Autotrophic, Hydrogen-Oxidizing, Denitrifying Bacteria in Groundwater, Potential Agents for Bioremediation of Nitrate Contamination

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Addition of hydrogen or formate significantly enhanced the rate of consumption of nitrate in slurried core samples obtained from an active zone of denitrification in a nitrate-contaminated sand and gravel aquifer (Cape Cod, Mass.). Hydrogen uptake by the core material was immediate and rapid, with an apparent Km of 0.45 to 0.60 μM and a Vmax of 18.7 nmol cm-3 h-1 at 30°C. Nine strains of hydrogen-oxidizing denitrifying bacteria were subsequently isolated from the aquifer. Eight of the strains grew autotrophically on hydrogen with either oxygen or nitrate as the electron acceptor. One strain grew mixotrophically. All of the isolates were capable of heterotrophic growth, but none were similar to Paracoccus denitrificans, a well-characterized hydrogen-oxidizing denitrifier. The kinetics for hydrogen uptake during denitrification were determined for each isolate with substrate depletion progress curves; the Km's ranged from 0.30 to 3.32 μM, with Vmax's of 1.85 to 13.29 fmol cell-1 h-1. Because these organisms appear to be common constituents of the in situ population of the aquifer, produce innocuous end products, and could be manipulated to sequentially consume oxygen and then nitrate when both were present, these results suggest that these organisms may have significant potential for in situ bioremediation of nitrate contamination in groundwater.

Nitrate is a common contaminant in shallow groundwater systems. Denitrification may be the process best suited to bioremediate nitrate contamination as it can utilize relatively large amounts of nitrate and it produces an innocuous end product. Studies have shown that denitrification does occur in nitrate-contaminated groundwater (3, 27, 32), but it is usually electron donor limited (25). Consequently, using denitrification to remediate nitrate contamination will necessitate the addition of a suitable electron donor to stimulate activity.

The utility of hydrogen-linked denitrification as a means to treat nitrate contamination in drinking water has been recognized in Europe (see reviews by Gayle et al. [7] and Mateju et al. [19]). Kurt et al. (15) used mixed populations of denitrifiers in bench-scale sand-bed reactors to study the process. They found that, in continuous operation, complete removal of 1.8 mM nitrate from tap water with no nitrite accumulation was accomplished in 4.5 h at 30°C. A pilot-scale industrial plant that uses hydrogen oxidation coupled with denitrification for drinking water production has been in operation in Monchengladbach, Germany, since 1986 (10). The plant produces about 50 m³ of drinking water h⁻¹, removing 0.74 mol of N m⁻³ h⁻¹. In a separate study in Belgium, hydrogenotrophic nitrate-reducing bacteria, some of which were denitrifiers, were isolated from a similar water treatment system and characterized (17). However, little is known about the occurrence and ecology of hydrogenotrophic denitrifiers in groundwater systems and whether there is any potential to utilize these organisms in situ to remediate nitrate contamination.

In this study we examined hydrogen oxidation by denitrification in a nitrate-contaminated sand and gravel aquifer. Hydrogen oxidizers appeared to be common constituents of the denitrifying population in the aquifer and had Km's sufficiently low that they could effectively compete for added hydrogen. These results suggest that hydrogen-stimulated denitrification may have significant potential for in situ remediation of nitrate-contaminated groundwater and that field studies of the process should be considered.

MATERIALS AND METHODS

Study site and sample collection. Sediments and groundwater were obtained from a nitrate-contaminated sand and gravel aquifer located on Cape Cod, Mass. The aquifer has been contaminated by the continuous disposal of treated sewage onto rapid infiltration sand beds for over 50 years. Much of the contaminant plume contains little or no oxygen and relatively high concentrations of nitrate (>1 mM) (16, 26). Consequently, denitrification is a prominent terminal electron-accepting process within certain zones of the plume (25, 27). The core material for this study was collected from such a zone.

Groundwater samples were obtained from multilevel samplers with a peristaltic pump (26). Samples were collected by overfilling 1-liter glass bottles; care was taken to minimize exposure of the sample to the atmosphere. Aquifer sediments were obtained with a 5-cm-diameter piston core barrel (4). Cores were collected in 1.5-m-long aluminum liners, cut into 0.3-m sections, and capped. All water and sediment samples were stored at 4°C prior to usage.

