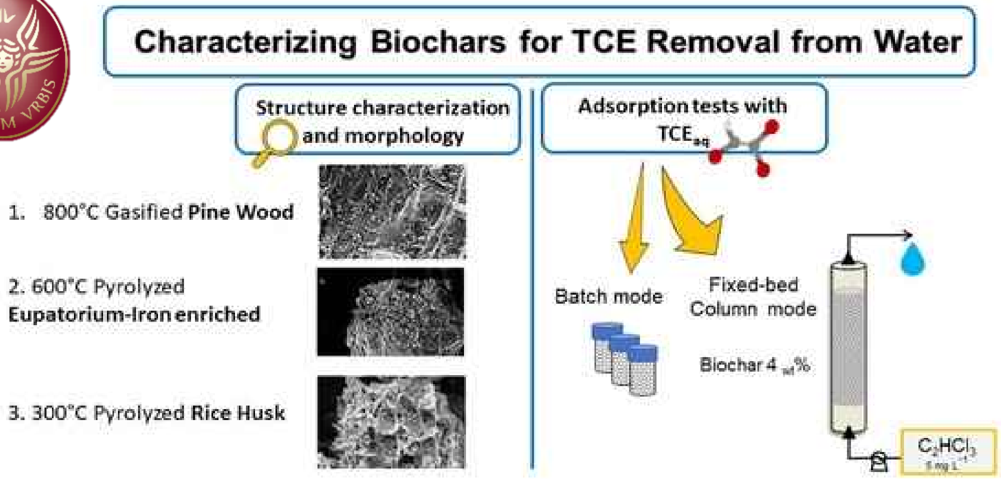
APPLICATIONS FOR POLLUTANTS REMOVAL

→ **Immobilization** of different classes of pollutants, thanks to the carbon structure and functional groups present on the surface

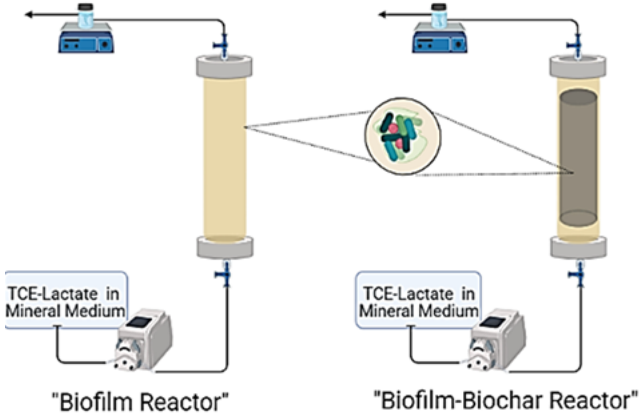
→ **Electron transfer capacity** which made biochar responsible for the degradation of several pollutants

Tan et al., 2015

Biochar in bioremediation technologies: previous experiences



Rossi et al., 2021

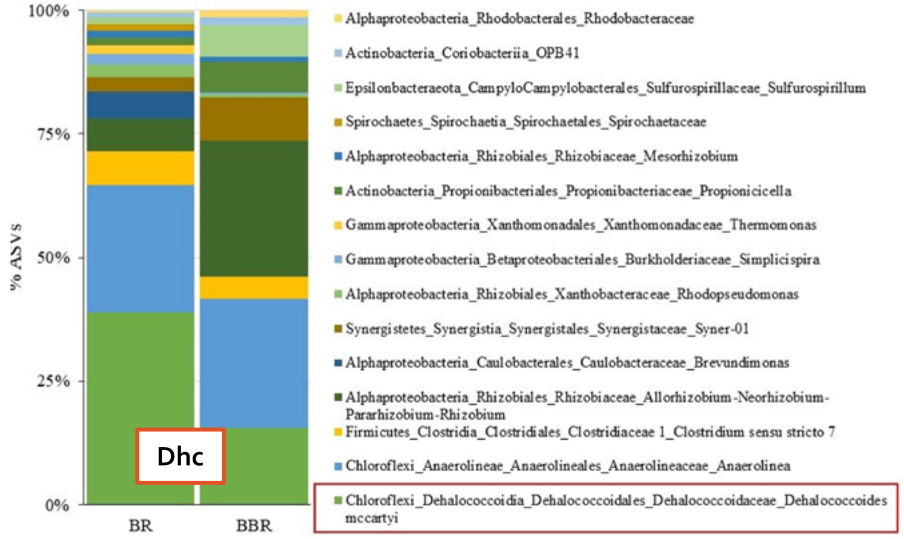


Rossi et al., 2022

Coupled Adsorption and Biodegradation of TCE on Biochar

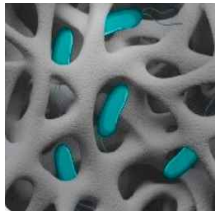
- TCE-dechlorinating consortium as inoculum
- Lactate as electron donor
- Biochar as bio-based material to sustain/enhance RD

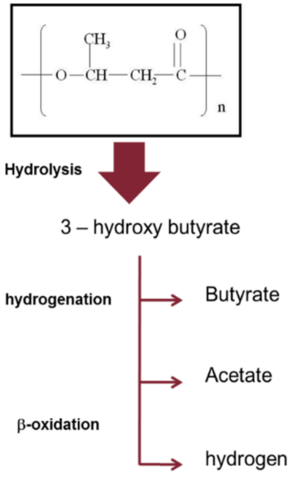
→*The role of Biochar for Dhc growth needs to be clarified*



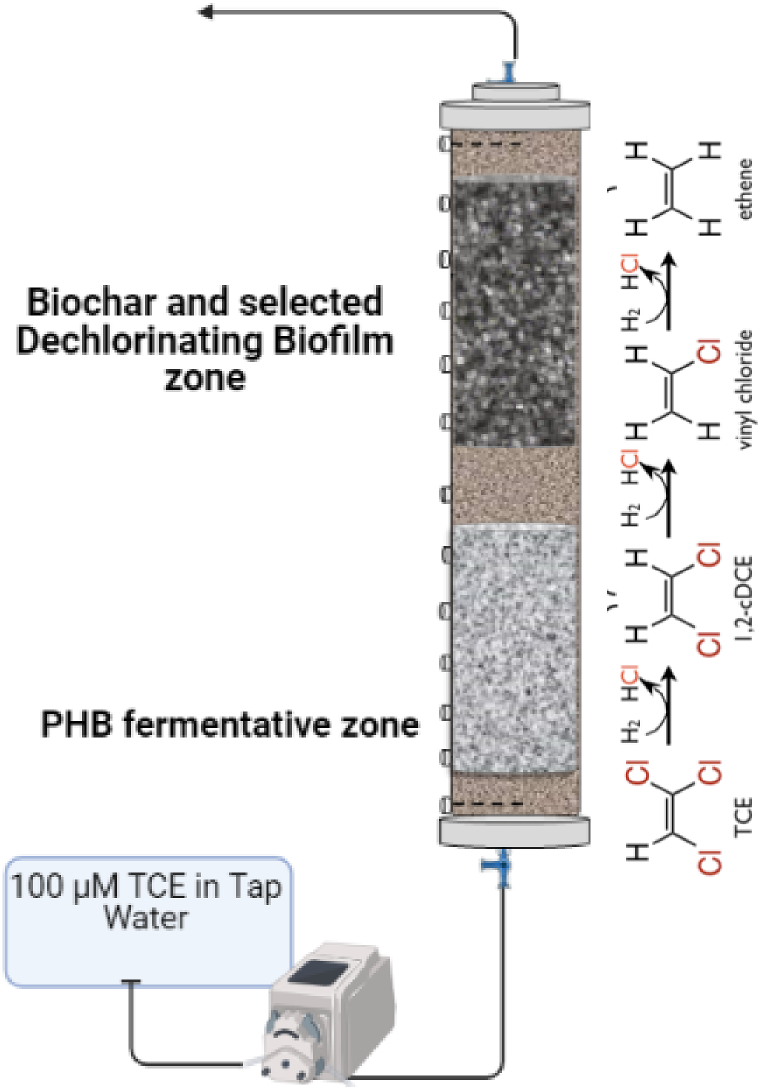
PHB/BIOCHAR Mini-pilot scale reactor to prompt TCE dechlorination

BIOCHAR
- Adsorbent material;
- Sustain growth/activity dechlorinating biomass



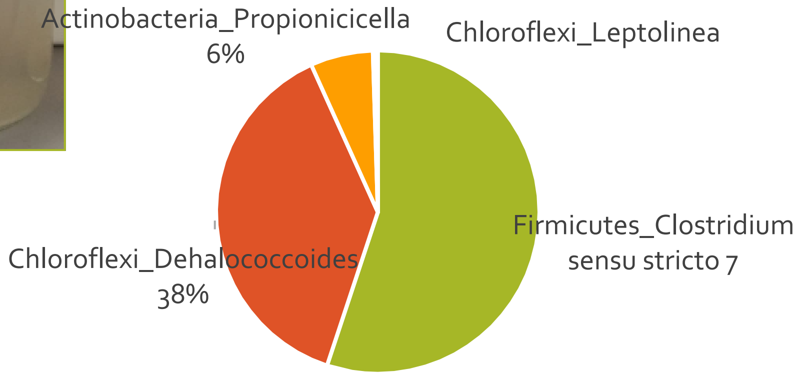


PHB
Source of electron donor



Reactor Working Conditions	
Flow rate (L/day)	**6**
HRT (day)	**1.8**
TCE in (mM)	0.1
Pore water velocity (cm/day)	76.4
Length (cm)	144

