

**MONITORING GENE EXPRESSION TO EVALUATE THE EFFECTIVENESS OF
OXYGEN INFUSION AT A GASOLINE-IMPACTED SITE**

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Evaluation of corrective actions designed to enhance biodegradation of petroleum hydrocarbons and fuel oxygenates should include chemical, geochemical, and microbiological lines of evidence. At gasoline-impacted sites, temporal monitoring and analysis of trends in dissolved benzene, toluene, ethylbenzene, xylene (BTEX) and methyl tert-butyl ether (MTBE) concentrations can be used to document contaminant loss and provide the first line of evidence. Likewise, temporal monitoring of geochemical parameters can reveal changes in redox status resulting from site activities, changes in electron acceptor availability, and can provide a second indicator of enhanced biodegradation. The third and potentially most direct line of evidence to evaluate the ability of a remediation technology to stimulate biodegradation is to quantify expression of the genes and activity of the organisms responsible for contaminant biodegradation. In the current study, quantitative polymerase chain reaction (qPCR) and reverse-transcription qPCR (RT-qPCR) were used to monitor microbial populations and gene expression to evaluate the effectiveness of an oxygen infusion system to promote aerobic biodegradation of BTEX and MTBE. During system startup and continuous operation, dissolved oxygen (DO) levels at the injection points were greater than 30 mg/L, contaminant concentrations decreased, and transcription of aromatic oxygenase genes (toluene dioxygenase and phenol hydroxylase) and activity of MTBE-utilizing strain *Methylibium petroleiphilum* PM1 increased by as many as four orders of magnitude in response to system operation. Moreover, aromatic oxygenase gene transcription and PM1 activity increased at downgradient locations despite the fact that DO levels in downgradient wells did not appreciably increase during system operation. Conversely, BTEX- and MTBE-utilizing populations, aromatic oxygenase gene transcription, and PM1 activity substantially decreased when the system was temporarily deactivated. Overall, traditional groundwater analyses combined with monitoring gene expression provided the three lines of evidence needed to conclusively demonstrate that the oxygen infusion system effectively promoted BTEX and MTBE biodegradation at the site.

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