Evidence Against Hydrogen-Based Microbial Ecosystems in Basalt Aquifers

Robert T. Anderson, Francis H. Chapelle, Derek R. Lovley*

It has been proposed that hydrogen produced from basalt–ground-water interactions may serve as an energy source that supports the existence of microorganisms in the deep subsurface on Earth and possibly on other planets. However, experiments demonstrated that hydrogen is not produced from basalt at an environmentally relevant, alkaline pH. Small amounts of hydrogen were produced at a lower pH in laboratory incubations, but even this hydrogen production was transitory. Furthermore, geochemical considerations suggest that previously reported rates of hydrogen production cannot be sustained over geologically significant time frames. These findings indicate that hydrogen production from basalt–ground-water interactions may not support microbial metabolism in the subsurface.

A better understanding of the energy sources that support microbial ecosystems in the deep terrestrial subsurface is needed because the deep biosphere is an important biogeochemical entity on Earth (*1*, *2*) and may serve as a model for life on other planets (*3*, *4*). The lack of light in the deep subsurface necessitates an ecosystem in which organic or reduced inorganic compounds are the energy source for deep subsurface microbes. Particulate organic matter that has been deposited with the sediments that form aquifers or organic matter that has been dissolved in ground water may support heterotrophic subsurface ecosystems (*1*, *5*). However, Stevens and McKinley proposed that H_2, rather than organic matter, was the primary energy source that supported microorganisms in deep basalt aquifers of the Columbia River basin (*6*). The hypothesized source of the H_2 was an abiological reaction between the basalt and the ground water, in which iron in ferromagnesium silicates reduced water to H_2 (*6*, *7*). If correct, this hypothesis changes the concepts of microbial life in the subsurface because it suggests that subsurface life can exist without a photosynthetic surface environment, which provides either organic electron donors for microbial metabolism or oxygen as an electron acceptor. This abiological reaction would not only alter our understanding of the environmental requirements for life on Earth but would also increase the possibility that other planets with surface environments that are not conducive to life could harbor subsurface microbial ecosystems.

To evaluate whether H_2 from basalt–ground-water interactions could support life in the subsurface, we collected basaltic aquifer material from the Snake River aquifer in Idaho and conducted a series of experiments to determine the conditions that would be favorable for H_2 formation (*8*). In a previous study (*6*), Snake River Plain basalt produced H_2 faster than any of the other basalts tested. When crushed Snake River Plain basalt was incubated with ground water (pH 8) from the Snake River aquifer under anaerobic conditions, there was no significant H_2 production from the basalt–ground-water interaction (Fig. 1).

We repeated this experiment, substituting a phosphate buffer at pH 6 for the ground water, because Stevens and McKinley used a pH 6 phosphate buffer in their experiments rather than ground water (*6*). Small amounts of H_2 were produced under these conditions (Fig. 1). The initial rate of H_2 production was more than three times as fast as any of the basalts examined by Stevens and McKinley, and the amount of H_2 produced within 120 hours (the longest incubations reported by Stevens and McKinley for active basalts) was about six times the previously reported number. We continued our incubations longer than the time reported by Stevens and McKinley and found that, with continued incubation, H_2 production stopped. To determine if H_2 production was product inhibited as H_2 accumulated, we flushed the H_2 from the headspace with N_2. However, there was little further H_2 production (Fig. 1). These results demonstrate that the lack of further H_2 production was not due to product inhibition but rather was due to a limited capacity for H_2 production from the basalt.

The amounts of H_2 produced in the pH 6 phosphate buffer were so small that any changes in the oxidation state of minerals in the basalt would be difficult to detect. The modest H_2 production and its transitory nature suggest that the H_2 production at pH 6 is an artifact due to the exposure of small amounts of reactive mineral surfaces during the crushing of the rock. For example, studies have demonstrated that grinding silicate-containing rocks results in the formation of silica radicals, which may react with water to produce H_2 (*9*). The suggestion that H_2 formation is an artifact from grinding is consistent with the finding that H_2 was produced faster and to a greater extent in our studies than in the study by Stevens and McKinley. The diameter of our crushed basalt particles (0.53 to 0.125 mm) was smaller than the size fraction (<0.250 mm diameter) used by Stevens and McKinley, and thus, our basalt is likely to have had a larger reactive surface area. If reactive surfaces generated by grinding are necessary for H_2 production, it is difficult to imagine how basalt fracturing at a rate that is sufficient to support H_2 production could be taking place in situ, with solid rock still existing 6 to 17 million years after basalt deposition.

To determine whether the difference in the rates of H_2 production between the incubations with ground water and the incubations with the pH 6 phosphate buffer were the result of a difference in pH, we incubated basalt in a phosphate buffer at pH 8. The rate of H_2 production with a pH 8 phosphate buffer was negligible (Fig. 1).

Thus, our studies demonstrated that small amounts of H_2 can be temporally produced in a basalt-phosphate buffer system at pH 6, as previously reported (*6*), and that H_2 was not produced at the environmentally relevant pH of 8. The pH dependence of H_2 production is significant because ground water in basalt

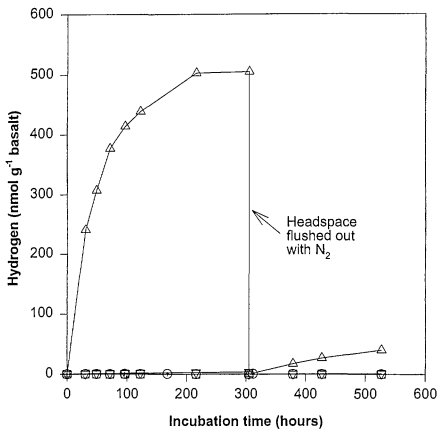

Fig. 1. Production of H_2 over time by Snake River Plain basalt when incubated with ground water collected from the Snake River aquifer or phosphate buffers (⊕, ground water with basalt; +, ground water alone; △, pH 6 phosphate buffer with basalt; ▽, pH 8 phosphate buffer with basalt; □, basalt alone; ○, buffer alone).