START UP with A TCE-dechlorinating consortium inoculated in the Biochar zone



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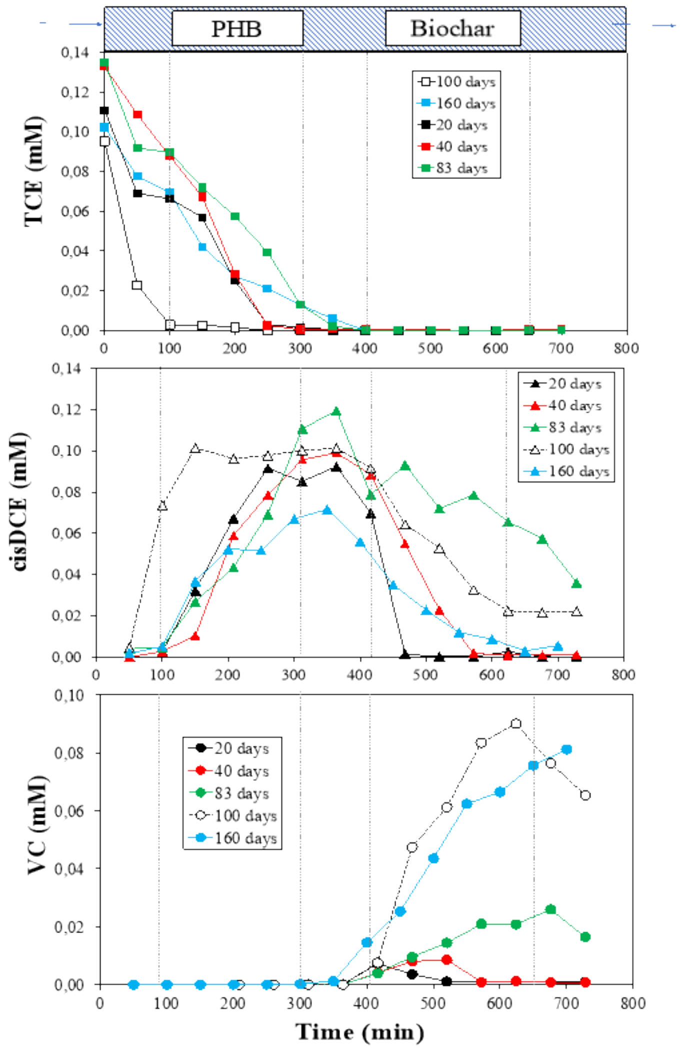
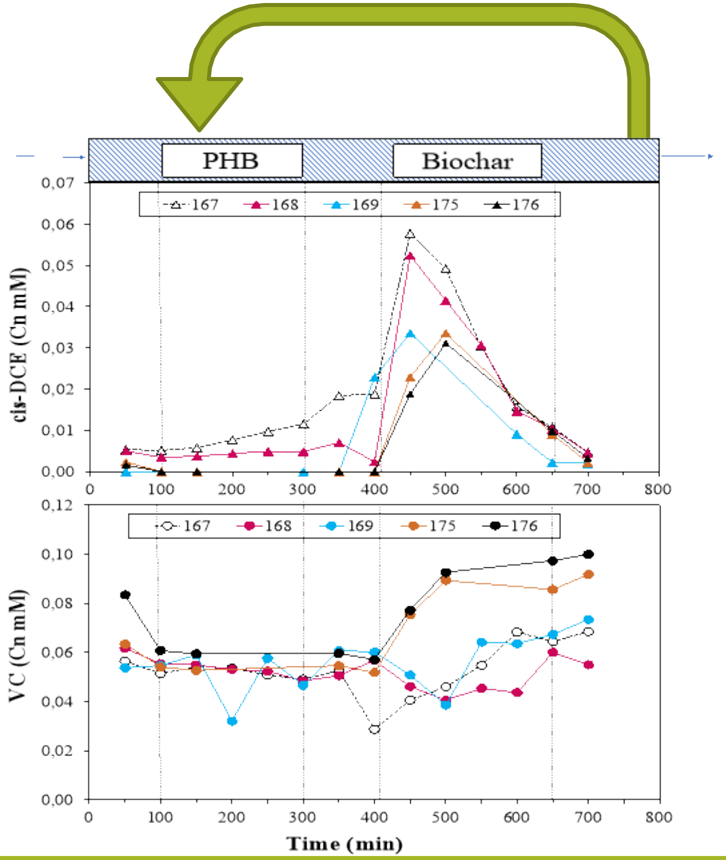
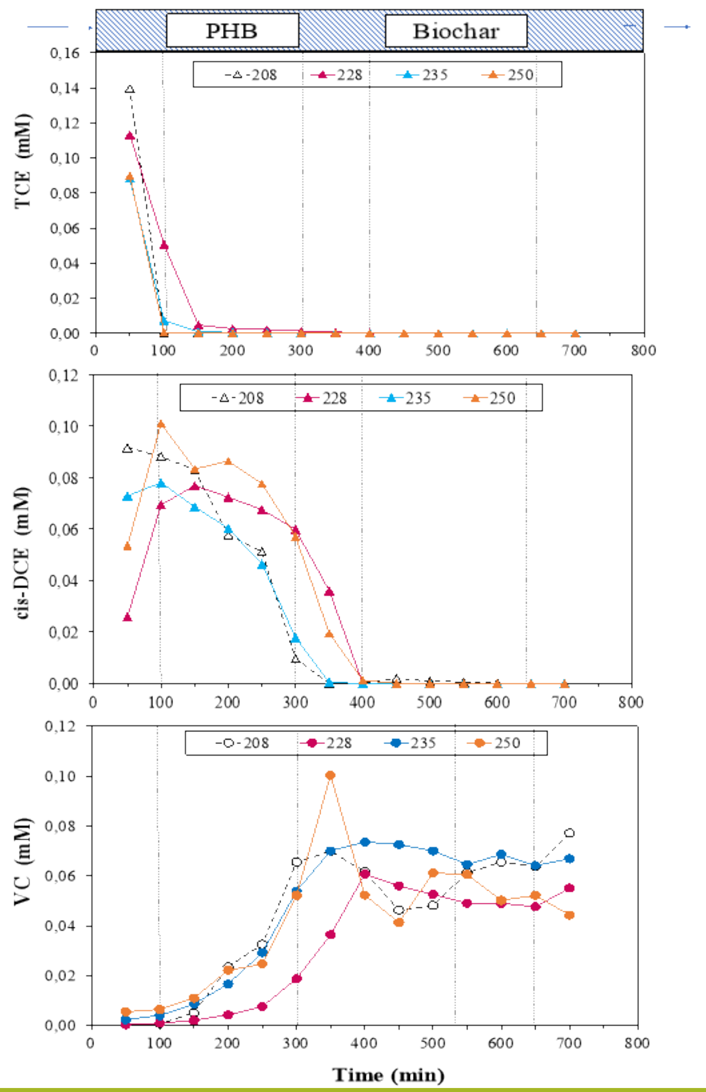
TAP WATER + TCE

OUTLET RICIRCULATION

ANAEROBIC MINERAL MEDIUM + TCE





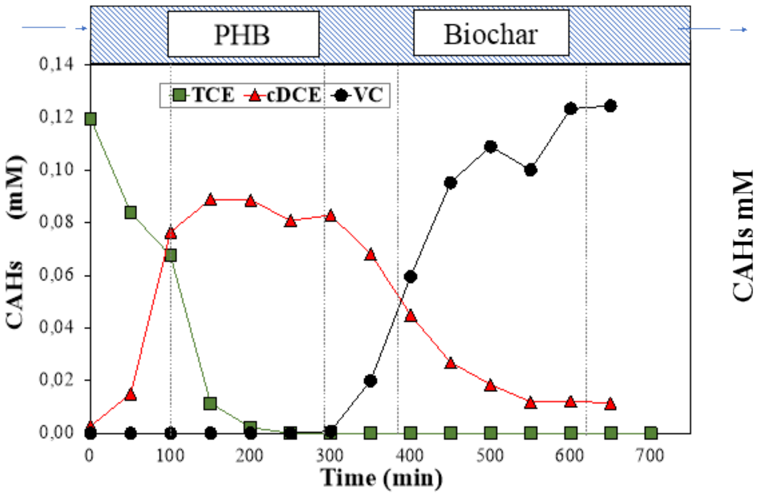


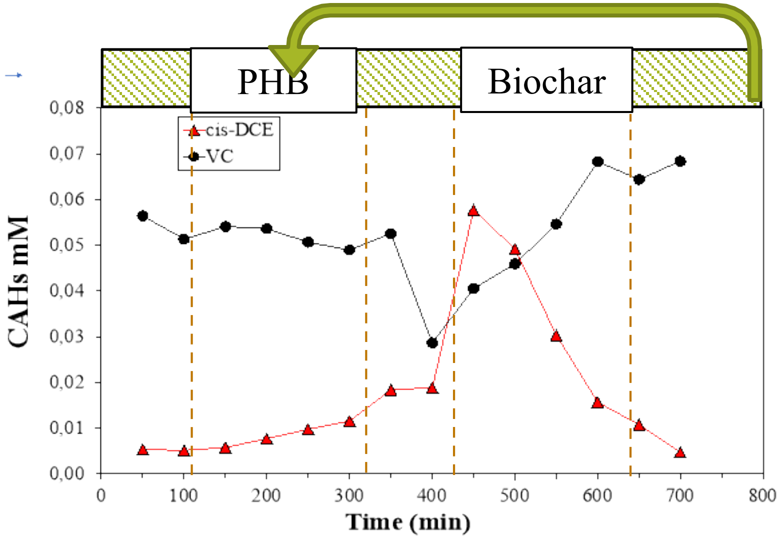
T1 (160 days) | T2 (180 days) | T3 (250 days)

