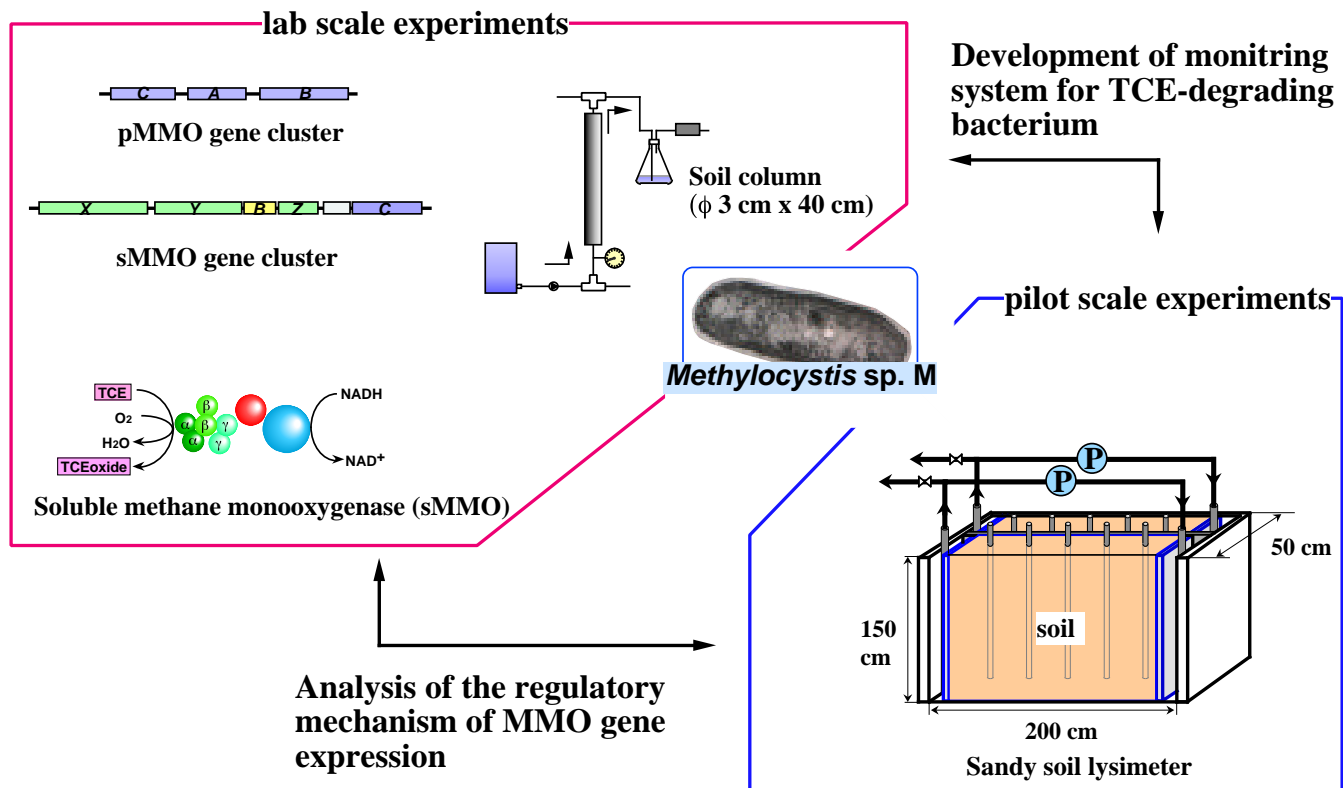


Development of Bioaugmentation Technologies for Cleaning Up Soil and Groundwater Contaminated with Trichloroethylene

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The contamination of groundwater and soil with volatile chlorinated aliphatic compounds, such as trichloroethylene (TCE), has become a serious environmental problem. While TCE has a large number of industrial applications as a solvent and degreaser, inadequate disposal techniques or accidental spillage have threatened drinking water supplies because of its toxicity and carcinogenicity. Various soil and groundwater clean-up technologies are now being developed. Physical and chemical methods such as soil incineration, vapor-extraction and pumping-up and treatment are very common approaches. Bioremediation is one of the most promising new technologies for cleaning up soil and groundwater contamination, because of its low cost and complete destruction of pollutants. However, we have not much experience on bioremediation in Japan.

Several studies of microbial aerobic degradation of TCE have shown that TCE can be degraded by toluene-oxidizing bacteria, phenol-oxidizing bacteria and methanotrophic bacteria. These TCE-degrading bacteria require some organic substrates, such as toluene, phenol and methane, to induce their TCE-degradation activity. Methane is less toxic to humans than aromatic compounds. Therefore, methanotrophs have been receiving attention for their possible application to the bioaugmentation of polluted soil and groundwater.

Methanotrophic bacteria are able to oxidize methane to methanol by the catalytic function of methane monooxygenases (MMOs). Two different types of MMO are known: a membrane-bound particulate enzyme (pMMO), which is found in all methanotrophs, and a soluble cytoplasmic enzyme (sMMO), which has been found in several strains. The substrate specificity of pMMO is narrow. In contrast, sMMO has broad substrate specificity and can oxidize a wide range of alkanes, alkenes and aromatic compounds, including TCE.

We have isolated a methanotroph, *Methylocystis sp. M*, which has high TCE-degradation activity, and studied on its TCE degradation characteristics in lab scale experiments. For effective application of *Methylocystis sp. M* to bioaugmentation, we have focused on following 3 subjects.

1. Development of monitoring system for TCE-degrading bacterium, *Methylocystis sp. M*, and Construction of simulation model of fate of *Methylocystis sp. M* in groundwater
2. Analysis of the regulatory mechanism of MMO gene expression, and Decision of optimum condition for expression of TCE-degrading activity
3. Development of efficacy measurement technologies and effective application methods of *Methylocystis sp. M* in pilot scale experiments (using sandy soil lysimeter)