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Denitrification with Pyrite for Bioremediation of Nitrate-Contaminated Groundwater

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ABSTRACT

In the last decades, nitrate pollution has become a major threat to groundwater quality. The consequences include health concerns and environmental impacts. Nitrate contamination is mainly derived from agricultural practices, such as the application of manure as fertilizer. The Osona area (NE Spain) is one of the areas vulnerable to nitrate pollution from agricultural sources. In this area, nitrate is derived from intensive farming activities and the high nitrate content results in a loss of water availability for domestic uses.

The most important natural nitrate attenuation process is denitrification. Denitrifying bacteria are generally heterotrophic and use carbon compounds as the electron donor. Nevertheless, a limited number of bacteria are able to carry out chemolithotrophic denitrification, and to utilize inorganic compounds. Several field studies have suggested by means of geochemical and/or isotopic data that denitrification in some aquifers is controlled by pyrite oxidation. However, the feasibility of pyrite-driven denitrification has been questioned several times in laboratory studies.

This thesis is concerned with the role of pyrite in denitrification and its potential use as a bioremediation strategy.

Earlier studies showed the occurrence of denitrification processes in a small area located in the northern part of the Osona region and suggested that sulfide oxidation had an important role in natural attenuation.

Therefore, the first part of this thesis deals with the characterization of the denitrification processes occurring in the Osona aquifer and their spatial and temporal variations. Denitrification processes linked to pyrite oxidation were identified in some zones of the studied area by means of multi-isotopic methods integrated with classical hydrogeological methods.

Nitrate removal from groundwater can be accomplished by the enhancement of in situ biological denitrification. One such bioremediation strategy is biostimulation, which involves the addition of suitable electron donors and/or energy sources to stimulate indigenous denitrifying microorganisms.

The second part of this thesis is devoted to clarify the role of pyrite as electron donor for denitrification and to evaluate the feasibility of a bioremediation strategy based on pyrite addition to stimulate native denitrifying bacteria. Nitrate consumption in experiments amended with pyrite and inoculated with Thiobacillus denitrificans demonstrated that this bacterium is able to reduce nitrate using pyrite as the electron donor. The efficiency in nitrate removal and the nitrate reduction rate depended on the initial nitrate concentration, pH and pyrite grain size. High nitrate removal efficiency was attained in long-term flow-through experiments under laboratory conditions similar to those found in slow-moving, nitrate-contaminated groundwater.

In addition, biostimulation experiments performed with sediments and groundwater from the Osona aquifer showed that the addition pyrite stimulated the activity of the indigenous microbial community and enhanced the nitrate removal. Furthermore, the long-term efficiency of the process was demonstrated. Hence, biostimulation with pyrite could be considered to remediate nitrate contamination in groundwater in future water management strategies, although further research is needed, especially at field scale.

It is critical for the success of the bioremediation strategy that not only the processes but also the microbial populations and their changes induced by the bioremediation treatment be well understood. The addition of pyrite resulted in an increase in the proportion of denitrifying bacteria and both autotrophic and heterotrophic denitrifiers were stimulated. Bacterial populations closely related to the Xanthomonadaceae might probably be the dominant autotrophic deni-

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trifiers that used pyrite as the electron donor in the biostimulated experiments.

The N and O isotopic enrichment factors associated with the pyrite-driven denitrification were computed and used to recalculate the extent of the natural nitrate attenuation in the Osona aquifer. This refinement becomes useful to predict the evolution of the contaminant in the aquifer, and it should be taken into account for potential implementation of induced remediation techniques. The isotopic approach was proved to be an excellent tool to identify and quantify natural denitrification processes in the field, and to monitor the efficacy of bioremediation strategies in the laboratory.

In order to improve the long-term performance of potential bioremediation strategies based on pyrite-driven denitrification, it is necessary to know the contribution of attached and free-phase denitrifying bacteria to this process in aquifers.

The last part of this thesis addresses the ability of T. denitrificans to grow and colonize pyrite surfaces. In the colonization experiments, attachment onto pyrite surface was required for at lest a small number of the cells in order to accomplish pyrite-driven denitrification. Nevertheless, both attached and planktonic cells probably contributed to the overall denitrification. However, the details of the relative roles of the two phases and the specific mechanisms remain to be addressed.

In conclusion, addition of pyrite to stimulate the activity of indigenous denitrifying bacteria could be considered in future water management strategies of nitrate remediation in groundwater, although further research is needed, especially at field scale. However, a significant limitation of this bioremediation strategy could be the release of toxic metals from pyrite oxidation, e.g. As and Ni could be present in minor amounts in the pyrite structure. Thus care should be taken with the source and characterization of the pyrite for its use as amendment. This bioremediation strategy could be optimized, promoting long-term activity, growth and survival of denitrifying bacteria and determining the optimum operational parameters (e.g. hydraulic retention time, nitrate loading rate, etc.) to achieve high efficiency in nitrate removal. Furthermore, more extensive understanding of denitrifying microorganisms and the environmental parameters controlling their activity is required.

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