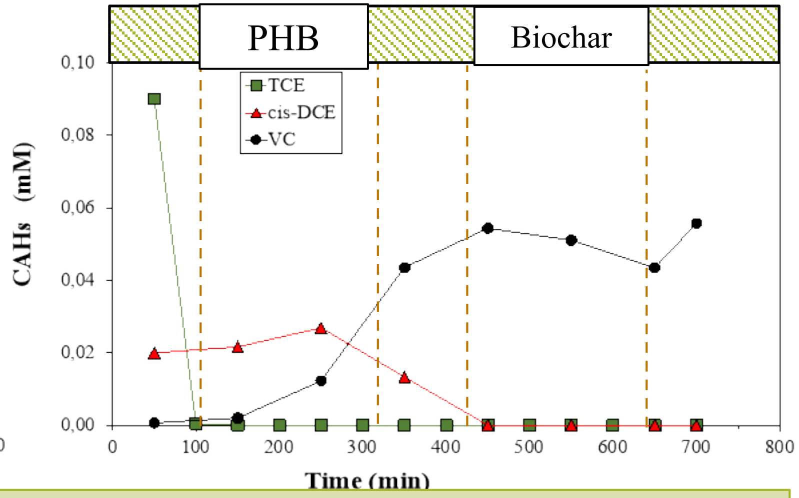
TAP WATER + TCE

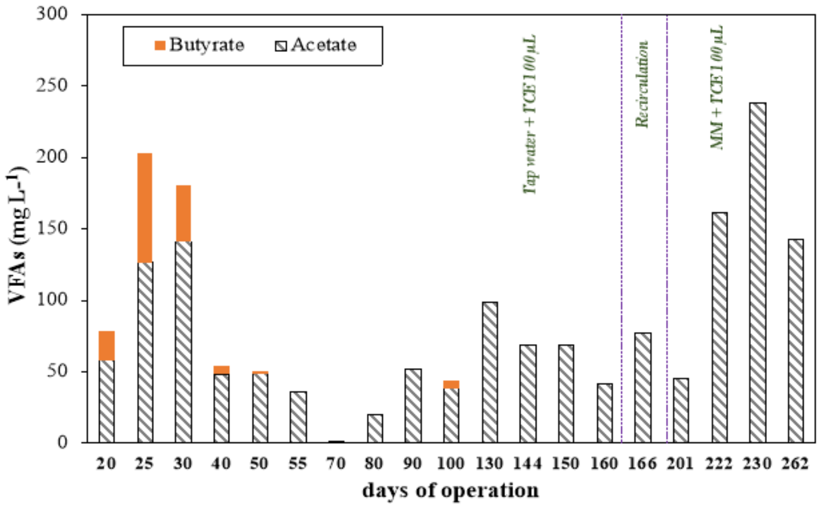
OUTLET RICIRCULATION

ANAEROBIC MINERAL MEDIUM + TCE



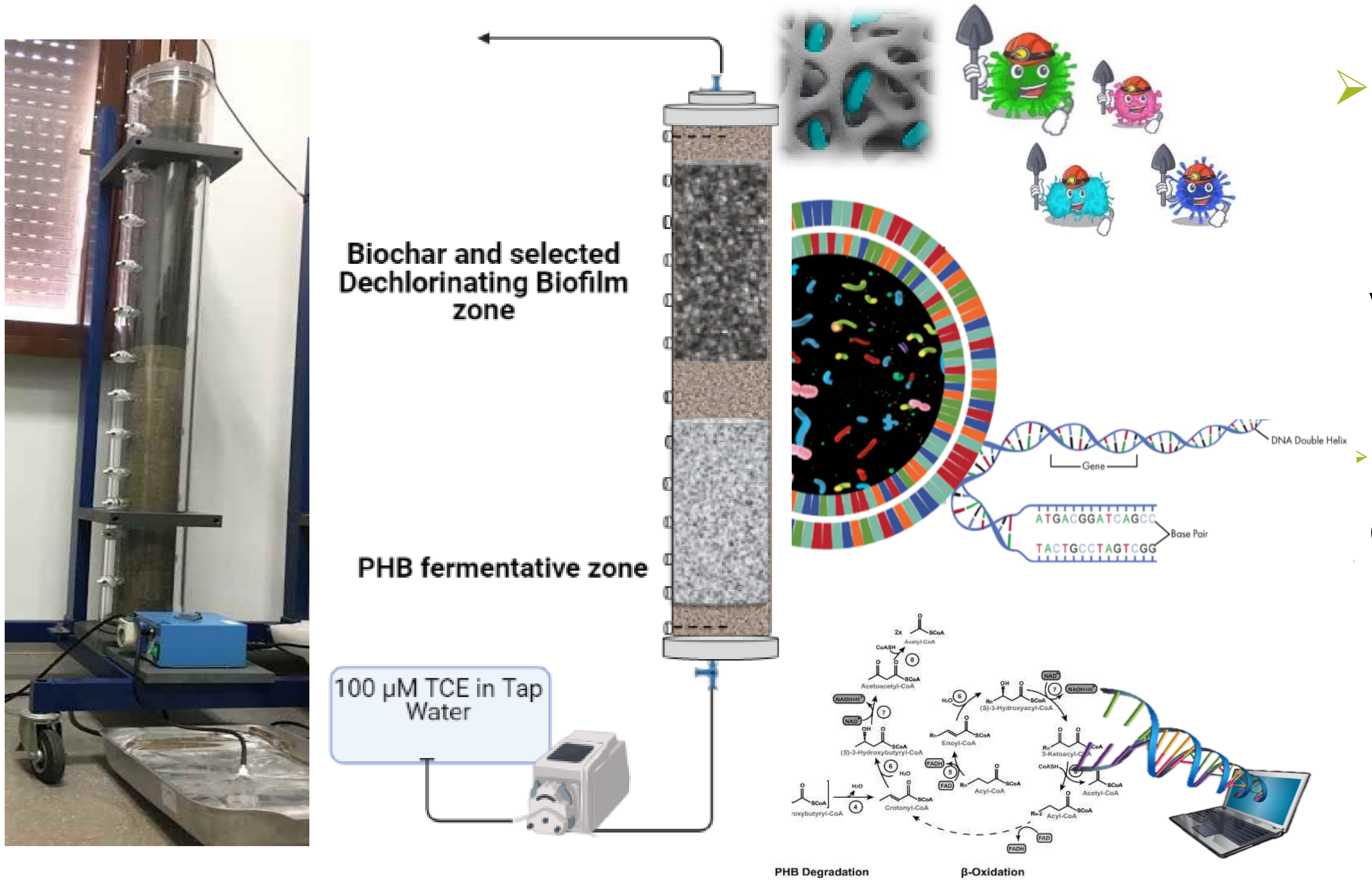






BIOMOLECULAR CHARACTERIZATION OF THE REACTOR

- 16S rRNA gene amplicon sequencing (MiSeq Illumina)
- Metagenomic analysis (MiSeq Illumina)
- Quantification of reductive dechlorination biomarkers' (digital droplet PCR)



Aim of the work

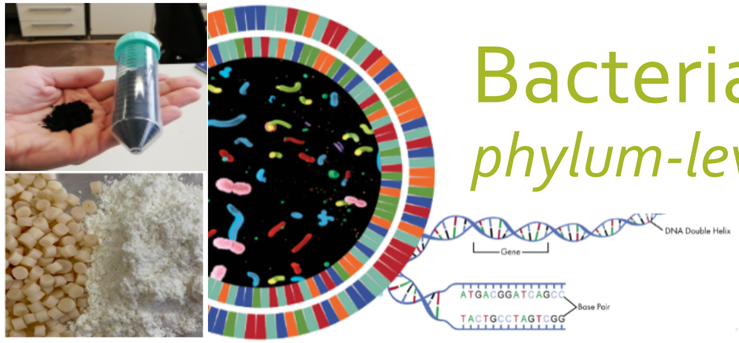
- Composition and dynamics of the bacterial communities established in the PHB and Biochar zone under variuos feeding conditions tested