R. T. Anderson, Department of Civil and Environmental Engineering, University of Massachusetts, Amherst, MA 01003, USA. F. H. Chapelle, U.S. Geological Survey, Columbia, SC 29210–7651, USA. D. R. Lovley, Department of Microbiology, University of Massachusetts, Amherst, MA 01003, USA.

*To whom correspondence should be addressed. E-mail: dlovley@microbio.umass.edu.

aquifers, including the Columbia River basalt aquifer, is buffered at an alkaline pH (*10*). The breakdown of basalt minerals that are unstable at low temperatures in the presence of ground water consumes H^+ and releases Ca^{2+} and HCO_3^- with the precipitation of $CaCO_3$. The combined processes of mineral dissolution and $CaCO_3$ precipitation buffer the ground water at about pH 8 (*10*). Thus, although small quantities of H_2 can be produced from freshly crushed basalt at pH 6, this result has no relevance to processes that actually occur in the aquifer because such a low pH is not found in basalt aquifers.

Furthermore, even if H_2 could be produced at realistic ground-water pH levels, the basalt does not contain enough reducing power to continually produce the amount of H_2 that is necessary to support a microbial community over the life of the aquifer. Reduced iron in the basalt has been proposed to be the source of electrons for H_2 production (*7*). Columbia River basalt typically contains 10 to 13% iron by weight (*11*). At the H_2 production rates that are suggested by the data of Stevens and McKinley (8 to 16 nmol day^{-1} g^{-1} of basalt) (*6*), all of the iron would be oxidized within several hundred years. Even if the basalt were composed solely of reduced iron, H_2 production would be exhausted in several thousand years. Thus, with the mechanism proposed by Stevens and McKinley (*6*), it would be impossible to produce enough H_2 to support a hydrogen-based microbial ecosystem at the present time, millions of years after the formation of the aquifer.

Stevens and McKinley have suggested that H_2 concentrations as high as 60 μM in the Columbia River basalt aquifer provide evidence for a H_2-based ecosystem (*6*). However, many of the hydrogen samples were taken with electrically driven pumps, which have been shown to overestimate ground-water H_2 concentrations (*12*). Furthermore, the concentration of H_2 in anaerobic microbial ecosystems is a function of the physiological characteristics of the microorganisms that consume the H_2 and gives no information on the rate of H_2 production or its source (*13*).

A more likely energy source for microorganisms living in the Columbia River basalt aquifer is organic matter. In contrast to the suggestion that this is an organic-poor environment (*6*), a report has indicated that the ground water contains significant quantities of dissolved organic carbon (DOC) (2 to 5 mg $liter^{-1}$) (*14*). This concentration of DOC is comparable to the concentration in other aquifer systems in which organic matter oxidation drives microbial metabolism (*15*).

Carbon isotope data are also consistent with the idea that organic matter oxidation serves as the primary source of energy for the microbial community in the Columbia River basalt aquifer. In the portion of the aquifer in which the sulfate reduction predominates, the ground waters are depleted in ^{13}C, which is consistent with organic matter oxidation being an important process in this environment (*6*). In the methanogenic portion of the aquifer, the enrichment of ^{13}C in dissolved inorganic carbon is similar to the enrichment observed in organic-rich marine sediments in which organic matter fermentation is the source of H_2 for methanogenesis (*16*).

Analysis of data (*14*) on the microbial community in the Columbia River basalt aquifer also supports the suggestion that organic matter degradation fuels the microbial community. Although initial culturing studies indicated that H_2-using anaerobes outnumbered heterotrophic microorganisms (*6*), culturing studies can provide a biased view of the microbial community because media do not often recover the dominant organisms in the environment (*17*). When the microbial populations in ground water from Columbia River basalt aquifer were investigated with molecular techniques that avoided the culture bias (*14*), the community structure was found to be similar to the structure in anaerobic ecosystems in which organic matter oxidation is important. For example, Archaea accounted for <3% of the total population in the methanogenic aquifer, which is similar to the proportion found in the rumen of cows, where the fermentation of organic matter to H_2 drives methanogenesis (*14*). Methanogenic bacteria, which are in the Archaea (*18*), can be expected to constitute a low proportion of the total population in the rumen because numerous fermentative bacteria are required to metabolize the organic matter to H_2 to support methanogenesis. However, if H_2 was being produced from basalt–ground-water interactions (eliminating organic fermentation as a major process), then methanogenic Archaea should constitute a greater relative proportion of the microbial population. This would be true even in the unlikely event that H_2 was first consumed by acetogenic bacteria, with a subsequent conversion of acetate to methane. In a similar manner, sulfate reducers accounted for <3% of the total population in the aquifer in which sulfate reduction predominated (*14*), whereas sulfate reducers would be expected to be a dominant population in an environment in which abiologically produced H_2 fueled the microbial ecosystem.

Thus, a combination of geochemical and microbiological studies has demonstrated that there is no significant evidence to support the hypothesis that H_2 produced from basalt–ground-water interactions is the primary energy source for the microbial ecosystem in basalt aquifers. The results presented here do not rule out the possibility that reduced gases emanating from deeper in the Earth could fuel deep subsurface microbial ecosystems, as has been previously suggested (*3*).

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