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ABSTRACT TITLE: Evolution of Methanogenesis during Biostimulation of Alluvial Sediments

ABSTRACT: We have been conducting a long-term column experiments to study the link between biostimulation and changes in permeability. Nine columns (4.8 cm ID, 30 cm long) were built with side ports at 2 cm intervals, which allow for dissolved species sampling and pressure change measurements along their vertical axis. The experiment has been operating now for approximately 700 days. One column is equipped with electrodes to determine the induced polarization (IP) and link this to changes in permeability and to IP measurements that have been conducted at Rifle during previous biostimulation experiments. Columns were filled with Rifle background sediment and the water being pumped into the columns is Rifle groundwater that is being shipped to the laboratory at monthly intervals. The experiment is divided into an acetate biostimulation phase of 488 days and in a post biostimulation phase that has now been operating for 212 days. Columns have been sacrificed at regular intervals during the biostimulation and the post-biostimulation period to analyze for solid-species changes and total microbiological quantification. Results to date have shown that there was a small and gradual (seemingly linear) increase in pressure over time during the first 200 days of biostimulation. Then, after about 220 days of biostimulation, we see a significant change in pressure along the longitudinal axis of the columns. This pressure increase occurred approximately 50 days after sulfate was completely reduced. Effluent analyses show that by this time, methane was near solubility. Microbiological analyses have shown that for the first 200 days of the biostimulation total bacterial numbers were similar to methanogen numbers (~ 10^8 cells/g). For columns sacrificed after 200 days of biostimulation, methanogen numbers were an order of magnitude higher than total bacteria. They have remained higher also troughut the post-biostimulation period. Although Rifle field experiments, focusing on dissolved groundwater samples have not reported significant presence of *Archaea*, these results, that focused on sediment analyses, seem to indicate that *Archaea*, in this case methanogens, might be a key organism competing for electron donors during biostimulation.

During the post biostimulation period, methane concentration in the effluent decreased within 20 days by 3 orders of magnitude, from solubility to about 10^{-6} M, and remained at that concentration without much change until this date.

In terms of permeability changes in response to biostimulation, we conclude that biogas formation has a higher impact than the precipitation of mineral phases and/or biomass buildup, and that these changes are reversible. Towards the end of the biostimulation period, the average permeability in the columns had decrease by one order of magnitude.

A conservative estimate of the acetate converted to methane over the 700-day duration of the experiment is 12% on a molar base. This is a lower-limit estimate, based on cumulative acetate consumed during the biostimulation period (488 days), and methane monitored in the effluent up to 700 days. It does not include any methane oxidation during the post stimulation phase, and there was a short period (~ 35 days) where methane gas in the effluent was not quantified.