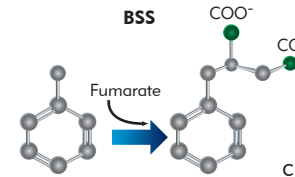
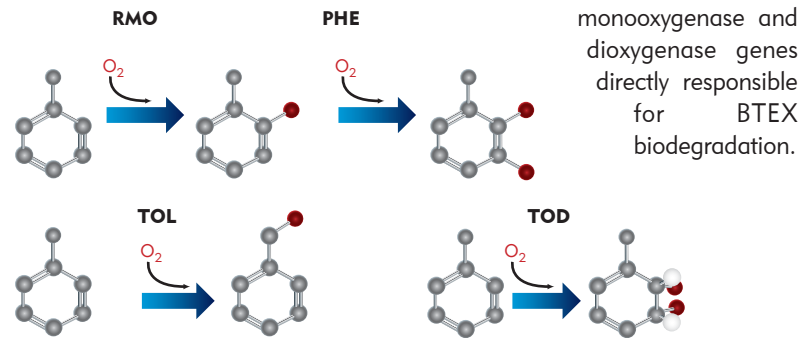




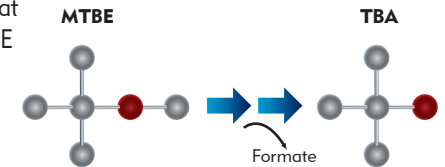
Detect and quantify bacteria responsible for biodegradation of BTEX and MTBE

Under aerobic conditions, some bacteria can catabolize aromatic hydrocarbons (benzene, toluene, ethylbenzene, xylenes (BTEX) and PAHs). The initial incorporation of oxygen catalyzed by aromatic oxygenase enzymes is often the rate-limiting step in aerobic BTEX bioremediation. Therefore, CENSUS® targets were developed for



BTEX compounds are also biodegraded under anoxic and anaerobic conditions. Although the catabolic pathways for anaerobic BTEX biodegradation are not as well characterized as the aerobic pathways, benzylsuccinate synthase (BSS) has been shown to be involved in the anaerobic biodegradation of toluene. CENSUS® can target the *bssA* gene encoding BSS to assess anaerobic BTEX biodegradation.

CENSUS® can target one of the few bacteria isolated that is capable of growth on MTBE (*Methylibium petroleiphilum* PM1) to evaluate the potential for aerobic MTBE bioremediation.

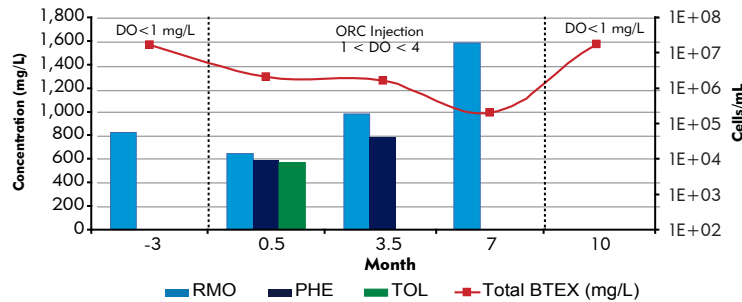


Target	Code	Contaminants	Environmental Relevance / Data Interpretation
Toluene Dioxygenase	qTOD	Benzene, Toluene Ethylbenzene	Catalyzes biodegradation of benzene and toluene by incorporation of oxygen into the aromatic ring
Phenol Hydroxylase	qPHE	BTEX	Catalyzes further oxidation of BTEX compounds Presence indicates the potential for aerobic BTEX biodegradation
Ring-hydroxylating Toluene Monooxygenases	qRMO	BTEX	Catalyzes the initial (and sometimes second) hydroxylation of BTEX compounds Presence indicates the potential for aerobic BTEX biodegradation
Toluene Monooxygenase	qTOL	Toluene, Xylene	Attacks toluene and xylenes at the methyl group
Naphthalene Dioxygenase	qNAH	Naphthalene	Catalyzes aerobic biodegradation of naphthalene and other PAHs by incorporation of oxygen into the aromatic ring
MTBE utilizing PM1	qPM1	MTBE	Targets <i>Methylibium petroleiphilum</i> PM1, one of the few bacteria isolated that is capable of growth on MTBE Indicative of the potential for aerobic MTBE biodegradation
Benzylsuccinate Synthase	qBSS	Toluene, Xylene	Targets gene encoding enzyme in anaerobic biodegradation of toluene Indicative of the potential for anaerobic BTEX biodegradation

