



## **Thermophilic nitrate-reducing microorganisms prevent sulfate reduction in cold marine sediments incubated at high temperature**

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Hydrogen sulphide produced during metabolism of sulphate-reducing microorganisms (SRM) is toxic, corrosive and causes detrimental oil reservoir souring. During secondary oil recovery, injecting oil reservoirs with seawater that is rich in sulphate and that also cools high temperature formations provides favourable growth conditions for SRM. Nitrate addition can prevent metabolism of SRM by stimulating nitrate-reducing microorganisms (NRM). The investigations of thermophilic NRM are needed to develop mechanisms to control the metabolism of SRM in high temperature oil field ecosystems. We therefore established a model system consisting of enrichment cultures of cold surface marine sediments from the Baltic Sea (Aarhus Bay) that were incubated at 60°C. Enrichments contained 25 mM nitrate and 40 mM sulphate as potential electron acceptors, and a mixture of the organic substrates acetate, lactate, propionate, butyrate (5 mM each) and yeast extract (0.01%) as potential carbon sources and electron donors. Slurries were incubated at 60°C both with and without initial pasteurization at 80°C for 2 hours. In the enrichments containing both nitrate and sulphate, the concentration of nitrate decreased indicating metabolic activity of NRM. After a four-hour lag phase the rate of nitrate reduction increased and the concentration of nitrate dropped to zero after 10 hours of incubation. The concentration of nitrite increased as the reduction of nitrate progressed and reached 16.3 mM after 12 hours, before being consumed and falling to 4.4 mM after 19-day of incubation. No evidence for sulphate reduction was observed in these cultures during the 19-day incubation period. In contrast, the concentration of sulphate decreased up to 50% after one week incubation in controls containing only sulphate but no nitrate. Similar sulfate reduction rates were seen in the pasteurized controls suggesting the presence of heat resistant SRM, whereas nitrate reduction rates were lower in the pasteurized experiment, suggesting either different populations of NRM or a population of NRM that was not resistant to the 80°C pre-treatment. These results demonstrate that thermophilic NRM exist in cold marine sediments from Aarhus Bay and can be enriched under appropriate conditions. Effective microbial control of SRM activity at high temperature in our Aarhus Bay sediment model system depends on the addition of nitrate to stimulate this group of microorganisms.