Hydrogenase Activity in Deeply Buried Sediments of the Arctic and North Atlantic Oceans

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The goal of this study was to measure hydrogenase activity in oceanic sediments of the Lomonosov Ridge (Arctic Ocean) and of the Porcupine Seabight (North Atlantic), and to understand how its distribution varied depending on geochemical and lithological characteristics of the sediment. Hydrogenase activity was found at all sites, and absolute values and downhole distributions varied widely within and between sites. At the Lomonosov Ridge, hydrogenase was below detection in the top 190 meters of sediment, but high levels were measured in the organic carbon-rich layers below this depth. Activity at Challenger Mound, Porcupine Seabight (Site 1317) was one to two orders of magnitude higher than the upslope or downslope sites (1318 and 1316, respectively), and was higher in the mound than below the mound base. Consistent with the interpretation of hydrogenase as an indicator of microbial activity, cells were present in all hydrogenase-positive samples or in nearby horizons. Cell-specific activity values were as much as 1000 fold lower than those of cultured Clostridium pasteurianum, with the values from the highest activity sediments approaching those of C. pasteurianum more closely. This suggests that little adaptation in hydrogenase activity might be necessary to support H₂ metabolism in deeply buried sediments that contain relatively high levels of substrates.

Keywords deep biosphere, hydrogen, hydrogenase, microbial activity, sediment

INTRODUCTION

It is well established that active microbial populations persist in marine sediments tens of millions of years old at depths of several hundred meters below the seafloor. Evidence of their existence comes from direct enumeration of cells, direct activity measurement by radiotracer assays (Parkes et al. 2000 and references therein), presence of amplifiable DNA sequences (Biddle et al. 2006; Inagaki et al. 2006), isolation of viable microbes (Cypionka et al. 2002; D’Hondt et al. 2004; Suß et al. 2004), and inference of activity from diffusion-reaction modeling of pore-water concentration profiles of potential substrates and products (D’Hondt et al. 2002, 2004). Cell enumeration, cultivation, and DNA sequence analyses have been instrumental in establishing the depth distribution, adaptations, and phylogenetic relationships of these populations (Smith and D’Hondt 2006). Obtaining direct measurements of their metabolism, however, has been difficult: with the exception of some ocean margin sites, microbial activity in subseafloor sediments is generally lower than the detection limit of available radioassays.

Knowledge of microbial activity, together with estimates of the active population size, can lead to important understanding of the adaptations that allow these organisms to maintain cellular function in habitats with extremely low energy sources. In our efforts to measure low levels of microbial activity in marine sediments, we have developed a sensitive tritium-based method to measure the activity of hydrogenase enzymes (Soffientino et al. 2006). This intracellular or membrane-bound enzyme catalyzes the lysis or formation of the covalent bond in molecular hydrogen (H₂), and is essential to all microbes that carry out H₂ metabolism, either as producers or consumers (Adams 1990; Vignais et al. 2001).

Hydrogen is an important metabolic intermediate in most, if not all, anaerobic communities (Wolin 1982): it links the activity of fermenters to that of terminal electron acceptor oxidizers (Lovley and Klug 1982), it is an important substrate for methanogens (Zeikus 1977), and it is the basis of kinetically and thermodynamically controlled competition between different groups of microbes, such as sulfate reducers and methanogens (Hoehler et al. 1998; Lovley and Goodwin 1988; Lupton and Zeikus 1984).

Given these considerations, it is surprising that only three studies have addressed the environmental distribution of hydrogenase. Schink et al. (1983) used a tritium-based assay to characterize in vivo hydrogenase activity of H₂-transforming microbes in culture as well as in water from acid, thermal and hypersaline environments. They presented evidence that hydrogenase activity per µg of cell protein in three pure cultures was proportional to the rate of H₂ production or consumption, and showed a depth-related association between hydrogenase,
zones of H₂ production and consumption, and viable counts of aerobic hydrogenotrophs in the water of a stratified hypersaline lake. The correspondence of hydrogenase with viable hydrogenotrophs was also later reported in two freshwater lakes (Schink and Zeikus 1984). Another study, by Goodwin et al. (1988), investigated hydrogenase activity in surface sediments of freshwater lakes with very different trophic regimes, and showed that activity increased with organic carbon (OC) input and H₂ turnover.