When combined with chemical and geochemical groundwater monitoring programs, CENSUS® results are valuable tools to determine the following:

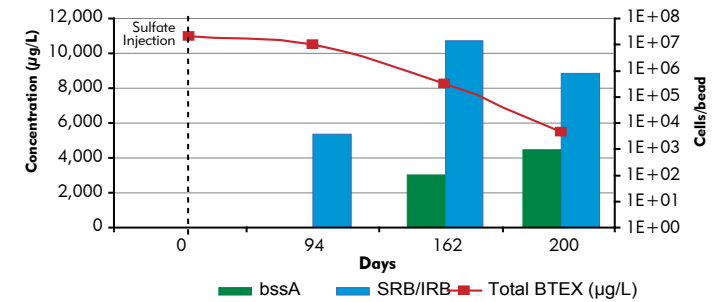
- feasibility of monitored natural attenuation (MNA)
- effectiveness of enhanced aerobic bioremediation (e.g. injection of oxygen releasing materials) of BTEX and MTBE
- potential for aerobic PAH biodegradation
- feasibility of anaerobic BTEX biodegradation (qBSS)
- effectiveness of enhanced anaerobic BTEX biodegradation (e.g. sulfate injection)

Aerobic BTEX Bioremediation



- Modeling of historical BTEX concentrations indicated that the dissolved BTEX plume was stable but not decreasing.
- RMO, a type of toluene monooxygenase gene, was detected in groundwater samples indicating the potential for aerobic BTEX biodegradation.
- However, dissolved oxygen (DO) concentrations were typically less than 1 mg/L suggesting that oxygen availability may have been limiting biodegradation under monitored natural attenuation (MNA) conditions.
- ORC® (oxygen releasing compound) was injected at the site (0 months).
 - DO increased to between 1 and 4 mg/L
 - BTEX concentrations decreased
 - RMO increased from 10⁴ to 10⁷ cells/mL
 - TOL and PHE gene copies increased from below detection levels to 10⁴ cells/mL
- ORC® was depleted after 7 months
 - DO decreased to less than 1 mg/L
 - BTEX concentrations increased
 - RMO, PHE, and TOL were no longer detected
- Incorporation of CENSUS® for aromatic oxygenase genes into the monitoring program provided an additional line of evidence indicating that the ORC® injection promoted aerobic BTEX biodegradation.

Anaerobic BTEX Bioremediation



- Natural attenuation of the central portion of a groundwater plume contaminated with BTEX had been limited due to its geology (fractured sedimentary bedrock) and significant underground utilities that prevented more traditional enhancement via aerobic biostimulation.
- Following sulfate injection to stimulate anaerobic bacteria and enhance BTEX biodegradation rates, concentrations of sulfate and iron reducing bacteria (SRB/IRB) increased four orders of magnitude between day 94 and day 162.
- Concentrations of the gene benzylsuccinate synthase (bssA) that encodes a key enzyme in the initial anaerobic breakdown of toluene and xylenes also showed a steady increase over time from below detection to ~10³ cells/bead.
- Sulfate injection increased the overall rate of degradation of BTEX components by more than two fold.
- Monitoring the CENSUS® results for the bssA gene provided a direct line of evidence indicating that the observed decreases in BTEX concentrations were a result of enhanced biodegradation.