Mixed Culture Hydrogenotrophic Nitrate Reduction in Drinking Water

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Abstract. Isolation and identification of the bacteria from a hydrogenotrophic reactor for the denitrification of drinking water revealed that several microorganisms are involved. Acinetobacter sp., Aeromonas sp., Pseudomonas sp. and Shewanella putrefaciens were repeatedly isolated from the hydrogenotrophic sludge and postulated to be of primary importance in the process. Nitrate reduction to nitrite appears to be a property of a diverse group of organisms. Nitrite reduction was found to be stimulated by the presence of organic growth factors. Thus, in a mixed culture, hydrogenotrophic denitrification reactor, NO₂⁻ formed by NO₃⁻-reducers can be converted by true denitrifiers thriving on organic growth factors either present in the raw water, or excreted by the microbial community. Mixotrophic growth also contributes to NO₂⁻ reduction. Finally, chemolithotrophic bacteria participate in the nitrite to nitrogen gas conversion.

Introduction

Hydrogen metabolism is widely distributed among various well-described physiological groups of bacteria, such as the methanogenic and acetogenic bacteria [49], the phototrophic bacteria [38, 48], the sulfate-reducers [69, 70], the organotrophic fermentative bacteria [15, 28], or the N₂-fixing bacteria [50]. Hydrogen is also reported to be a driving mechanism for the microbial degradation of micropollutants, such as atrazine, in soils and drinking water [67].

The aerobic hydrogen bacteria belong to different taxonomic groups. They are characterized by the ability to obtain energy by oxidation of gaseous hydrogen via an electron transport chain and concomitant synthesis of cell material by the reductive assimilation of CO₂ via the Calvin cycle, i.e. to grow chemolithoautotrophically [2]. They are all facultative autotrophs with one exception, Hydrogenobacter thermophilus, which is an obligate autotroph [10]. The hydrogen bac-
teria all show a versatile metabolism; many of them, especially those regarded as *Pseudomonas* and *Alcaligenes*, are characterized by the ability to use a wide spectrum of carbon substrates [10].

Anaerobic growth on hydrogen is associated with dissimilatory nitrate reduction and confined to only a few species [10]. Two different pathways of dissimilatory nitrate reduction can be distinguished. In the first, nitrate is converted to gaseous products such as N₂ or N₂O, a process called denitrification. In the second, nitrate is converted to NH₄⁺ [14, 59]. So far, only *Paracoccus denitrificans* and closely related strains have been found to be able to denitrify nitrate, nitrite, or nitrous oxide under hydrogenotrophic conditions [2, 4, 34]. The hydrogen oxidizers *Alcaligenes eutrophus* and *Hydrogenophaga (Pseudomonas) pseudoflava* are able to grow heterotrophically as denitrifiers with nitrate as an electron acceptor. Growth under autotrophic anaerobic conditions with nitrate, however, is minimal or absent in these species [2]. Hydrogen is also a potential electron donor for dissimilatory nitrate reduction to ammonium, a process first observed in the highly reducing environment of the rumen [22, 31, 37] and later in representatives of the genera *Clostridium* [65], *Desulfovibrio* [6], *Wolinella* [11] and *Campylobacter* [60].

*Paracoccus denitrificans* is one of the most intensively studied denitrifying microorganisms due to its nutritional versatility [59, 65]. Its well-known capability to reduce nitrogenous compounds with hydrogen is at the basis of several new approaches for denitrification of drinking water [20, 26, 35, 39, 58]. The concentration of nitrate in ground and surface water shows an increasing trend. The use of hydrogen as a reductant for denitrification is currently under investigation in a 1.0 m³ h⁻¹ pilot plant on a water production center fed from a storage reservoir with nitrate-rich surface water [39]. The hydrogenotrophic treatment concept offers a number of important benefits, such as food-grade quality of the reductant, process reliability, low excess sludge production and no need for intensive monitoring or biological post-treatment to control and remove residual reductant [20, 26, 39].

In-depth insight about the hydrogenotrophic reduction of oxidized nitrogen compounds relates to only a few organisms [59]. To our knowledge, this is the first report about the ecology, stability, and dynamics of a mixed culture, hydrogen consuming denitrification system. To study the bacterial populations of the pilot-reactor, microorganisms were enriched and purified from the in-situ biomass and the reactor effluent under different conditions. Isolates have been tentatively identified and the importance of hydrogen as a microbial reductant and energy source has been assessed.

**Methods**

**Sampling Procedure**

A simplified scheme of the hydrogenotrophic denitrification system is shown in Fig. 1. The process–technological aspects have been described elsewhere [39]. A mixture of H₂ and CO₂ (97.5:2.5) was recirculated and diffused from the bottom of the left column, in counter-current with the nitrate-rich surface water. The main denitrifying activity occurred in this part of the plant. The water subsequently entered an upflow column where, in the bottom part, the residual dissolved H₂ was