These studies suggest that hydrogenase activity should be widespread in environments where H₂ metabolism occurs, and that it might be an informative indicator of microbial processes in anoxic sediments. In this paper we present the first measurements of hydrogenase activity in deeply buried marine sediments, collected through Integrated Ocean Drilling Program (IODP) Expedition 302 to the Lomonosov Ridge, Arctic Ocean, and through IODP Expedition 307 to the Porcupine Seabight, on the southwestern Irish continental margin.

**MATERIALS AND METHODS**

**Hydrogenase Assay**

Hydrogenase activity was measured as described in Soffientino et al. (2006). Briefly, the assay measures the rate of isotopic exchange between dissolved tritiated hydrogen (³H₂) and water. A sediment sample is slurried with sterile, anoxic seawater in a glass syringe, and a tritiated H₂/N₂ headspace is added. The syringe is incubated at room temperature (22 ± 1°C) with shaking for 2 h or more. A paired sample slurried with saturated mercuric chloride (HgCl₂) solution serves as negative control.

Subsamples of the slurry (∼100 μl) are withdrawn every 30 min, shaken under a vacuum for 15 min to remove unreacted ³H₂, centrifuged, and the amount of ³H₂O determined by liquid scintillation counting. Linear regression of tritiated water activity versus time yields a rate of tritium incorporation in water that is proportional to the total concentration of hydrogenase in the sample and is interpreted as “potential” activity. The rate in Bq/time/vol is converted to nmol H₂ exchanged/time/g sediment using the sample weight, porosity, volume of seawater added, and specific activity of the tritiated headspace.

Samples from Challenger Mound (Site 1317) were assayed as described in Soffientino et al. (2006); samples from the other Porcupine Seabight Sites (1316 and 1318) and from the Arctic Ocean, however, were run using a different source of ³H₂, and with a different method of measuring the tritiated headspace specific activity. These modifications are described next.

**Sources of ³H₂**

The method described in Soffientino et al. (2006) relied on reacting tritiated sodium borohydride with acid to produce ³H₂. In view of the large number of samples at hand we sought a more convenient and economical way to produce the ³H₂/N₂ headspace mixture. A manifold was constructed (Figure 1) to store, dilute, and dispense ³H₂ gas to the incubation syringes.

The manifold was assembled from a 1-l 304 stainless steel cylinder with threaded 17 mm openings on either end, one of which was fitted with a high-purity, stainless steel diaphragm valve, and the second fitted with a removable adaptor and plug. The diaphragm valve was connected to a system of two 3-way and two 4-way, high purity ball valves that deliver the ³H₂/N₂ stock from the cylinder to a secondary headspace reservoir for dilution with cold H₂/N₂ (20% v/v H₂, ultra-high purity) and from this container to the sample via a loading port (Fig. 1).

Since most hydrogenases from anaerobes are very sensitive to O₂, the system was designed so that the sample syringe stopcocks are flushed with H₂/N₂. The secondary reservoir consists of a 100 or 500 ml gas-tight syringe into which the ³H₂/N₂ stock can be diluted to the desired specific activity before being loaded to the samples. The parts were mounted on a perforated steel plate, and connected through compression fittings with 1.6 mm diameter stainless steel tubing (Figure 2).

![FIG. 1. Manifold for storing, diluting, and dispensing ³H₂/N₂ gas. A, stainless steel cylinder; B, diaphragm valve; C, adaptor fitting with plug; D, E, 3-way switch valve; F, G, 4-way switch valve; H, sample loading port; I, secondary reservoir; K, tubing loop.](image1)

![FIG. 2. Rear view of manifold. A, B, 3-way switch valves; C, D, 4-way switch valves; E, bulkhead connection to tubing loop; F, supply of ultrapure, non-radioactive H₂/N₂ mixture; G, connection to secondary reservoir; H, bulkhead connection to loading port.](image2)
The total concentration of H₂ (20% by volume) was chosen for two reasons: first, because it results in a dissolved H₂ concentration much larger than most hydrogenase half-saturation constants (Adams 1990, and references therein), a necessary condition to interpret the measurements as potential activity; and second, because it resulted in a high enough H₂ specific activity to give the assay enough sensitivity for most of our samples, within the total H₂ possession limits imposed by our radioisotope license.