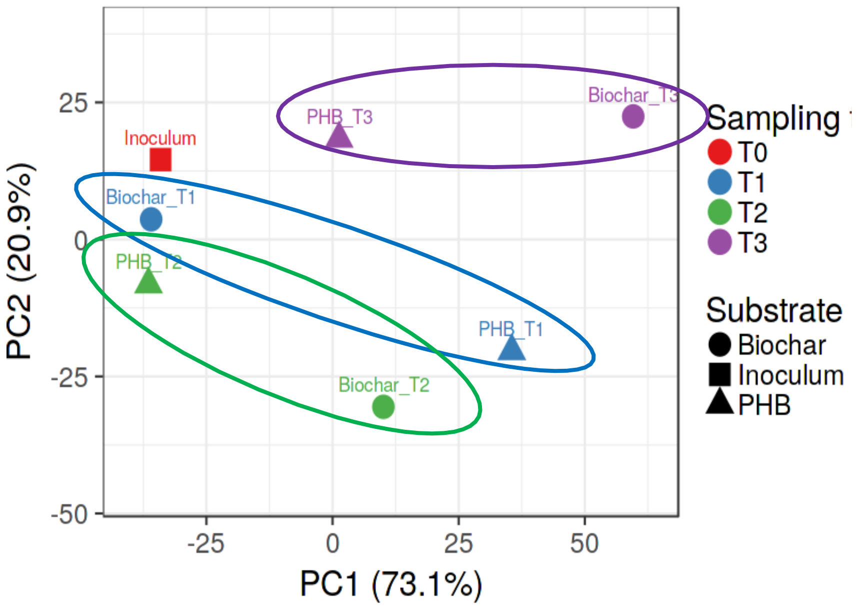
- Shed light on the functional role of the main bacterial components in the system
  - Bacteria involved in the PHB-to-butyrate/acetate/H2 transformation
  - Role of Biochar for Dhc growth/activity