Incubations with aquifer sediments. Activity assays were conducted by sediment slurry incubations. Cores were extruded into an anaerobic glovebag and mixed, and 50-ml aliquots were dispensed into tared 125- or 250-ml Erlenmeyer flasks. Groundwater (50 or 150 ml) was added to the flasks, which were then stoppered, weighed, and flushed for 30 min with He. For the nitrate consumption experiment (250-ml flasks), 1.5 ml of an anoxic solution of 200 mM sodium formate was added to one set of duplicate flasks and 15 ml of H₂ was added to a second set. The flasks were incubated with shaking at 30°C. Ten-milliliter aliquots of water were removed daily.* Corresponding author. Mailing address: Water Resources Division, U.S. Geological Survey, 3215 Marine Street, Boulder, CO 80303. Phone: (303) 541-3032. Fax: (303) 447-2505.
from each flask and replaced with 10 ml of He. The aliquots were filtered (0.45-μm-pore-size filters) and frozen. For the hydrogen consumption experiment, 50 or 60 μl of H₂ (84 kPa) was added at various times to the flasks. The flasks were placed on their sides and incubated at 30°C with vigorous shaking. At 15- to 30-min intervals, 10 to 200 μl of headspace gas was removed with a gas-tight syringe and assayed for H₂. For denitrification, rates of activity were determined by the acetylene block technique (25).

**Medium and MPN determination.** The medium used for this study (designated HOD) was a mineral salts medium consisting of (in grams per liter): MgSO₄·7H₂O, 0.2; NaNO₃, 0.8; K₂HPO₄, 5.0; KH₂PO₄, 3.94; NaNO₃, 1.7 (0.08 for most-probable-number [MPN] determination); and trace element solution, 0.5 ml. The trace element solution consisted of (in grams per liter): MnCl₂·4H₂O, 0.2; ZnSO₄·7H₂O, 0.1; Na₂MoO₄·2H₂O, 0.1; CuSO₄·5H₂O, 0.02; and CoCl₂·6H₂O, 0.002. The medium was adjusted to pH 7, autoclaved, and flushed with N₂ while being cooled. Then the medium was transferred to a glovebag and 20 ml of filter-sterilized, 0.03% FeCl₃ was added per liter.

Five-tube MPN determinations were used to enumerate hydrogen-oxidizing, formate-oxidizing, and heterotrophic denitrifying microorganisms in aquifer sediments. Core material (200 g) was added to 200 ml of HOD medium in an anaerobic glovebox, 0.5 ml of filter-sterilized 10% Polysorbate-80 was added, and the sediment slurry was agitated for 10 min. Serial dilutions were made from the slurry, with either 10 mM sodium formate, ~35 kPa hydrogen, or one-half-strength nutrient broth plus nitrate as the substrate. Acetylene was added to all bottles (15% headspace volume), which were then incubated at 30°C for 4 to 7 weeks. Results were scored according to the criteria specified by Tiedje (31).

**Enrichment and isolation procedure.** The initial enrichments were for both hydrogenotrophic and methylo trophic denitrifiers. They were prepared by placing 40 cm³ of core material from a denitrifying zone and 60 ml of groundwater (collected from the same location as the core) into 125-ml Erlenmeyer flasks and adding filter-sterilized solutions of sodium nitrate (final concentration, 5 mM) and methanol (final concentration, 10 mM). The flasks were stoppered and incubated at 30°C until the nitrate concentration was nearly depleted. Then 5 ml of each enrichment was transferred to 150-ml serum bottles containing 30 ml of HOD mineral medium plus 25 mM methanol in an anaerobic glovebox containing an atmosphere of N₂, H₂, and CO₂ (85:10:5). The serum bottles were stoppered, 30 ml of H₂ was added by syringe, and the bottles were incubated at 30°C. Growth was determined by the development of turbidity and loss of nitrate. After four to six successive transfers, it was determined that methanol was not required for growth and it was subsequently omitted from the enrichment medium. After an additional three transfers without methanol, the enrichments were streaked onto agar plates of HOD medium and incubated in H₂ (35 kPa). Colonies were picked and transferred to liquid HOD medium plus H₂. Transfers from liquid to plate to liquid were repeated until colony and cell morphology were uniform on two successive cycles. Isolates were maintained both anaerobically in liquid HOD medium plus H₂ and aerobically on nutrient agar slants.

