

## Treatment Strategies for Cold Groundwater Contaminated with High Levels of Nitrate and Ammonium, Using Nitrification, Denitrification, and Anammox

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### ABSTRACT

The issue of nitrogen pollution in aquifers as a result of human activity is a serious concern, as it not only compromises the quality of groundwater but also poses a threat to public health. In situ bioremediation represents a promising approach to addressing this issue, but the challenge lies in the specific conditions of low temperatures and the presence of both high nitrate concentrations and ammonia, which remains a relatively underexplored area. In this study, we explored the feasibility of various bioremediation strategies utilizing the activity of microbial communities involved in nitrification, denitrification, and anammox processes to treat highly contaminated groundwater at a temperature of 10°C. At lower levels of pollution (1 g/L nitrate, 0.17 g/L ammonium), the strategy of nitrification followed by denitrification was successful in removing both ammonium and nitrate. At higher pollution levels (7.1 g/L nitrate, 0.3 g/L ammonium), a strategy of partial denitrification/anammox, enhanced by bioaugmentation with a mesophilic anammox community (PD/AMX+B), was found to be feasible. Both strategies could completely eliminate N species in a period of 80-87 days, which was reduced to 51-52 days in the second cycle. *Nitrospira* and *Nitrosomonas* were responsible for nitrification, whereas denitrification and anammox processes were mediated by *Tolumonas*, *Acidovorax*, *Pseudomonas*, *Nocardoides*, and *Candidatus Kuenenia*. For the PD/AMX+B approach, both real-time polymerase chain reaction assays and functional gene prediction analysis confirmed a continuous increase in anammox metabolic activity, despite low temperatures, confirming the success of the bioaugmentation approach. The results obtained provide a deep understanding of the sustainable bioremediation strategies for cold aquifers heavily contaminated with oxidized and reduced nitrogen compounds.

**Keywords:** bioremediation, aquifers, nitrogen pollution, bioaugmentation

### References:

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