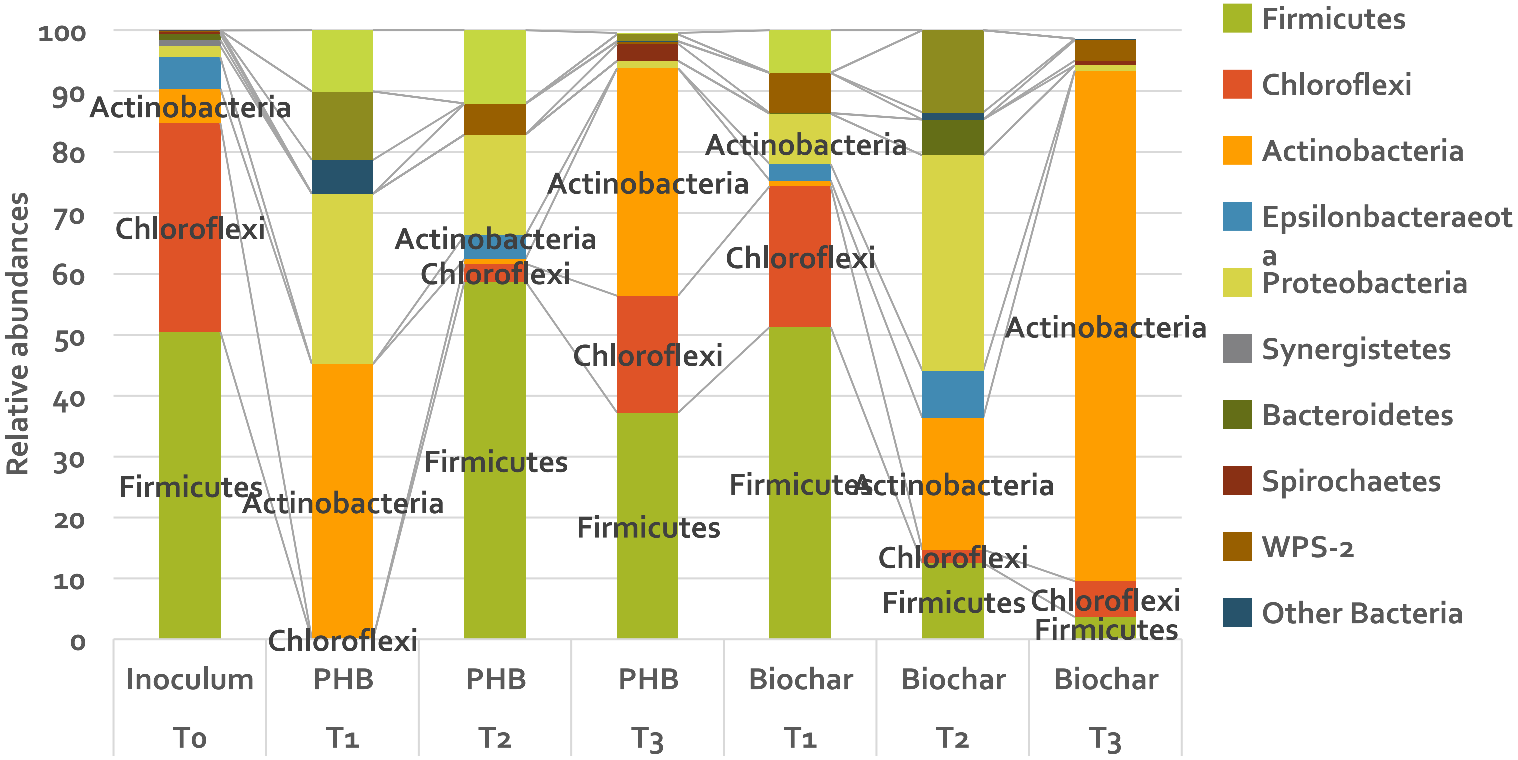
- Dhc biomonitoring overtime

Bacterial community composition and dynamics

phylum-level



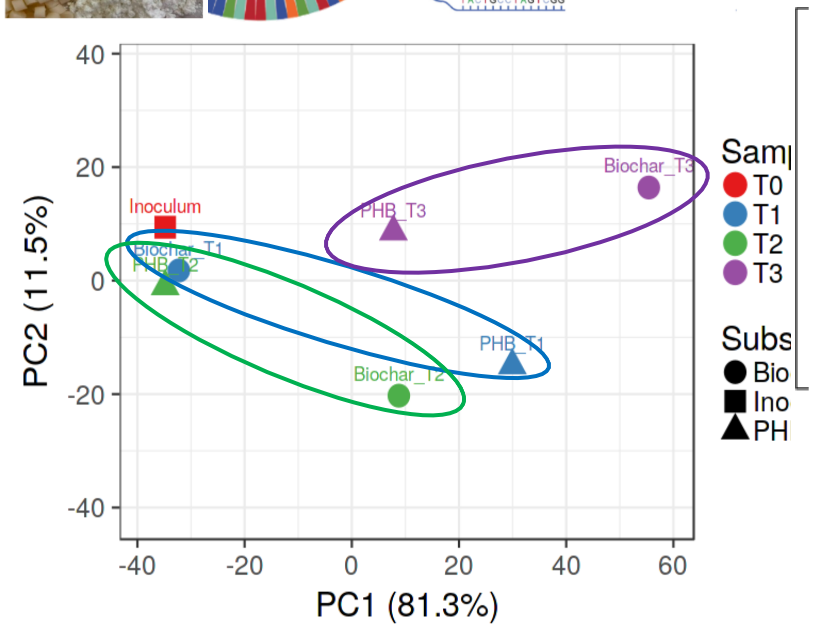


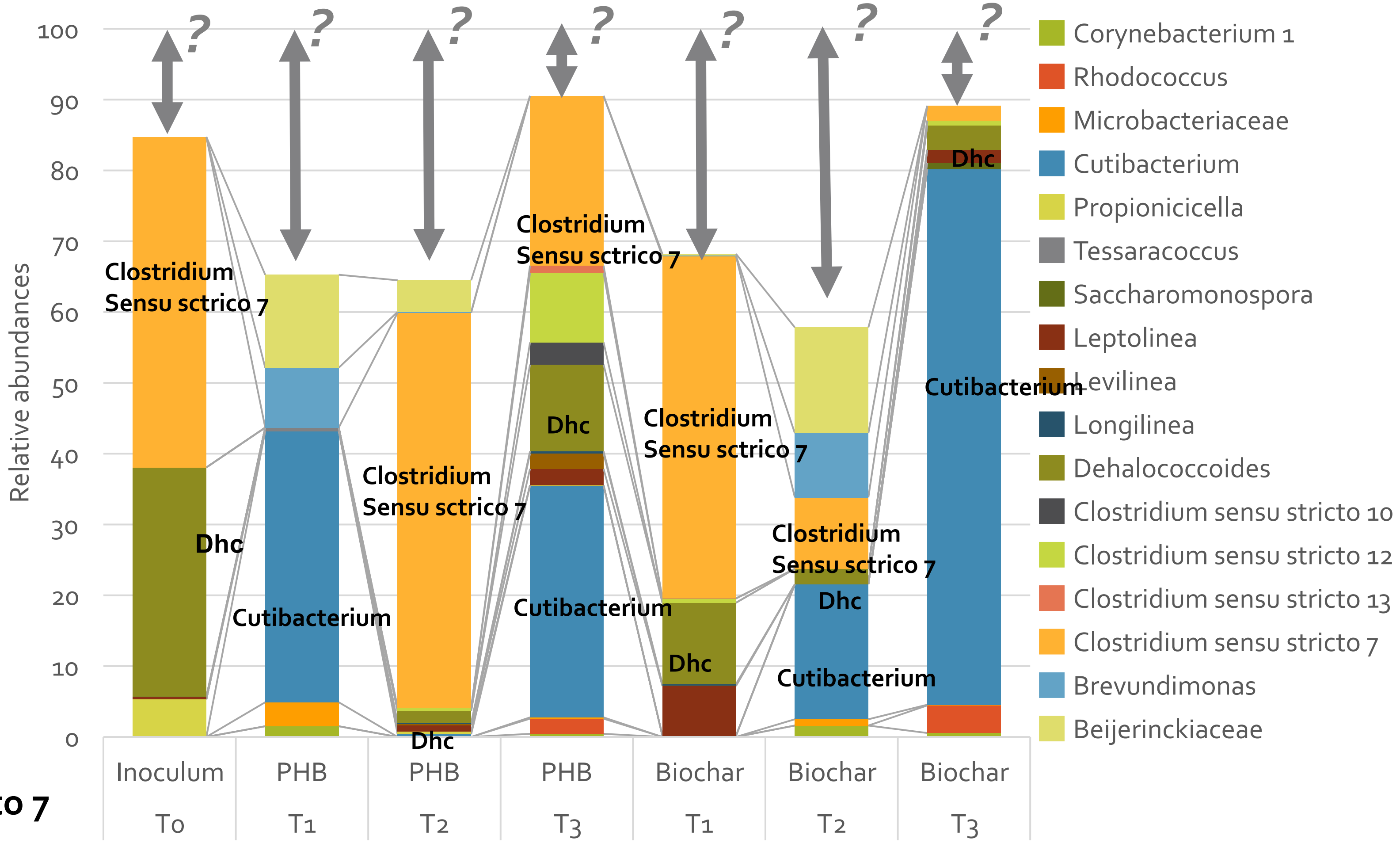


- **Inoculum:** core microbiome composed by *Firmicutes*, *Chloroflexi* → dominated the PHB/Biochar reactor fed with TapWater (and after ricirculation) at T1-T2
- **PHB zone**: effect of ricirculation on the microbial structure→ fermentative *Actinobacteria* shifted to fermentative *Firmicutes* and *Chloroflexi* were introduced in the fermentative zone → *T3* increment of dechlorinating *Chloroflexi (effect of the anaerobic mineral medium feeding)*
- **Biochar zone**: at T1 core microbiome of the inoculum → *Firmicutes* shifted to *Actinobacteria* that increased at T2 and then T3 in the biochar zone.

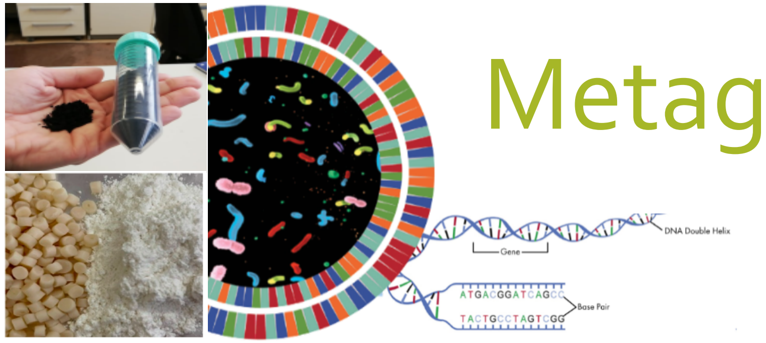
Bacterial community composition and dynamics

genus-level

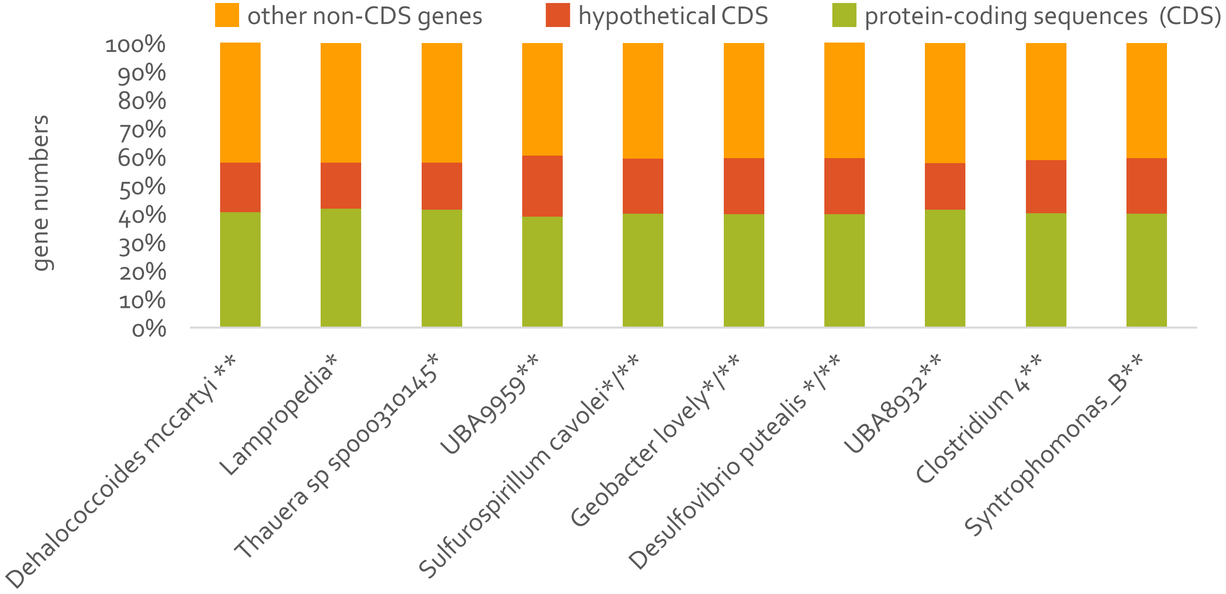




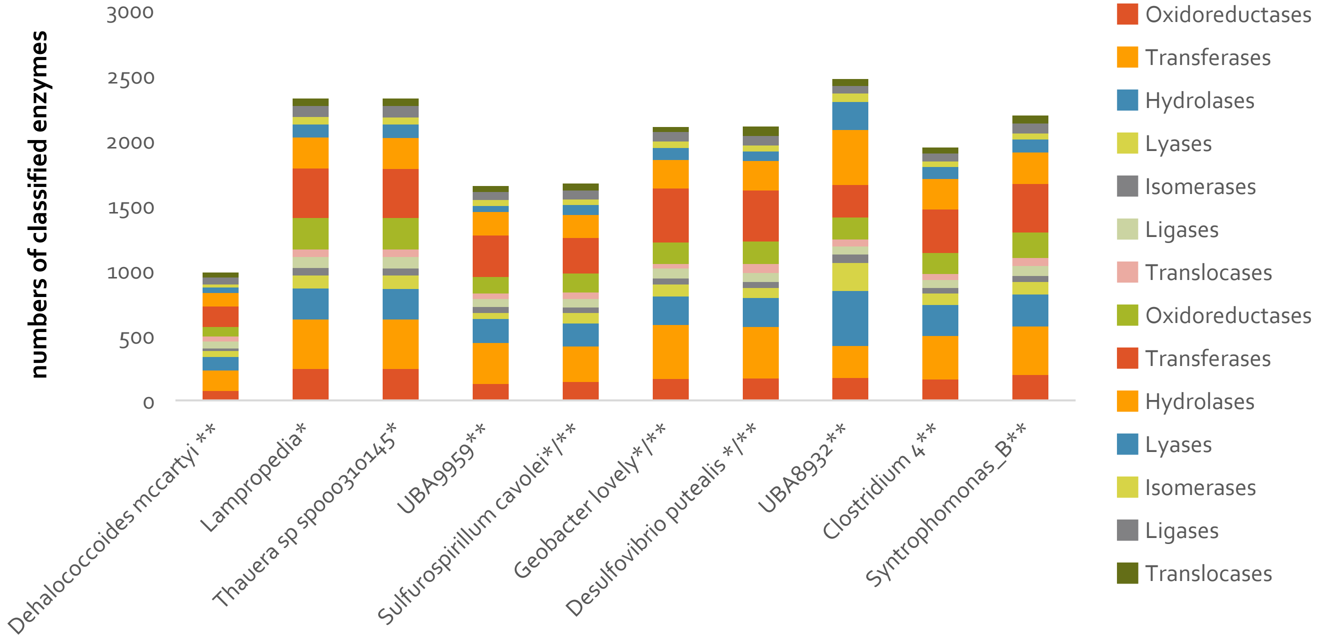
- **Most representative fermenters**
  - **Firmicutes_Clostridium sensu stricto 7**
  - **Actinobacteria_Cutibacterium**
- **Dechlorinating bacteria: Dehalococcoides mccarty**
  - **PHB zone:** Dhc stablished after the tap water ricirculation and increased with the anaerobic mineral medium feeding (T2, T3)
  - **Biochar zone**: Dhc Established immediately but decreased both after the tap water ricirculation (T2) and feeding with the anaerobic mineral medium (T3)



