Geophysical Research Abstracts, Vol. 8, 08498, 2006
SRef-ID: 1607-7962/gra/EGU06-A-08498

Competititive dissimilatory iron reduction during *in situ* bioprecipitation of metals

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In situ precipitation of metals from contaminated groundwater by acceleration of biogeochemical processes that may occur naturally, is a promising sustainable technology to remediate sites polluted by inorganics. This technique uses the stimulation of the natural ability of sulphate reducing bacteria to indirectly precipitate the metals as metalsulphides by injection of organic substrates. "Sustainable" implies that remediation will be faster, more economical and more environment friendly and offers a long term solution, because final metal concentrations in groundwater are lower, no solid waste is produced and no hazardous chemicals are being used. However, often these aquifers contain high concentrations of Fe, presumably present as Fe(III) minerals which may compete with the *in situ* bioprecipitation (ISBP) process by sulphate reducing bacteria (SRB). Based on thermodynamics, microbes are supposed to use the electron acceptors resulting in the highest energy yield. Once oxygen is completely used or becomes limiting, microbes utilize nitrate, followed by iron and later on sulphate. Nevertheless, in natural environments, microbe–soil/sediment–water interactions can be far more complex, and multiple metabolic pathways can occur simultaneously.

Dissimiliatory iron reduction (DIR) has indeed often been assumed to be a competitive process in our studies since high concentrations of Fe -likely to be $Fe(II)_{aq}$ at neutral or slightly acidic pH- have been measured in the groundwater used in our experiments. Although the formed ferrous iron might have a beneficial impact on several elements like Cr reduction, this dissolved Fe often means an additional request for sulphide and might result in mixed or coprecipitated metal-sulphide precipitates. In our laboratory feasibility studies have been conducted using both microcosm and column experiments containing aquifer material derived from different metal contaminated sites mainly located in the Flemish part of Belgium to evaluate the *in situ* bioprecipitation (ISBP) process. Different substrates including molasses, HRC®, and lactate were used to stimulate the biological activity and to determine its impact on metal removal, DIR and the dominating microbial population. The aim of this study was to provide molecular evidence based on the use of specific primers in quantitative PCR to explain the observed physico-chemical reactions. Besides monitoring parameters like pH, ORP, metals including Fe, we applied PCR-DGGE with specific primer sets targeting either the *dsrB* (dissimilatory sulfite reductase β-subunit)-genes or the 16S rRNA gene fragments of *Geobacteraceae* on samples derived from the microcosms/columns to study the competition between sulphate and Fe-reducing bacteria and the effect on the precipitation of heavy metals. Results of both analyses of metals as well as of molecular application of selected primers will be presented.