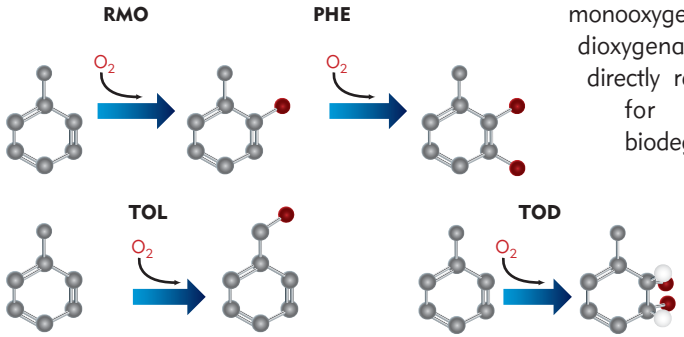
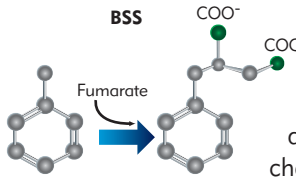
# miCENSUS®

MOLECULAR BIOLOGICAL TOOL

*Detect and quantify bacteria responsible for biodegradation of BTEX and MTBE*

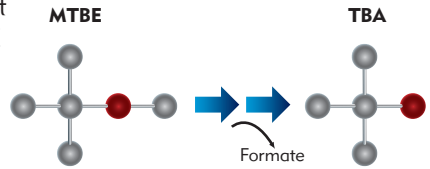
Under aerobic conditions, some bacteria can catabolize aromatic hydrocarbons (benzene, toluene, ethylbenzene, xylenes (BTEX) and PAHs). The initial incorporation of oxygen catalyzed by aromatic oxygenase enzymes is often the rate-limiting step in aerobic BTEX bioremediation. Therefore, CENSUS® targets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




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 *bss*A 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.

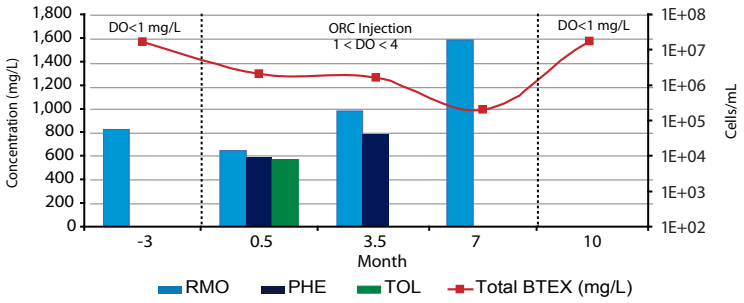



| Target | Code | Contaminants | Environmental Relevance / Data Interpretation |
|---|---|---|---|
| Ring-hydroxylating Toluene Monooxygenases | qRMO | BTEX | Catalyzes the initial (and sometimes second) hydroxylation of BTEX compounds<br>Presence indicates the potential for aerobic BTEX biodegradation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

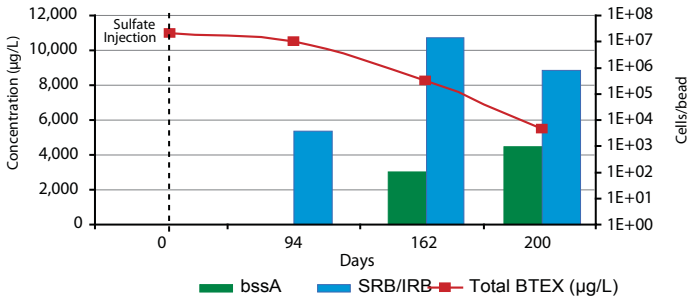
## Aerobic BTEX Bioremediation




- 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 $10^4$ to $10^7$ cells/mL
  - TOL and PHE gene copies increased from below detection levels to $10^4$ 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.

CENSUS ®
MOLECULAR BIOLOGICAL TOOL

## 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 ~$10^3$ 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.

microbialinsights
www.microbe.com
ERR www.erraus.com.au
Unit 59/148 Chesterville Road
Moorabbin Vic 3189 Australia
Phone: +61 3 9555 3800