Application of Selective Reaction Monitoring (SRM) Proteomics to Quantify Reductive Dehalogenase Peptides (RDases) in Microbial Consortium SDC-9

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Background/Objectives. Mass spectrometry-based proteomic strategies are of increasing interest as means to assess and to predict chlorinated solvent biodegradation activity in groundwater aquifers. Many organism- and process-specific biomarker genes for monitoring reductive dechlorination have been identified, and the vinyl chloride reductive dehalogenase (RDase) genes *bvcA* and *vcrA* serve as biomarkers for ethene formation at sites impacted with chlorinated ethenes. Whereas the abundance of RDase genes alone provides a measure of reductive dechlorination potential, the quantitative assessment of RDase proteins can provide information about actual reductive dechlorination activity and potentially inform about dechlorination rates. A selected reaction monitoring (SRM) proteomics assay was developed to identify and quantify biomarkers specific to the degradation of chlorinated ethenes. The bioaugmentation consortium SDC-9, which can degrade a variety of different chlorinated ethanes and ethenes, was used to develop and test the SRM approach.

Approach/Activities. A liquid chromatography – mass spectrometry (LC-MS) targeted proteomic approach was used to detect and quantify biomarkers related to degradation of chlorinated ethenes. In this study, triple quadrupole MS was used to isolate the peptide ion of interest and the derived fragment ion(s). This enables the precise quantification of peptides in a complex biological background, and thus indirectly the corresponding proteins present in the samples. The application of this approach allowed the simultaneous analysis of multiple proteins of interest including the quantification of a selected set of RDase peptides.

Results/Lessons Learned. In the present study, the SRM approach was applied to detect and quantify selected RDase peptides in SDC-9 grown with chlorinated ethenes. Several elements of the proteomic process such as internal controls, external controls, retention time, collision energy, presence of carrier proteins and protein recovery were optimized during the proteomic analyses. The SRM approach was then applied to determine the lower level of detection and quantification of selected RDase peptides and finally to quantify native peptides expressed by the SDC-9 culture. The results of this assay demonstrated that LC-MS protein detection is a sensitive and accurate method to quantify expressed proteins.