Carrier-free H₂ (American Radiolabeled Chemicals, Inc.) in a break-seal vial was loaded into the cylinder in order to prepare the H₂/N₂ stock. The break-seal vial ground glass fitting was cut off, and a stir bar wrapped in teflon tape so it just fit the glass tube was inserted ∼3 cm from the breakable septum. The vial was then inserted in the cylinder, always held horizontally to avoid sliding the stir bar into the septum and prematurely liberating the tritium. The removable adaptor was then installed. A vacuum line was connected to the fitting, and the cylinder was pressurized to 5 atm, to avoid gas flowing, the vacuum tube was removed and the cylinder closed with the plug. The cylinder was pressurized to 5 atm, to obtain a H₂ activity of ∼7.5 × 10⁶ Bq (0.2 mCi) per ml of gas. The cylinder was closed and the vial was broken by turning the cylinder vertically and tapping it on a piece of wood to slide the stir bar into the septum to liberate the H₂.

Measurement of H₂ Specific Activity

The procedure as described in Soffientino et al. (2006) employed a 20 ml septum-sealed vial containing pellets of palladium on alumina to convert a measured volume of H₂/N₂ headspace to tritiated water at room temperature. This method proved unreliable and was substituted with catalysis by an incandescent platinum wire. For this purpose, a two-neck glass vessel (∼30 ml volume) was custom-built from a 2.5 cm diameter glass tube (Figure 3). One of the necks was fitted with o-ring sealed electrical leads, the other with a 10-mm diameter injection septum. The ends of the electrical leads were connected with a 5 cm loop of 0.1 mm diameter platinum wire. Voltage was gradually increased until the platinum wire glowed bright orange (∼5 V); a 1-ml volume of headspace was injected through the septum and allowed to react for 3 min; the power was then turned off, and 5 ml of distilled water were injected into the vessel and shaken for 1 min to trap the H₂O vapor. Fifty µl of water were withdrawn for scintillation counting. The specific activity of the gas (Bq/mol H₂) was calculated as described in Soffientino et al. (2006).

To allow comparison of hydrogenase activity between sites assayed with the different methods, the Challenger Mound data were normalized by the ratio of the headspace specific activities obtained with Pt and with the Pd method. The ratio (6.13) was determined by measuring a batch of tritiated headspace in triplicate with both procedures.

To determine the effect of freezing and thawing on sediment hydrogenase, sulfidic coastal sediment was collected by hand-coring as described in Soffientino et al. (2006), stirred with anoxic sterile seawater under a stream of N₂, and divided into six 1-ml aliquots, half of which were frozen for 3 hours at −80°C, and half refrigerated at 4°C. Subsamples (100 µl) from each aliquot were assayed in the same run, yielding mean activities of 1.51 × 10⁻⁸ and 1.78 × 10⁻⁸ mol H₂/ml slurry/min for the frozen and unfrozen samples, respectively. The means were not statistically different (t-test P = 0.24).

Cell Abundance Data and Cultures

Published cell abundance data were used to calculate cell-specific hydrogenase values. Data for the Lomonosov Ridge sediments are from Kallmeyer et al. (2008). Most cell abundance data is from horizons within 2 m of the one that was assayed for hydrogenase activity. Data for the Porcupine Seabight were obtained from the Expedition 307 report (Ferdelman et al. 2006).

Clostridium pasteuriunum was purchased from the American Type Culture Collection (ATCC no. 6013) and grown in Reinforced Clostridial Broth (Difco # 1808-17-3) in Hungate tubes at 37°C. One hundred µl of late exponential culture was diluted in 5 ml of phosphate buffer (pH 7.2) and assayed for hydrogenase as described; cell concentration was determined by serial dilution and plate counting.

Sites Description

Sediments from the Lomonosov Ridge Site were obtained during the IODP Arctic Coring Expedition (ACEX) in the summer of 2004. A total of 428 m of sediment were recovered from five holes at three sites (Backman et al. 2006). All sites were within a 16 km transect near 87.5° N, 139° W. Based on stratigraphic correlation, the cores are treated as a single sediment column and depths are reported in meters composite depth (mcd).