The isolates were characterized with Oxi/Ferm Tubes (Roche Diagnostics, Nutley, N.J.), by cellular fatty acid composition (Microbial Identification, Inc., Newark, Del.), and by the capacity to grow aerobically on 13 selected organic compounds (each at 50 mM) as the sole source of carbon and energy.

**Culture experiments.** Progress curves of hydrogen consumption were used to assess the kinetics of hydrogen uptake by the HOD isolates. Denitrifying starter cultures growing in HOD medium plus H₂ were inoculated into 100 ml of the same medium in 500-ml flasks, 100 ml of H₂ was added, and the flasks were incubated with shaking at 30°C. The cultures were grown to mid-logarithmic phase and then diluted to an optical density (600 nm) of 0.05 with fresh HOD medium. Fifteen-milliliter aliquots of the diluted culture were placed in replicate 150-ml serum bottles, which were stoppered and flushed for 10 min with He. Then 6.5 ml of CO₂ and 0.2 to 0.4 ml of H₂ were added to the bottles, which were placed on their sides in a 30°C incubator with vigorous shaking. Hydrogen concentrations in the headspace of each bottle were measured over time.

Growth curves were conducted with some of the isolates to examine autotrophic hydrogen consumption when both oxygen and nitrate were available as electron donors. Mid-log-phase cultures were inoculated into replicate 500-ml flasks containing 150 ml of HOD medium. The flasks were stoppered, and 4.5, 40, and 55 ml of CH₄, O₂, and H₂, respectively, were added to each. The flasks were incubated with shaking at 30°C. H₂ (75 ml) or CO₂ (20 ml) was added whenever concentrations fell below 25% of the initial values. Methane was used as an internal standard to quantify pressure changes within the flasks, and all calculations were adjusted accordingly.

**Estimation of kinetic parameters.** Aqueous hydrogen concentrations were calculated with the solubility coefficients given by Gordon et al. (9). Kinetic parameters for hydrogen uptake were calculated with a linearized form of the integrated Michaelis- Menten equation (equation 15 in reference 23). The precisions of the Kₘ and Vₘₐₙ values were assessed with a fourth-order Runge-Kutta numerical approximation of the Michaelis-Menten equation and the initial substrate concentration (DIFFEQ; MicroMath Scientific Software, Salt Lake City, Utah) as described by Sulfita et al. (28). The theoretical substrate depletion curves that were generated were visually compared with the observed data. Relatively small changes in the Kₘ and Vₘₐₙ values for the numerical approximation resulted in computed progress curves that did not match the observed data. For the sediment incubations, the kinetic parameters were calculated a second time with the Runge-Kutta approximation of the differential form of the Michaelis-Menten equation fitted with a term (R) for the rate of endogenous substrate production (equation 3 in reference 23).

**Analytical techniques.** Nitrate, nitrite, and ammonium concentrations were determined colorimetrically with a flow-injection analyzer (Lachat Instruments, Mequon, Wis.). Nitrous oxide was measured by gas chromatography with an electron capture detector (4). Oxygen, carbon dioxide, and methane were analyzed with a Carle analytical gas chromatograph (model ACG-311; Hach Co., Loveland, Colo.) equipped with thermal conductivity and flame ionization detectors connected in series. Hydrogen was analyzed on the same gas chromatograph by connecting a reducing gas detector (model RGD; Trace Analytical, Stanford, Calif.) in series with the thermal conductivity detector and using a 0.3-cm-diameter, 1.8-m-long column of Carbosieve S-II. Bacteria were enumerated with acridine orange staining and epifluorescence microscopy (26). All gases used for cultures and incubations were O₂-free.

