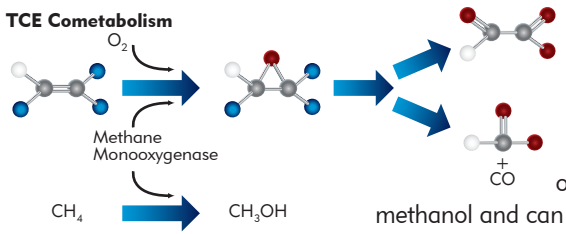
CENSUS – Cometabolism of Chlorinated Ethenes

mi CENSUS®
MOLECULAR BIOLOGICAL TOOL

Detect and quantify methanotrophs and other bacteria capable of aerobic cometabolism of chlorinated solvents

Under aerobic conditions, several different types of bacteria including methane-oxidizing bacteria (methanotrophs), ammonia-oxidizing bacteria, and some toluene/phenol-utilizing bacteria can cometabolize or co-oxidize trichloroethene (TCE), dichlorethene (DCE), and vinyl chloride (VC). In general, cometabolism of chlorinated ethenes is mediated by mono-oxygenase enzymes with "relaxed" specificity that oxidize a primary (growth supporting) substrate and co-oxidize the chlorinated compound.



In the presence of methane, for example, methano-trophs produce methane mono-oxygenases which oxidize methane to methanol and can also co-oxidize TCE.

For aerobic cometabolism to be a primary treatment mechanism, three key factors must be present or supplied:

- A primary (growth supporting) substrate
- Oxygen
- Bacteria capable of producing non-specific monooxygenases

Although a variety of primary substrates including toluene, phenol, propane, and ammonia can foster production of different monooxygenases capable of cometabolic oxidation, methane is frequently the most readily available primary substrate.

Most methanotrophs are only capable of producing particulate methane monooxygenase (pMMO) which is capable of aerobic cometabolism but often at lower rates. Other methanotrophs are capable of producing both pMMO and soluble methane monooxygenase (sMMO) enzymes which in general are believed to be capable of greater rates of aerobic cometabolism.

Microbial Insights has developed CENSUS targets for quantification of:

- **total methanotrophs (qMOB) and**
- **soluble methane monooxygenase (qsMMO) genes expressed by a subset of methanotrophs**

Target	Code	Contaminants	Environmental Relevance / Data Interpretation
Methanotrophs	qMOB	TCE, DCE, VC	Targets two types of methane oxidizing bacteria (methanotrophs) Indicates the potential for cometabolic oxidation of TCE
Soluble Methane Monooxygenase	qsMMO	TCE, DCE, VC	Targets the soluble methane monooxygenase gene Soluble methane mono-oxygenases are generally believed to support faster cometabolism of TCE
Propane Monooxygenase	qPPO	TCE, DCE, VC	Propane can be added as a primary substrate to promote cometabolic oxidation of TCE
Toluene Dioxygenase	qTOD	TCE, DCE, VC	Applicable at mixed waste sites where BTEX and TCE are co-contaminants When expressed, toluene dioxygenase is capable of cometabolism of TCE
Ring-Hydroxylating Toluene Monooxygenase	qRMO	TCE, DCE, VC	Applicable at mixed waste sites where BTEX and TCE are co-contaminants When expressed, toluene monooxygenases are capable of cometabolism of TCE

The combination of the qMOB and qsMMO provides quantification of total methanotrophs and those specifically capable of producing sMMO to aid in the evaluation of aerobic cometabolism as a treatment mechanism.

As mentioned previously, a variety of substrates can support cometabolism of TCE. Propane addition has been shown to stimulate propane utilizing bacteria also capable of aerobic cometabolism. Likewise, the relaxed specificity of some aromatic oxygenases also permits cometabolism of chlorinated ethenes. Depending on site conditions and corrective actions taken, these pathways may also be involved in TCE degradation.

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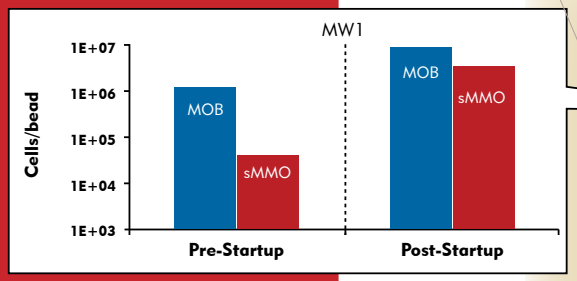
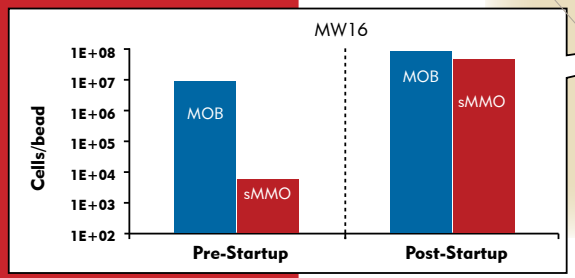
Cometabolic TCE Biodegradation