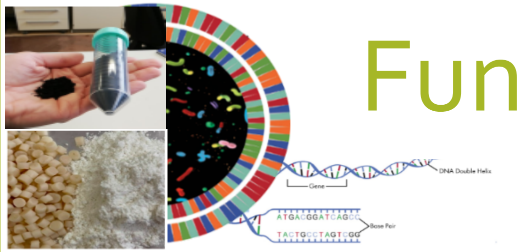
Metagenome

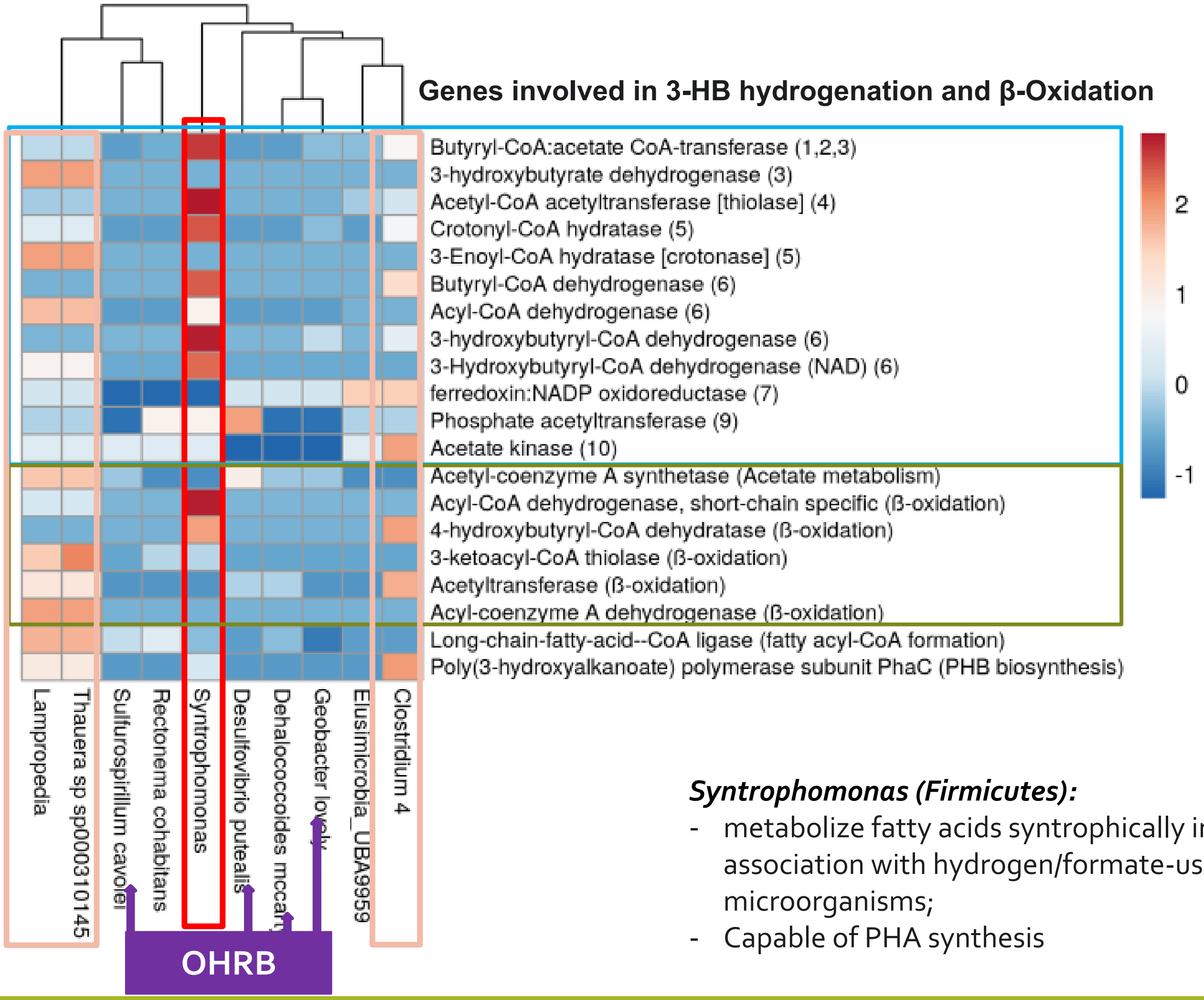


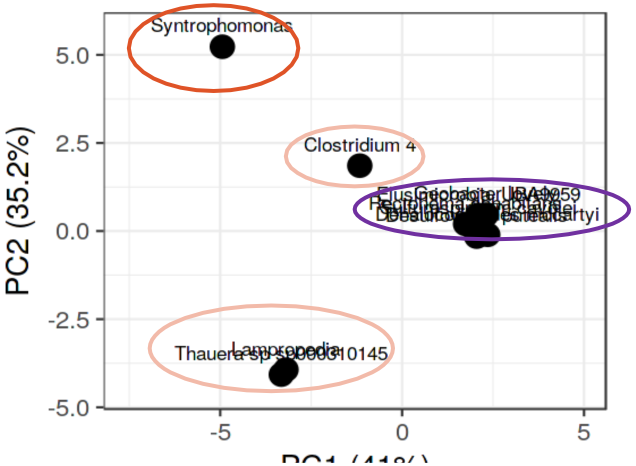
Bin	Affiliation	Coverage PHB*/Biochar**
Bin1	**Dehalococcoides mccartyi**	0/151
Bin2	**Lampropedia**	218/82
Bin3	**Thauera sp spoo310145**	274/46
Bin4	**Elusimicrobia_UBA9959**	16/56
Bin5	**Sulfurospirillum cavolei**	27/25
Bin6	**Geobacter lovely**	10/8
Bin 7	**Desulfovibrio putealis**	26/28
Bin8	**Rectonema cohabitans**	2/28
Bin9	**Clostridium 4**	4/11
Bin10	**Syntrophomonas_B**	11/36