**RESULTS**

**Sediment experiments.** The groundwater in the zone of the contaminant plume from which all the samples for this study
were taken had a specific conductance of 410 μS cm⁻¹ and contained 0.1 mM dissolved organic carbon, no detectable dissolved oxygen, 1.1 mM nitrate, 0.04 mM nitrite, and 2.3 μM nitrous oxide. When this groundwater was incubated in the absence of oxygen in flasks containing aquifer core material obtained from the same location and the same depth, a slow, but measurable, decrease in nitrate concentration was evident (Fig. 1). At 30°C, the endogenous rate of nitrate consumption was 4.2 nmol (cm² of sediment)⁻¹ h⁻¹, and after 9 days 34% of the nitrate lost was present as nitrite. The addition of formate or hydrogen significantly stimulated nitrate consumption, to the extent that all or nearly all of the nitrate was depleted by 9 days (Fig. 1). The maximal rates of nitrate consumption were 57.8 and 23.1 nmol (cm² of sediment)⁻¹ h⁻¹ for the formate and hydrogen additions, respectively. Ammonium was not produced during these incubations.

The kinetics of hydrogen consumption by aquifer sediments was examined in similar incubations with much smaller quantities of added hydrogen. Progress curves of hydrogen uptake by these sediments demonstrated rapid and immediate uptake of the added hydrogen (Fig. 2A) that was repeatable with successive additions of different amounts of hydrogen. These incubations were vigorously agitated to prevent phase-transfer limitations. The linear relationship of the transformed data (Fig. 2B) indicates that consumption of hydrogen in the incubations followed Michaelis-Menten kinetics rather than a first-order transfer of hydrogen across the gas-water interface. On the basis of this linear model, the Kₘ for hydrogen uptake was 0.04 μM and the Vₘₐₓ was 1.1 nmol (cm² of sediment)⁻¹ h⁻¹. Applying the same data to a nonlinear model containing a term for endogenous hydrogen production (R) resulted in a slightly lower Kₘ (0.45 μM), the same Vₘₐₓ, and an endogenous rate of production of 0.6 nmol (cm³ of sediment)⁻¹ h⁻¹, which was about 3% of the uptake Vₘₐₓ. Simultaneous incubations using separate aliquots of the same sediment determined a denitrification rate of 0.9 nmol of N₂O produced (cm³ of sediment)⁻¹ h⁻¹ by the acetylene blockage technique (with no added hydrogen).

MPN estimates of heterotrophic denitrifying bacteria were 91 (g of moist sediment)⁻¹ for samples taken from the active zone of denitrification within the aquifer. There were also low, but measurable, populations of denitrifiers that could grow and utilize either hydrogen or formate as the sole electron acceptor (7 and 3% of the heterotrophic denitrifiers, respectively).

**Hydrogen-oxidizing cultures.** Enrichments for hydrogen-oxidizing denitrifying bacteria were prepared with core material collected from a denitrifying zone at two different locations within the aquifer. Nine isolates were obtained from these enrichments and designated strains HOD 1 to 9. All of the isolates were gram-negative rods, all could grow autotrophically on hydrogen and nitrate via denitrification, and all could grow heterotrophically either as denitrifiers or as oxygen respirers. Denitrification was determined by the ability to produce nitrous oxide in the presence of acetylene; acetylene did not prevent autotrophic growth on hydrogen. The isolates could also grow autotrophically when incubated aerobically with hydrogen. However, after being maintained on nutrient agar slants, HOD 7 lost its autotrophic capacity but could consume hydrogen when the growth medium was supplemented with 2% nutrient broth. A partial characterization of the HOD strains is given in Table 1. None of the strains could produce indole from tryptophan or hydrolyze urea. Cellular fatty acid analysis suggested that HOD 1 and HOD 4 were closely related, possibly as subspecies or identical strains, though they did differ in their ability to grow on succinate (Table 1).

**FIG. 1.** Consumption of nitrate by aquifer sediments when slurried with groundwater collected from the same location (endogenous) or amended with 2 mM formate or 20 kPa of hydrogen. Flasks were incubated at 30°C. Error bars represent ± 1 standard deviation.