These sediments contain three main lithologic units. Unit 1 extends from the seafloor to about 220 meters composite depth (mcd) and ranges in age from present to middle Eocene, with
a 20 Ma hiatus around 200 mcd. It is divided into 6 subunits on the basis of color, texture, and composition. Sediments are mainly silty clay, with biogenic carbonate ooze towards the top and bottom, and OC increasing with depth from <0.3% to ~1% in subunit 1/5 with a maximum of 6% in subunit 1/6. Unit 2 comprises the interval between 220 and 310 mcd, ranging in age from ~46 to 52 Ma. Sediments are microlaminated, biosiliceous clays and oozes, containing 2–4% OC. Unit 3 is found between ~310 and 405 mcd, comprising ages between 52 and 56 Ma, and consisting of mud-bearing biosiliceous oozes with microlaminated zones and 1–2.5% OC content. Fifteen samples from Unit 1 and three from Unit 2 were obtained for microbiological analyses.

Sediments from the Porcupine Seabight (~51°N, 11°W) were obtained in the spring of 2005 during IODP Expedition 307 (Ferdelmann et al. 2006). Three Sites were cored along a transect that included Challenger Mound (Site 1317), a downslope (Sites 1316), and an upslope Site (1318). Water depth at these locations was between 500 and 1000 m. Samples were obtained roughly every 10 m intervals from two or more drill holes within each site (see below for specifics), and their depth is given as meters below seafloor.

Challenger Mound consists of two main lithostratigraphic packages: Unit 1, Pliocene-Pleistocene in age, extending from the seafloor to an unconformable, consolidated boundary at ~130 meters below seafloor (mbsf) that defines the mound base. Sediments are made up of clays and 15 to 75 wt.% carbonate with abundant coral fragments, arranged in layers of floatstone (40%), rudstone (50%), and wackerstone (15%). Unit 2, Miocene in age, extends from ~130 to 260 mbsf, and consists of siltsand and sandstone facies with ~25–75% carbonate but few or no coral fragments. Samples came from Holes 1317A (15 samples, Unit 1) and 1317D (14 samples, Unit 2).

Site 1316, downslope from the mound, includes 3 lithostratigraphic units: Unit 1 (middle-late Pleistocene) extending from the seafloor to 45 mbsf; Unit 2 (Pleocene-late Pleistocene) from 45 to 55 mbsf; and Unit 3 (early-middle Miocene) from 55 mbsf to the bottom of the Hole (140 mbsf). The unconformable boundary separating Units 2 and 3 is correlated with the mound base at Site 1317. Sediments consist mostly of silty clay and clayey silt layers, with 20–60% carbonate but few coral fragments. Five samples of Unit 1 and one sample of Unit 2 were obtained from Hole 1316B; six samples of Unit 3 came from Hole 1316C.

Site 1318, upslope from the mound, is also composed of 3 distinct units: Unit 1 (Pleistocene, 3 subunits) composed of silty and sandy clay with ~15% carbonate, extending to ~80 mbsf; Unit 2 (Pleistocene), composed of fine sand and 20–50% carbonate, extending to ~86 mbsf; and Unit 3 (Miocene, 3 subunits), composed of alternating layers of silty and sandy clay with variable amounts of carbonate (15–70%), extending from 86 mbsf to the bottom of the hole at 245 mbsf. Six Unit 1 and two Unit 3 samples came from Hole 1318A; four additional Unit 3 samples came from Hole 1318B.

**Sample Collection and Handling**

Samples from IODP Expedition 307 were collected on board the R/V JOIDES Resolution using standardized clean handling protocols. Five-cm long sections of the intact core were cut, capped, immediately sealed in airtight bags with O2 scrubbers, and frozen at ~80°C. Samples from Expedition 302 were collected onboard the RVIB Vیدar Viking and sampled directly from the end of the core sections using sterile, 50 cc plastic syringes with the luer end cut off. The syringes were sealed in airtight bags with O2 scrubbers, and frozen at ~80°C.

At the time of the assay, the samples were removed from the bags and split with hammer and chisel while still frozen. The outer 2 cm of the core sections were pared off and discarded. Fragments totaling 3–6 g were taken from the center, weighed, and placed in a 50-ml glass syringe barrel fitted with a stopcock. The syringe barrel was mounted vertically on a manifold delivering a stream of ultrapure N2, to keep the samples anoxic during thawing and slurring. Ten ml of 0.2 µm filtered, autoclaved, anoxic seawater was added to the syringe and the sediment was slurried using a glass rod. The syringe piston was fitted into the barrel while maintaining the flow of N2 to avoid trapping air. The stopcock was closed, the syringe was removed from the manifold and turned upward, and the trapped N2 bubble was removed. Twenty-five ml of tritiated headspace mixture was added and the syringes were incubated as described above.

