Emulsified vegetable oil stimulates sulfate-reducing communities for U(VI) reduction at a contaminated aquifer via pyrosequencing of dissimilatory sulfite reductase genes

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Sulfate reducing bacteria (SRB) are predominant and play an important role in accelerated, sustained reduction of U(VI) during bioremediation at contaminated sites, but the SRB community diversity, composition and structure are poorly understood. In this study, 454 pyrosequencing of dissimilatory sulfite reductase genes (*dsrA*) was used to analyze 44 groundwater samples collected from a U(VI)-contaminated aquifer (Oak Ridge, TN) undergoing bioremediation with an amendment of emulsified vegetable oil (EVO). Acetate was detected as a representative of the intermediates (e.g. H_2, CO_2, glycerol, propionate, butyrate) from EVO biodegradation, which stimulated the reduction of significant electron acceptors (sulfate, nitrate, Fe, Mn) and U(VI) in the seven downgradient wells during the entire experimental period of 269 days. A total of 82,537 sequences were obtained, and a total of 15,084 OTUs were defined at a 90% similarity level among all 44 samples. While a majority of the sequences (84%) were founde to be *Deltaproteobacteria*, 0.4% were related to *Desulfotomaculum* in *Firmicutes,* and a considerable portion (21% at the genus level) was novel *dsrA* sequences. Significantly different SRB communities (P<0.01) were observed after EVO amendment. While novel *dsrA* sequences were predominant (98%) in the samples before EVO addition, *Desulfovibrio* and to lesser degrees, *Desulfobacterium* and *Desulfococcus* species overwhelmingly dominated the SRB communities after EVO injection. A temporal dynamics of these organisms relative to EVO amendment was observed, which was coordinated with the succession of microbial communities as well as EVO biodegradation and U(VI) reduction. When both species relative abundance and relative frequency of occurrence were taken into account, *Desulfovibrio* (60-80% of the SRB community) and *Desulfococcus* (20%) were indicator species from 4 to 31 days, and *Desulfobacterium* (50%) characterized the community at 80 days when extensive EVO degradation and U(VI) reduction occurred. *Desulfovibrio* species remained important indicators by 269 days when acetate production and U(VI) reduction were still detected, suggesting a long-term modification of the community. The indicators of the non-EVO samples were aligned with cultured *Desulfotomaculum* strains and the *Firmicutes*-like sequences recovered in this study, suggesting that *dsrA* genes of non-EVO U(VI)-contaminated environments may be phylogenetically distant from those enriched by EVO amendment. A regression analysis showed a significant influence of *Desulfovibrio* dominance over the U(VI) concentration in groundwater (P<0.001). *Desulfovibrio* species could play an important role in the accelerated and sustained U(VI) reduction at this site. Further analysis of *in situ* activity of *Desulfovibrio* and their relationship with U (VI)-reduction should provide more information for bioremediation of this contaminated DOE site.