Progress curves with dilute suspensions of mid-logarithmic-phase cultures were used to determine the kinetics of hydrogen uptake by the HOD isolates (Fig. 3). When grown as an autotrophic denitrifier, the Kₘ for hydrogen uptake for HOD 8 was 0.38 to 0.79 μM for replicate determinations (Fig. 3, Table 2). These values were similar to the apparent Kₘ for hydrogen uptake by the Cape Cod aquifer sediments. Overall, the Kₘ's for the HOD isolates and a strain of *Paracoccus denitrificans* ranged from 0.30 to 1.50 μM, except for HOD 7, which had much higher Kₘ and Vₘₐₓ values (Table 2). The minimum thresholds for hydrogen for HOD 2 and HOD 8 were less than the detection limit for hydrogen, which was 0.017 Pa (0.11 nm).

When grown autotrophically on hydrogen and carbon dioxide, HOD 8 could sequentially use oxygen and then nitrate as electron acceptors (Fig. 4A). After the initial aerobic growth, nitrite consumption began when the oxygen partial pressure had decreased to 2 kPa (22 μM). The shift to denitrification was marked by a 10- to 15-h lag in growth and a plateau in oxygen concentration, with the remainder of the oxygen being consumed during the period when nitrate was the primary electron acceptor. There was no nitrite accumulation at any time and no change in pH. The rate of hydrogen consumption was greatest from 35 to 42 h during logarithmic aerobic growth and from 65 h onward, after the oxygen had been consumed. A lower intermediate uptake rate was observed during mixed-phase growth (Fig. 4B). The same trend was evident for carbon dioxide consumption. Sixty percent more hydrogen (per mole) was used for denitrification (based on the 75- to 79-h interval when oxygen was depleted) than for aerobic growth (based on the 35- to 42-h interval). The overall stoichiometries were 9.0:2.6:1 for H₂-O₂-CO₂ and 10.3:1:8.1 for H₂-NO₃-CO₂.

**DISCUSSION**

It has been well established that hydrogen production and consumption are important steps for organic matter mineralization in environments within which sulfate reduction or methane production is the predominant terminal electron-accepting process (18). However, much less is known about hydrogen metabolism coupled to denitrification, either in natural settings or in pure cultures. *P. denitrificans* can grow
autotrophically as a hydrogen-oxidizing denitrifier (1) and has been studied extensively because of its suggested similarity to mitochondria (13). Only a few other known bacteria can oxidize hydrogen and denitrify (though not necessarily simultaneously), such as three of the Hydrogenophaga species (33) and Alcaligenes eutrophus (21), although autotrophic growth by the latter is reported to be very slow or absent (1). Hydrogenase activity under denitrifying conditions has also been reported for Rhizobium japonicum (20) and Azospirillum brasiense (30). More recently, Liessens et al. (17) isolated several strains of nitrate-reducing organisms that could oxidize hydrogen, a small subset of which were denitrifiers but none of which were P. denitrificans, nor was P. denitrificans found in a survey of denitrifiers isolated from world soils (6).

The results from this study clearly demonstrate that a denitrification-based microbial community, in a groundwater environment, can readily utilize hydrogen as an electron donor. This capacity appears to be directly attributable, at least in part, to the denitrifiers themselves. We were able to isolate hydrogen-oxidizing denitrifiers from the aquifer and to determine that a portion of the heterotrophic denitrifying population could autotrophically utilize hydrogen for growth. It also seems likely that an even larger fraction of the denitrifiers in the aquifer possess the physiological capacity to oxidize hydrogen when growing heterotrophically, though this was not specifically examined. Denitrification is often electron donor limited in groundwater (25, 27). This situation arises because pristine subsurface environments are characterized by being oligotrophic, but when contaminated, nitrate loading frequently exceeds organic carbon loading. The result is atypical of denitrification in most surface waters or soils, which are usually more electron donor rich relative to available nitrate (5, 14). For example, dissolved organic carbon in the nitrate-containing zone of the Cape Cod aquifer is only 2 to 2.5 mg of C per liter (27, 29), and the organic carbon content of the sand and gravel matrix is <0.1% (2). Therefore, autotrophic denitrification may be much more prevalent in groundwater environments. Pyrite oxidation has been reported as a dominant denitrifying mechanism in groundwater in Germany (3) and Denmark (22).