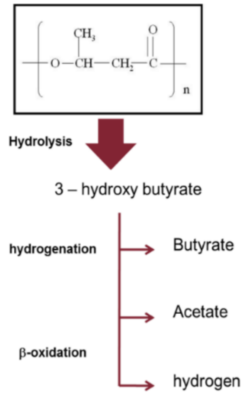


Functional prediction



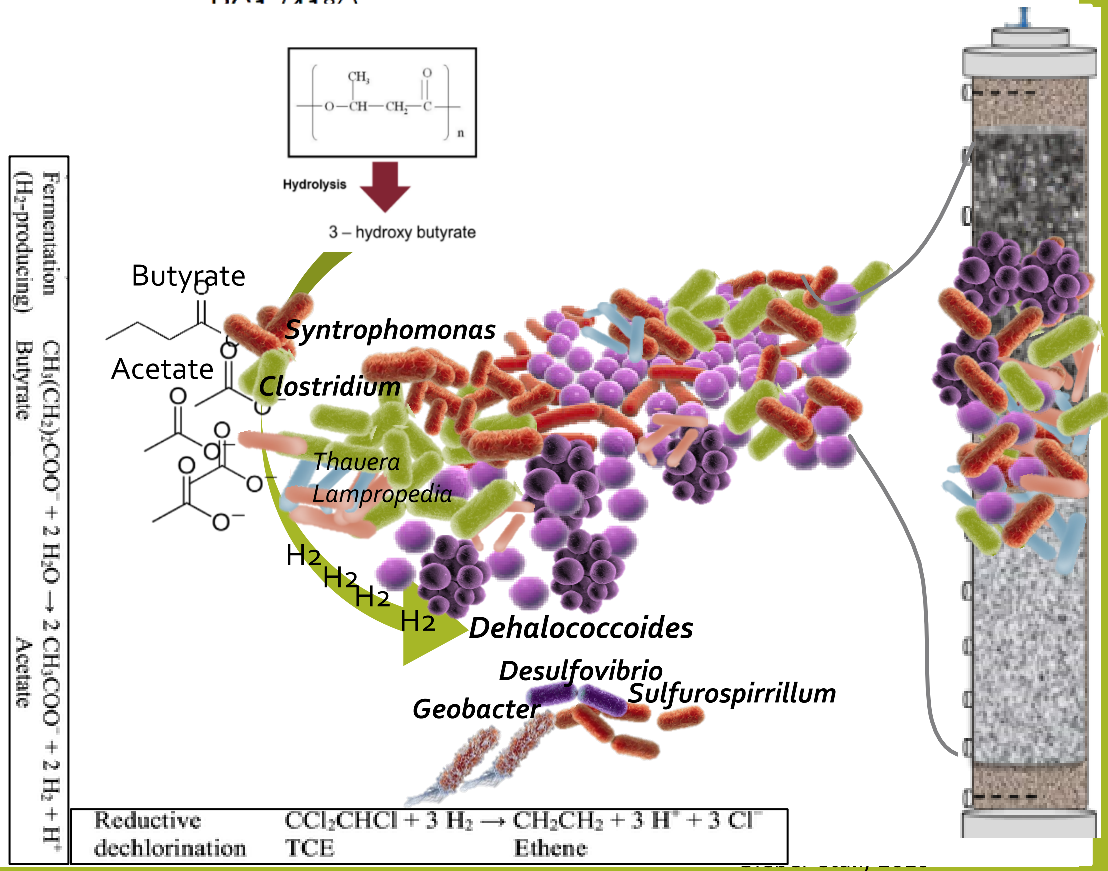




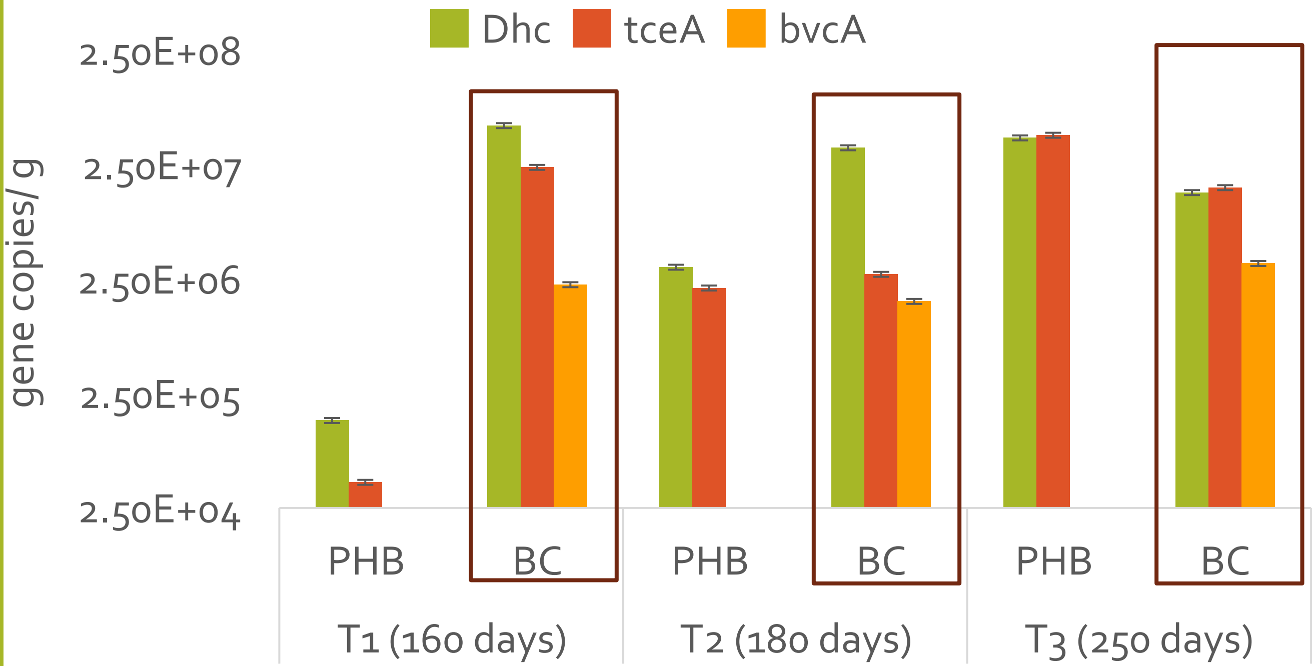


Syntrophomonas (Firmicutes):

- metabolize fatty acids syntrophically in association with hydrogen/formate-using microorganisms;
- Capable of PHA synthesis



Dhc quantification



Dhc also found in the PHB zone→ probably some «backdiffusion» towards PHB zone (?)

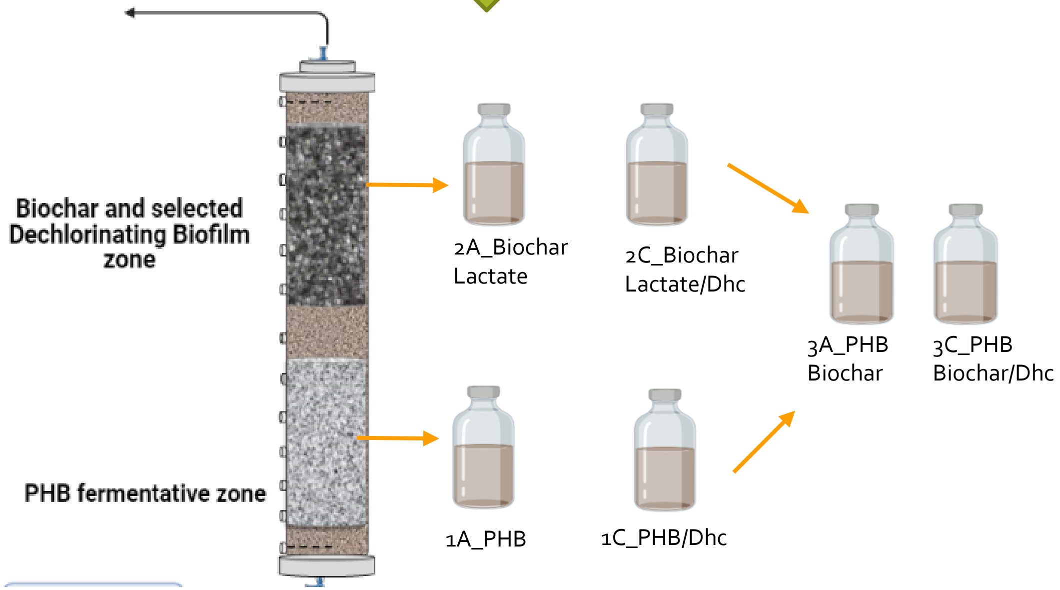
After the ricirculation, *Dhc* increased in the PHB zone

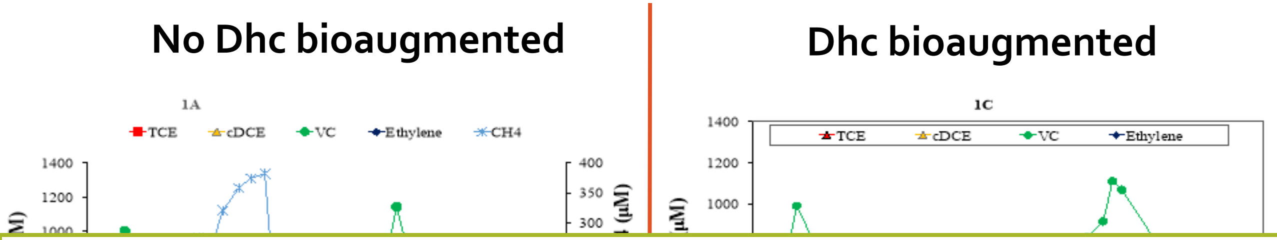
The anaerobic mineral medium positively affected *Dhc* abundance

- *Dhc* actively grows on Biochar → Biochar sustains biofilm-formation but does not provide nutritional requirements for growth and RD activity → no electron trasfer systems known from biochar to *Dhc* cells!
- *Dhc tceA*-carrying strains were the most abundant and *bvcA* was found only in the BC zone → in line with VC formation

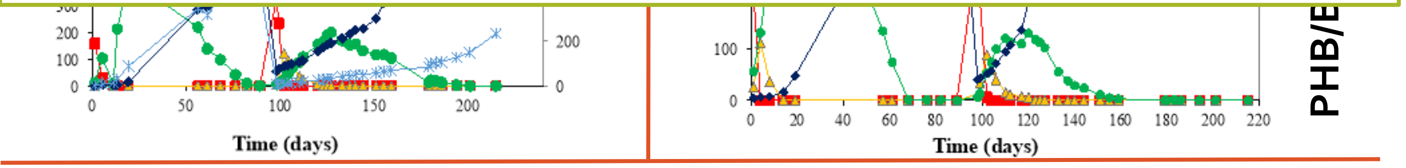
Why RD stalled at VC??