**Statistical Analysis**

Hydrogenase activity was calculated by linear regression of water tritium scintillation counts against time. Two or three replicates were run for each sample. Because of sediments heterogeneity, the detection limit of the assay was defined for each sample as the upper 95% confidence limit (CI) of the regression slope for the mercury-killed paired control. A sample’s activity was significant if the lower 95% CI of the “live” sample slope was greater than the upper 95% CI of the killed control.

Comparisons of mean activity between sites, or between different units or geochemical zones within or between sites, were done by t-test or by one-way ANOVA followed up by the t-test where appropriate.

**RESULTS**

**Hydrogenase Activity**

Hydrogenase activity and its depth distribution varied widely within and between sites (Figures 4 a, e, h, and k).

Among the Porcupine Seabight sites, activity was measured in all samples tested. Mean activity was significantly greater at Challenger Mound (Site 1317) than at the upslope Site 1318 (1.15 vs. 0.12 nmol H2/g/min, P < 0.01) or downslope Site 1316 (1.15 vs. 0.45 nmol H2/g/min, P < 0.01). Mean activities at the upslope and downslope Sites were not statistically different from each other (P = 0.10).
FIG. 4. Hydrogenase distribution and geochemical profiles in sediments of the Arctic Ocean (a-d), and Porcupine Seabight Sites 1316 (e-g), 1317 (h-j), and 1318 (k-m). Hollow symbols in a, e, h, and k are not significantly different from HgCl-killed control. Symbols of different color represent different holes within a site. Error bars are one standard deviation.
At Challenger Mound (Site 1317), relatively low values, around 0.6 nmol H$_2$/g/min, were measured in the top 50 mbsf; activity increased 10-fold to a maximum around 90 mbsf, declined several-fold to a local minimum at 125 mbsf, and climbed again to a single-point maximum at 133 mbsf. Below this depth, values declined by two orders of magnitude to a minimum of 0.018 nmol H$_2$/g/min at 152 mbsf, increasing again 10-fold at 0.05 nmol H$_2$/g/min at 270 mbsf, and remaining relatively constant from this point to the deepest sample at 265 mbsf (Figure 4h).

Site 1316, downslope from Challenger Mound, had much lower activity and a different depth distribution. The highest activity was measured in the topmost sample at 0.018 nmol H$_2$/g/min at 265 mbsf, remaining around 0.05 nmol H$_2$/g/min at 221 mbsf, declining 10-fold to a relatively constant value of 0.005–0.01 nmol H$_2$/g/min by 20 mbsf. Values again increased 5- to 8-fold around 100 mbsf, remaining around 0.1 nmol H$_2$/g/min to the bottom of the Hole (Figure 4k).

In the Lomonosov Ridge sediments, activity was below background (∼0.005–0.01 nmol H$_2$/g/min) from 12 to 169 mcd. Significant activity was first measured within Unit 1/5 at 193 mcd, and reached a maximum of 80 nmol H$_2$/g/min at 270 mcd within Unit 2 (Fig. 4a).

**Correlation with Lithological Features**

Comparison of hydrogenase profiles and lithostratigraphy was done to understand whether changes in activity were associated with particular stratigraphic boundaries, facies, or mineral components.

At Challenger Mound (Site 1317), differences in activity between the two major lithostratigraphic units were apparent (Figure 4h). Average activity in the mound body (Unit 1, above 130 mbsf) was significantly higher than in the mound base (Unit 2, below 130 mbsf; 1.6 vs. 0.45 nmol H$_2$/g/min, P < 0.01). Although considerable textural and compositional variability is present within each of these Units (Ferdelman et al. 2006), no small-scale correlation with hydrogenase was evident.

The profiles of Sites 1316 and 1318 show no differences in hydrogenase activity associated with specific lithostratigraphic units, facies, or mineral composition. No organic carbon data is yet available for any of the Porcupine Seabight Sites.

Hydrogenase activity was undetectable in the Lomonosov Ridge sediments subunits 1/1 to 1/4 (∼5–170 mcd). The appearance of significant hydrogenase activity was associated with the transition into Unit 1/5, at 193 mcd. Activity increased further in Unit 1/6, and reached a maximum of 80 nmol H$_2$/g/min within Unit 2 at 280 mcd. Organic carbon content increased in parallel with hydrogenase, reaching ∼1% within Unit 1/5 and oscillating between 2 and 4% in the layers below (Stein 2007).