While the goal of this study was not necessarily to identify the hydrogen-oxidizing organisms that were isolated from the aquifer, it is interesting that none of the isolates were similar to P. denitrificans (Table 1), nor were they yellow-pigmented Hydrogenophaga spp., the genus that harbors most of the other hydrogen-oxidizing denitrifiers (33), and they do not match any of the hydrogen-oxidizing Alcaligenes species in terms of compounds that can be utilized as sole carbon and energy substrates for growth (1). All of the isolates in this study, except HOD 7, are capable of reducing nitrate beyond nitrite when grown autotrophically and therefore most closely resemble group D of the hydrogen-oxidizing organisms isolated by Liessens et al. (17) from a hydrogen-amended water treatment system. HOD 7 was able to grow on a much broader range of organic substrates than the other HOD strains but only grew mixotrophically on hydrogen after the first few transfers. Hydrogen-stimulated denitrification appears to be a good candidate for in situ bioremediation of nitrate-contaminated groundwater. The end products of the process, N₂ and H₂O, are innocuous, and hydrogen oxidizers were an established component of the indigenous population of denitrifiers in the sand and gravel aquifer in this study. Nitrate contamination is a widespread phenomenon in groundwater throughout North America and Europe and, in many cases, in the presence of oxygen. All of the known hydrogen-oxidizing denitrifiers, including the organisms isolated here, can also oxidize hydrogen aerobically. Thus, it may be possible to use hydrogen to sequentially consume first the background oxygen and then the contaminant nitrate. The fraction of the hydrogen-oxidizing population that can use both electron acceptors should be able to switch from oxygen to nitrate with very little lag time (Fig. 4). For either electron acceptor, the addition of hydrogen targets a very specific microbial population and there would be very little competition from other unwanted processes, such as might occur if an organic compound were added (e.g., see reference 19). Furthermore, as a secondary result, increases in subsurface biomass should also be minimal.

Effective bioremediation is facilitated if the kinetics and the stoichiometry of the process of interest are known. The V_max for hydrogen uptake by the aquifer core material was very low when compared with that of sulfate-reducing and methane-producing sediments taken from productive surface water systems (for a summary, see Table IV in reference 8), but only about 40-fold lower than aerobic hydrogen uptake by a sandy soil (24). This difference is due generally to a much lower microbial biomass in the groundwater environment (12, 26). The K_m for hydrogen uptake is also lower than for those same
surface water sediments (Table IV in reference 8). Lovley and Goodwin (18) suggested that the half-saturation constant for hydrogen uptake should be lower for nitrate-reducing systems than for sulfate-reducing and methane-producing environments, and these results support that conclusion. The implication is that groundwater denitrifiers will be extremely competitive when hydrogen is added for remediation. The average hydrogen uptake \( K_m \) for all of the HOD isolates is 0.9 \( \mu M \). The close agreement with the \( K_m \) for the aquifer core material suggests that the HOD strains are representative of the indigenous hydrogen oxidizers in the denitrifying zone of the aquifer. The hydrogen uptake kinetics (both \( K_m \) and \( V_{\text{max}} \)) determined for \( P. \text{denitrificans} \) (Table 2) were within the range of values for the HOD strains and agreed well with the values determined previously for this organism (\( K_m = 0.97 \mu M, V_{\text{max}} = 3.75 \text{ nmol cell}^{-1} \text{ h}^{-1} \)) by Haring and Conrad (11) when it was grown as a denitrifier. Interestingly, there was little difference in the kinetic parameters when oxygen was the electron acceptor (11).

