# PhD DISSERTATION PROJECTS

XXIII WORKSHOP ON THE DEVELOPMENTS IN THE ITALIAN PhD RESEARCH ON FOOD SCIENCE, TECHNOLOGY AND BIOTECHNOLOGY
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# *Biodegradation of chlorinated ethenes and aromatic compounds in contaminated aquifer*

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The aim of this PhD project is to analyse the bacterial population in a site contaminated by different compounds and subjected to different biostimulation treatments through permeable reactive barriers technique. In particular, bacterial populations involved in the degradation of chloroethenes and aromatic compounds are investigated by environmental genomics and by cultivation technique.

## Biodegradazione di eteni clorurati e composti aromatici in un acquifero contaminato

Lo scopo del progetto di dottorato è analizzare e caratterizzare la popolazione microbica in un sito contaminato da diversi composti e soggetto a diversi trattamenti di biostimolazione attraverso la tecnica delle barriere permeabili reattive. In particolare, saranno investigate le popolazioni batteriche coinvolte nella degradazione dei composti clorurati e aromatici attraverso tecniche di genomica ambientale e tecniche di coltivazione.

## 1. State-of-the-Art

Chloroethenes are important contaminants all over the world. They are widely used as solvents in industrial activity and in different household products as dry-cleaning solvents and paint products. Because of their toxicity, in the last years, their used is decreasing. Their presence in the environment is due to inadequate disposal methods in the past (Beamer et al. 2012; Moran et al., 2007). They are not only a human activity product, but they are produced by different natural process (for example volcanic eruptions) (Gribble, 1992; Gribble, 1994; Abrahamsson et al.,1995). For this reason, the presence of bacteria that can degrade these compounds was hypothesized.
Nowadays, four degradative pathways are known.

- anaerobic reductive dechlorination: chloroethenes are used as electron acceptors and hydrogen as electron donor. Each chloride atom is replaced with a hydrogen atom, allowing the degradation from perchloroethene (PCE), via trichloroethene (TCE), dichloroethene (*cis*-DCE) and vinyl chloride (VC) down to ethene. Dechlorination rate decrease with the decrease of the number of chloride atoms, for this reason is common an accumulation of *cis*-DCE and VC in contaminated sites (Bradley, 2003; Smidt & de Vos, 2004; Futagami et al., 2008; Schmidt & Tiehm, 2008; Abe et al., 2009).
- aerobic oxidative degradation: chloroethenes can be used as carbon and energy source for the bacterial growth under aerobic conditions. Aerobic oxidative degradation is more efficient in the degradation of chloethenes with a low number of chlorine substituent.
- anaerobic oxidative degradation: Only DCE and VC are involved in this pathway of degradation. It is improved in particular conditions like Fe(III)-reducing conditions, Mn(IV)-reducing conditions and in presence of humic acid (Bradley & Chapelle, 1996; Bradley et al., 1998).
- co-metabolic biodegradation: chloroethenes are degraded by bacteria that don't use these compounds as carbon source but use the same enzyme involved in the degradation of the carbon source. Co-metabolic biodegradation is widely founded in aerobic conditions but in anaerobic conditions it is not well documented. In aerobic co-metabolic degradation, known co-substrate are ammonium, cumene, ethane, ethene, phenol, propane, methane and toluene. Anaerobic co-metabolic degradation is less efficient than anaerobic reductive dichlorination.

A possible efficient method of bioremediation is the use of sequential anaerobic-aerobic system to improve all chloride ethenes degradation pathways.

Aromatic hydrocarbons that are widely found in contaminated site are: benzene, toluene, ethylbenzene and xylenes. They are called BTEX. They are the products of release of compounds by petrochemical industry. BTEX have higher water solubility. They are carcinogenic and neurotoxic to humans (Lueders, 2017).
Two different BTEX degradation pathways are known: aerobic and anaerobic. BTEX aerobic degradation is well studied. Instead, the research of anaerobic degradation of these compounds is increasing in the last years.
During aerobic degradation, sequential oxidations produce from aromatic ring a catechol structure that is degraded into Krebs cycle intermediates (Harayama & Rekik, 1993).
Anaerobic BTEX degradation starts with a "fumarate addition" to methyl group of BTEX compound (Fuchs et al., 2011; Rabus et al., 2016). Then, CoA is added and the ring is cleavage. A followed oxidation-like reaction produces acetyl-CoA that enters into Krebs cycle.

## 2. PhD Thesis Objectives and Milestones

PhD thesis project is composed by different phases summarized into the following Gantt diagram in Table 2:

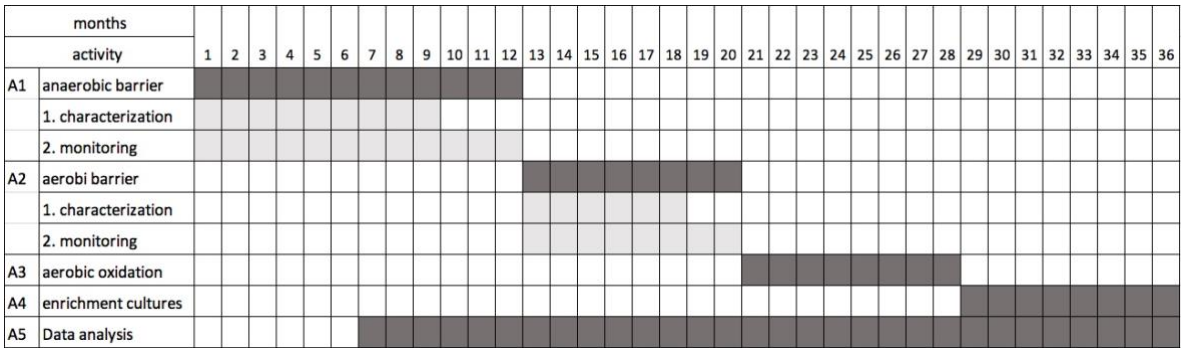
A1) **Monitoring of degradative activity and characterization of microbial community involved in reductive dehalogenation at the site.** Next generation sequencing are applied to analyse the structure of the microbial community. Quantification of phylogenetic and functional biomarkers is achieved by Real Time q-PCR of environmental DNA. Laboratory-based microcosms are used to validate microbial degradative activity.

A2) **Monitoring of degradative activity and characterization of microbial community involved in aerobic aromatic biodegradation.** Microbial isolation of BTEX degrading bacteria is achieved by enrichment technique. Monitoring of BTEX degrading population at the site is performed by designing new molecular probes to be used in q-PCR reactions.

A3) **Focus on the aerobic oxidative degradation of vinyl chloride.** Due to scarce information, bacterial populations that can oxidize vinyl chloride in aerobic conditions will be enriched and studied. A collaboration with a foreign institute of research is forecasted.

A4) **Data elaboration**

**Table 2** *Gantt diagram for this PhD thesis project.*

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