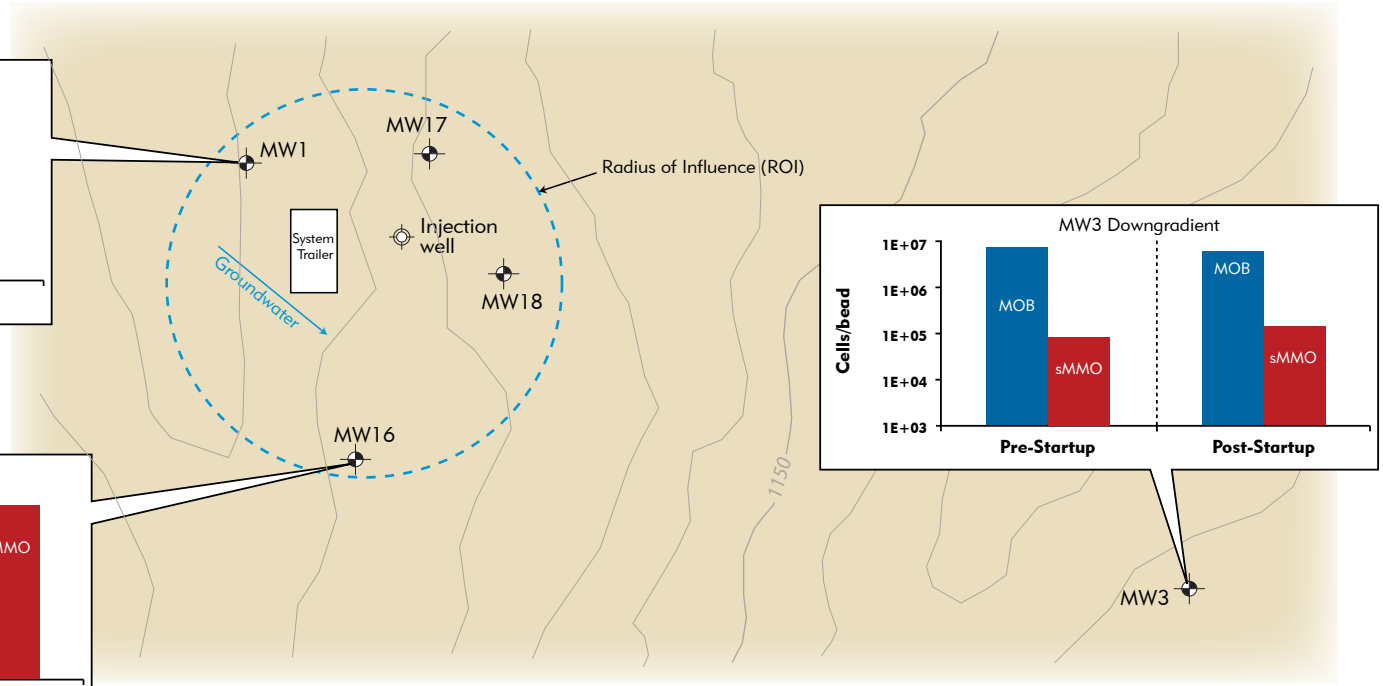
- A pilot study using Bio-Trap® Samplers and quantification of methanotrophs was designed to evaluate the efficacy of a remedial system designed to stimulate cometabolic biodegradation of TCE.
- The technology consisted of injecting a gaseous mixture of methane (carbon source), air, nitrous oxide, and triethyl phosphate to promote growth and activity of methanotrophs.
- Injection operations were performed at one well for approximately 3 months.
- Bio-Trap® Samplers were deployed in surrounding wells located ~30 ft, 40 ft, and >150 ft from the injection well prior to and during system operation.
- Recovered Bio-Trap® Samplers were analyzed for total methanotrophs (qMOB) and Type II methanotrophs capable of producing soluble methane monooxygenase (qsMMO).
- Within the radius of influence (ROI) of the injection system (MW1 and MW16),
  - total methanotrophs increased by 1-2 orders of magnitude,
  - soluble methane monooxygenase genes increased by over 4 orders of magnitude, and
  - TCE concentrations decreased.
- Outside the system ROI (MW3),
  - no significant changes were noted in methanogens or methane monooxygenase genes and
  - TCE concentrations did not decrease appreciably.

Conclusions:

- Loss of TCE corresponded to increases in methanotroph populations and soluble methane monooxygenase gene copy numbers.
- The remediation technology stimulated growth of methanotrophs and promoted cometabolism of TCE.







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