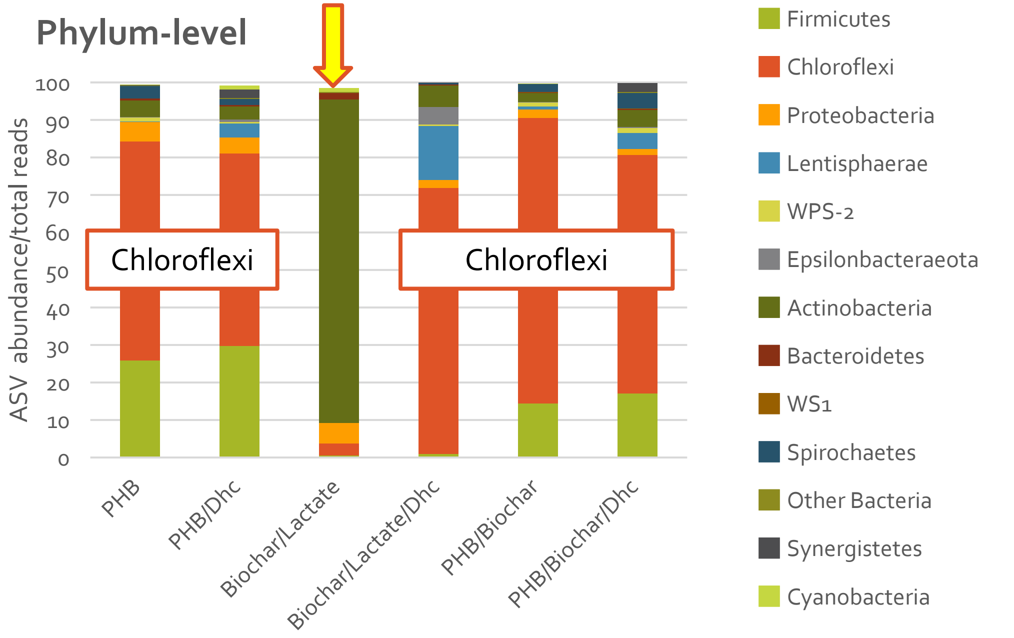
MICROCOSM STUDY

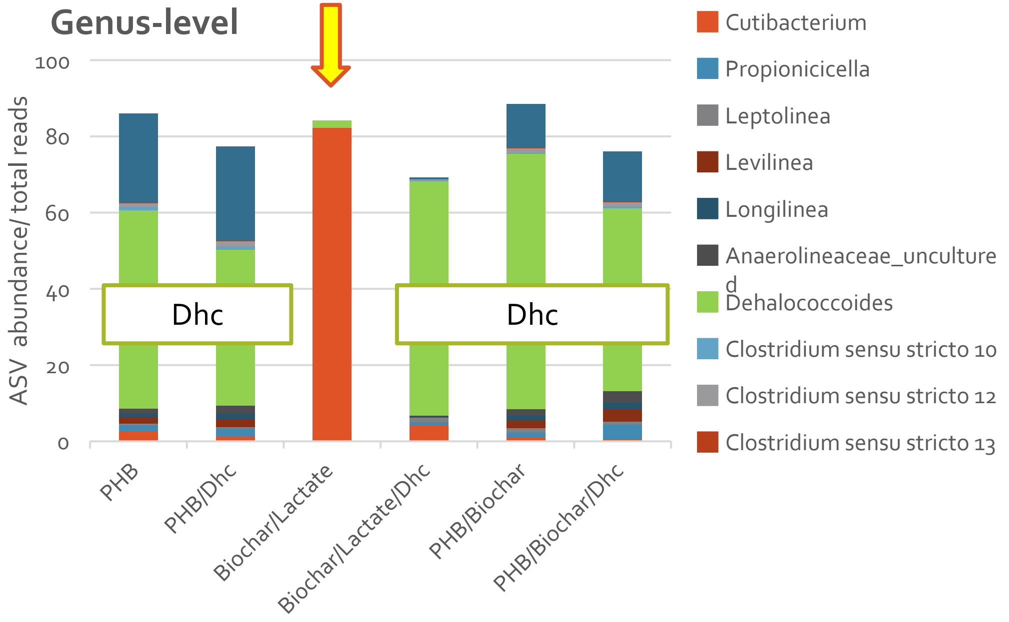




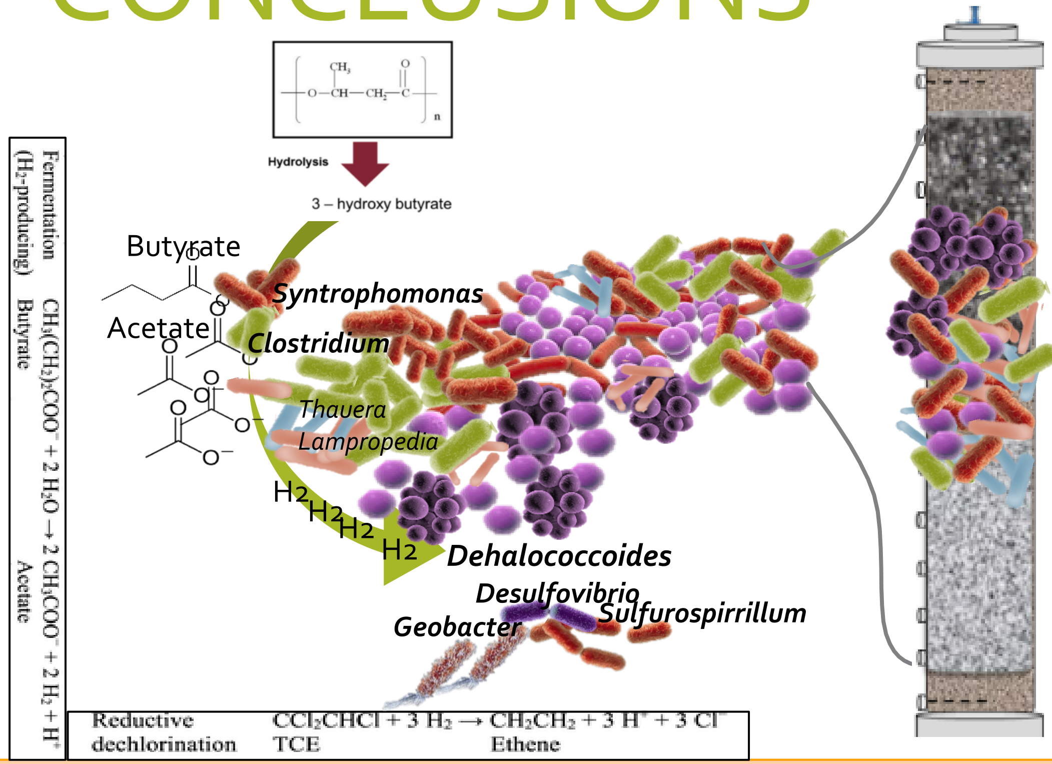
- ✓ TCE-to-ethylene was succesfully completed in all the microcosms set-up from the mini pilot PHB/Biochar reactor;
- ✓ *Dhc* dominated the microbial community also in the non-bioaugmented microcosm;
- ✓ *Clostridium* as the main PHB-fermentative bacteria, except for Biochar microcosm (*Cutibacterium*);
- → HRT of the mini pilot PHB/Biochar reactor lowered *Dhc* activity in VC-to-ethylene step
  - ❖ *Dhc strains usually grow slowly with a doubling time of 1 to 2 days and have complex nutritional requirements!*







CONCLUSIONS



❖ ***SOME ISSUES TO BE ADDRESSED IN THE NEXT ACTIVITIES***

- *Screening of the genes coding for hypothetical proteins in the extracted genomes*
  - *Reductive dehalogenases of the OHRB-community*
  - *PHB depolymerase*

- *Evaluation of the optimum HRT, by balancing reactor performances and management (e.g. volume of the feeding medium), with Dhc growth-requirements (e.g. doubling time, nutritional supply by the microbial community) to fulfill the complete TCE dechlorination to ethylene*

✓ **Bio-based circular economy** spans various multidisciplinary areas, including the utilization of biological resources for bioremediation purposes → many efforts are still needed to fully exploit its **advantages in the bioremediation technologies.**

✓ **PHB and Biochar** have great potential as bio-based materials to sustain and support **Reductive Dechlorination processes**

✓ The mini-pilot scale PHB/Bioreactor successfully prompted RD processes:

→ **A core microbiome established in the PHB/Biochar reactor**

- __PHB zone__: fermentation to butyrate/acetate/H2 sustains the growth and activity of *Dhc* and other OHRB in the system (*Sulfurospirillum, Geobacter, Desulfovibrio*)
- __Biochar zone__: biomaterials that support the "biofilm-formation" of *Dhc* → once *Dhc* colonized the PHB zone, it prefers the direct availability of electrons derived from fermentation products
- The ricirculation of tap water from Biochar to PHB zone and then the feeding with the anaerobic mineral medium, **prompted RD activity and growth of *Dhc*, the latter preferentially established in the PHB zone**

✓ In the reactor, RD stalled at VC probably because of the low HRT → microcosm study demonstrated the whole TCE-to-ethylene dechlorinating capability of the reactor-microbial community

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