Development of Multiple Reaction Monitoring (MRM) Proteomic Assay for the Detection of Methyl Tertiary-Butyl Ether (MTBE) Degradation Peptides

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Background/Objectives. The presence of high concentrations of methyl tertiary-butyl ether (MTBE) is usually a result of leaks from underground storage tanks used to store gasoline containing this additive. Since MTBE is a persistent and slow degrading contaminant, its presence may cause threat to human health and the environment. Thus, MTBE contamination of soil and groundwater poses a problem to Remediation Project Managers (RPMs) while cleaning up their sites.

Several bacterial species have the ability to perform complete conversion of MTBE into completely benign products. The microbial characterization of complex sites contaminated with MTBE with tools complementary to qPCR (proteomics and metagenomics) provide important information which can be used to prioritize and design more successful remediation strategies. New advanced molecular tools such as targeted proteomics based in multiple reaction monitoring (MRM) assay have a great potential to provide important details on the biochemical processes involved in the degradation of contaminants. This information can be used to document the natural attenuation of MTBE and/or to design more effective bioremediation strategies to accelerate the rate of biotransformation under anaerobic conditions (via co-metabolic processes) or under aerobic conditions (via bioaugmentation and direct mineralization).

Approach/Activities. LC-MS-targeted proteomics approaches, based on MRM has been proven to be effective for the analysis of markers related to degradation of chlorinated ethenes. In an MRM experiment, the two mass analyzers of a triple quadrupole mass spectrometer are used to isolate the peptide ion of interest and the derived fragment ion(s). This enables the precise quantification of peptides in complex biological background, and thus indirectly the corresponding proteins present in the samples. The applicability of the method to multiplex analytes allows the panels of proteins across multiple samples to be quantified by monitoring the signals of a specific transition (precursor – fragment ion pairs) during a predefined elution window. Here, we propose a protein-based diagnostics tool that offers a viable alternative for direct quantification of proteins involved in degradation of MTBE and its degradation products such as tertiary butyl alcohol (TBA).

Results/Lessons Learned. In the present study, the primary results obtained via discovery shotgun proteomics identify a variety of peptides that may serve as biomarkers of MTBE degradation. In the next steps, targeted proteomics was used to develop accurate detection of specific peptides in environmental samples. The data presented demonstrate validity of a step wise approach in which 16S sequencing and gene-specific sequencing aid targeted proteomic in detection of key peptides involved in the biodegradation of MTBE.