

Nitrogen, carbon, and sulfur isotope fractionation during heterotrophic and autotrophic denitrification reactions

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In batch culture experiments, we studied the isotope fractionation of nitrogen in nitrate, carbon in dissolved inorganic carbon, and sulfur in sulfate during heterotrophic and autotrophic denitrification of two bacterial strains (*P. aerofaciens* and *T. denitrificans*). Heterotrophic denitrification experiments were conducted with trisodium citrate as electron donor, autotrophic denitrification experiments were carried out with iron disulphide as electron donor. For heterotrophic denitrification experiments a complete nitrate reduction was accomplished, however bacterial denitrification with *T. denitrificans* is a slow process in which the degree of denitrification achieved in seventy days was 60 %. In the former experiment, systematic change of $\delta^{13}\text{C}_{DIC}$ with increase of DIC was observed during denitrification (enrichment factor ϵ_N was -2.3 ‰), suggesting the contribution of C of trisodium citrate. No SO_4^{2-} and $\delta^{34}\text{S}_{\text{SO}_4}$ changes were observed. In the latter experiment, clear fractionation of $\delta^{13}\text{C}_{DIC}$ during DIC consumption and $\delta^{34}\text{S}_{\text{SO}_4}$ during sulfur use of FeS_2 -S (around 2 ‰) were confirmed through denitrification ($\epsilon_N = -12.5$ ‰). The results of this batch experiment study are useful to understand the anaerobic bacterial denitrification processes in contaminated groundwater flow systems where a carbon source and/or pyrite are present. However, in natural aquifers, other anaerobic microbial activities such as sulfate reduction and methanogenesis would take place after or in the middle of the progress of the denitrification reaction, which play a decisive role changing isotope ratios of carbon and sulfur. Nevertheless, obtained results can be applicable in environments where complex simultaneous anaerobic reactions would not occur after, in the middle of the denitrification reaction, or at organic poor land that prevent further heterotrophic bacterial reactions to proceed.

Keywords: N-C-S stable isotope ratios, batch culture experiments, groundwater, denitrification