**Correlation with Geochemical Profiles**

In the Lomonosov Ridge sediment sequence, the profiles of sulfate, alkalinity, ammonium, and dissolved Fe$^{2+}$, Mn$^{2+}$ (Figure 4b, c, and d) suggest that microbial activity is present throughout the sediment column, despite the apparent absence of hydrogenase down to 190 mcd. The appearance of hydrogenase activity below 190 mcd coincides with relatively high alkalinity and ammonium concentrations, but does not correlate with changes in the profiles of iron, manganese, or sulfate.

Zones of organoclastic sulfate reduction at the three Porcupine Seabight Sites are evident from the concave down sulfate and concave up alkalinity profiles within the top 10–15 mbsf at Sites 1316 and 1318 (Figs 4f and 4l, respectively), and within the top 50 mbsf at Site 1317 (Figure 4i). As shown in Figures 4e, 4k, and 4h, hydrogenase is present in these zones, although activity does not appear to be enhanced relative to other regions. At Sites 1316 and 1318, the meandering sulfate profiles below 15 mbsf suggest that those sediments may not be at steady state with respect to transport of dissolved substances. The local sulfate maxima around 30 and 150 mbsf at Site 1318 (Figure 4l), for example, cannot be easily explained by chemical or biological processes, and might have been caused by periods of relatively high sedimentation rate. Zones of sulfate reduction, however, can still be inferred at this site by examining the profiles of alkalinity and methane concentration.

Deeper in the sediment, a zone of sulfate-dependent methane oxidation can be inferred from depletion of methane, concave down sulfate profile, and presence of a local maximum in alkalinity below 80 mbsf at Site 1316 (Figures 4f and g), and between ∼150 and 200 mbsf at Site 1317 (Figures 4i and j). Hydrogenase was present in these zones at both sites, and the mean values do not differ significantly (1316, n = 6; 1317, n = 5; P = 0.14). Although no change in activity from the overlying zones is apparent at Site 1316 (Figure 4e), mean hydrogenase activity decreased ∼25-fold with respect to the sediments above it at Site 1317 (Figure 4h).

**Cell-Specific Hydrogenase Activity**

Cell-specific activity is shown in Figure 5. Values spanned 3 orders of magnitude, with most of the variability in the ratio being contributed by hydrogenase. The relative standard deviation was 5.5 and 0.7 for hydrogenase and cell abundance, respectively. All but one datum were below the value calculated for *C. pasteurianum*. Most of the Challenger Mound (Site 1317) and Lomonosov Ridge samples are within one order of magnitude or less of *C. pasteurianum*.

**DISCUSSION**

In this paper we present the first measurements of hydrogenase activity in deeply buried oceanic sediments. The samples came from two widely different environments: the clay sediments of the Lomonosov Ridge, Arctic Ocean, collected during
that the activity of a coastal, sulfidic sediment is not severely decreased by freezing, it needs to be demonstrated whether the activity from different sedimentary environments is similarly stable or is affected differently, as this might lead to biased comparisons. Studies are also needed to determine how much, if any, of the sediment activity is extracellular, as was found to be the case in soil (Häring et al. 1994), and whether the kinetic properties of this fraction are different from those of the cell-associated activity (Häring and Conrad 1994). It is also not known how closely hydrogenases’ temperature optima match the in situ temperature, and how wide the optimum range might be. The optima of carbohydrate-hydrolyzing enzymes in shallow sediments, for example, match the average in situ temperature fairly closely (Arnosti and Jørgensen 2003). If this were the case for hydrogenases, it would render necessary the use of temperature-controlled incubations for different depth ranges, at least for sediment columns with a strong geothermal gradient such as those found in tectonically active regions.

With regard to the results presented here, both Lomonosov Ridge and Porcupine Seabight sediments exhibited a 2–3°C/100 m geothermal gradient (Backman et al. 2006; Ferdelman et al. 2006), typical of regions away from plate boundaries. The seafloor temperature, however, was very different at the two locations, being −0.5°C at the Lomonosov Ridge, and about 10°C at the Porcupine Seabight. From this we can estimate that most of our Porcupine Seabight samples were assayed at a temperature (room, about 22°C) at most 10–12°C above in situ, while most of the Lomonosov Ridge, which would have been assayed at as much as ∼22°C above. It is possible, therefore, that our failure to detect significant activity in many of the Lomonosov Ridge samples might have been at least in part caused by our assaying these possibly psychrophilic enzymes at too high a temperature.