In summary, the microbial population in the portion of a sand and gravel aquifer in which denitrification was the predominant terminal electron-accepting process could readily utilize hydrogen or formate and had a relatively low \( K_m \) for hydrogen uptake. Hydrogen-oxidizing denitrifying bacteria were subsequently isolated from the aquifer, most of which could grow autotrophically on hydrogen with either nitrate or oxygen as the electron acceptor. Given the relative abundance of these organisms in the aquifer and the fact that their high affinity for hydrogen coupled with their autotrophic capacity makes them relatively easy to specifically target in situ, hydrogen-stimulated denitrification may be a useful option for bioremediating nitrate-contaminated groundwater. Utilization of the above approach will depend on the extent to which nitrate accumulates, the amount of hydrogen (or possibly formate) that can be added to the subsurface, and the degree to which dispersion can be enhanced or facilitated. We are currently conducting field experiments using natural gradient tracer tests to address these issues.

![FIG. 3. Progress curve of hydrogen consumption by a mid-log-phase denitrifying culture of HOD 8. The solid line is a theoretical curve calculated from the initial substrate concentration and the kinetic parameters obtained from the linear transformation (\( r^2 = 0.98 \)) of the data (inset).](http://aem.asm.org/)

### TABLE 1. Characteristics of hydrogen-oxidizing denitrifying bacteria isolated from nitrate-contaminated groundwater

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<sup>a</sup>Strains tested for growth: Gu, glucose; Xy, xylose; Me, methanol; Su, sucrose; Fr, fructose; Fo, formate; Ci, citrate; Ac, acetate; Py, pyruvate; Lc, lactate; Sc, succinate; Gm, glutamate; and Le, leucine.

### TABLE 2. Kinetic parameters for hydrogen uptake by cultures of hydrogen-oxidizing denitrifying bacteria with nitrate as the electron acceptor

<table>
<thead>
<tr>
<th>Strain&lt;sup&gt;c&lt;/sup&gt;</th>
<th>( K_m ) (( \mu M ))</th>
<th>( V_{\text{max}} ) (fmol cell&lt;sup&gt;-1&lt;/sup&gt; h&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>( r^2 ) &lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOD 1</td>
<td>0.88</td>
<td>6.14</td>
<td>0.97</td>
</tr>
<tr>
<td>HOD 2</td>
<td>0.70</td>
<td>2.42</td>
<td>0.95</td>
</tr>
<tr>
<td>HOD 3</td>
<td>0.54</td>
<td>2.49</td>
<td>0.97</td>
</tr>
<tr>
<td>HOD 4</td>
<td>1.50</td>
<td>5.24</td>
<td>0.99</td>
</tr>
<tr>
<td>HOD 5</td>
<td>0.30</td>
<td>3.53</td>
<td>0.82</td>
</tr>
<tr>
<td>HOD 6</td>
<td>0.65</td>
<td>3.57</td>
<td>0.98</td>
</tr>
<tr>
<td>HOD 7</td>
<td>3.32</td>
<td>13.29</td>
<td>0.98</td>
</tr>
<tr>
<td>HOD 8&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.38</td>
<td>2.13</td>
<td>0.98</td>
</tr>
<tr>
<td>HOD 9</td>
<td>0.79</td>
<td>1.85</td>
<td>0.98</td>
</tr>
<tr>
<td>P. denitrificans ATCC 17741</td>
<td>0.77</td>
<td>1.33</td>
<td>0.99</td>
</tr>
</tbody>
</table>

<sup>a</sup>Cell growth and uptake assays were done in an autotrophic medium except for HOD 7, for which the medium was supplemented with 2% nutrient broth.

<sup>b</sup>Correlation coefficient for the linearly transformed progress curve data (see Fig. 3, inset) for the mean of replicate determinations for 12 to 24 time points.

<sup>c</sup>Results from replicate experiments are shown for HOD 8 and 9.

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We thank D. R. LeBlanc, coordinator of the Cape Cod site, for field and technical assistance. J. Robinson for helpful discussions, and D. Lovley, F. Chapelle, and J. Robinson for manuscript reviews. This study was funded by the U.S. Geological Survey Toxic Substances Hydrology Program.

REFERENCES


FIG. 4. Autotrophic growth of HOD 8 with oxygen and nitrate used sequentially as electron acceptors. (A) Oxygen and nitrate concentrations and OD of the growth medium; (B) cumulative amounts of hydrogen and carbon dioxide consumed.