Hydrogenase showed considerable variation between and within sites. At the Porcupine Seabight, hydrogenase was measured throughout the sediment column, consistent with the presence of microbial cells (direct counts) in all sediments examined, as well as with inferred microbial activity from geochemical profiles in large portions of the sediment column (Ferdelman et al. 2006). In the Lomonosov Ridge sediments, on the other hand, hydrogenase activity was undetectable in the top 190 meters of sediment, but was measured at high levels below this depth. However, cell abundance (Kallmeyer et al. 2008) and concentration profiles of reduced manganese and iron show evidence of activity in the top 175 meters of sediment. The potential effects of assaying at too high a temperature were discussed above, but in any case, microbial activity should be extremely low in this region, judging from the rather large vertical extent of the manganese and iron reduction zones, together with the very low levels of OC (<0.3%).

The most conservative interpretation of this absence of hydrogenase, therefore, is that activity was probably present, but that our assay was not sufficiently sensitive to detect it. The sudden appearance of hydrogenase activity in sediment units...
below 190 meters followed a compositional change from OC-poor clays to OC-rich silty sediments. This is consistent with the report of Goodwin et al. (1988), who showed that hydrogenase (and H$_2$ turnover) was positively correlated to organic loading of surface sediments in lakes with different degrees of eutrophication. It is somewhat curious that an increase in cell abundance did not also parallel the hydrogenase increase in the OC-rich Units 1/5, 1/6, and 2 of the Lomonosov Ridge sediments (Kallmeyer et al. 2008), since a positive correlation between microbial abundance and OC content has been shown for surficial marine sediments (Deming and Baross 1993). A direct test of the relationship between hydrogenase and OC content was unfortunately not possible, because measurements were made on horizons separated by as much as 20 m.

Among the Porcupine Seabight sites, higher activity was present at Challenger Mound (Site 1317). One of the main objectives of IODP Expedition 307 was to characterize the biogeochemistry of Challenger Mound, to understand whether biological activity had implications for the mechanism of mound genesis (Ferdeman et al. 2006). While the hydrogenase results reinforce the view that the mound is an area very distinctive from its immediate surroundings, there is no obvious indication of processes that might account for these differences in the sedimentological and geochemical data that we examined. Future studies involving hydrogenase measurement and concomitant assessment of community diversity and composition will be helpful in determining what variables drive large hydrogenase excursions such as those present at the Unit 1/2 boundary of Challenger Mound (Site 1317).

It was surprising to find that many cell-specific activity values from the Lomonosov Ridge and Challenger Mound sediments were on average within five-fold or less of $C.\ pasteurianum$ in laboratory culture. The Lomonosov Ridge measurements in particular come from high OC layers, suggesting the possibility that little physiological adaptation might be needed for microbes to utilize H$_2$ in deep sediments that contain an ample supply of organic matter. Furthermore, if we consider that only about 30% of direct counts might be active cells (Schippers et al. 2005), that some hydrogenase activity might have been destroyed by freezing, and that we might have underestimated the activity of pressure- and temperature-adapted enzymes by assaying at surface temperature and pressure, many more of the hydrogenase/cell values could be close to $C.\ pasteurianum$. On the other hand, if sediment bulk hydrogenase were to comprise a significant amount of extracellular activity, as it does in soils (Häring et al. 1994), the cell-specific activity reported here would represent somewhat of an overestimate.

In conclusion, we have shown that hydrogenase activity is present in large portions of deeply buried sediments from the Arctic Ocean and the Northeastern Atlantic. The co-occurrence of microbial cells in all samples corroborates our interpretation of these measurements as indicative of ongoing microbial processes. The hydrogenase assay appears to be a promising tool to detect low microbial activity in the deep subsurface, because it measures the catalytic activity of enzymes involved in the metabolism of H$_2$, which is ubiquitous in anoxic sediments. Its potential to inform us about the metabolic adaptations of deeply buried microbial communities will benefit from studies of hydrogenase activity distribution within the sedimentary matrix, its temperature optima in different environments, and its relationship withcell metabolic rate and growth from experimental systems with relevant environmental conditions.

REFERENCES


