

Hydrogen-driven subsurface lithoautotrophic microbial ecosystems (SLiMEs): do they exist and why should we care?

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One of the keys to success of many anaerobic ecosystems is the process of syntrophic intercellular hydrogen transfer. This process facilitates the overall reaction by end-product removal, taking advantage of a wide variety of organisms that are able to use hydrogen directly as an energy source by uptake hydrogenases. Thus, the issue is not whether there are hydrogen-driven processes or communities but whether there are hydrogen-driven communities that exist and persist independently of the products of photosynthesis (so called subsurface lithoautotrophic microbial ecosystems, or SLiMEs). It is the proof of long-term independence from photosynthesis and its products that is the most difficult issue to establish, and perhaps the most important one with regard to searching for SLiMEs both on and off our planet. Although the evidence is not yet unequivocal, a growing body of evidence supports the existence of SLiME-like communities: if they exist, the implications are immense with regard to understanding subsurface environments on Earth, looking for present day analogs of early Earth and the search for life in other worlds.

Introduction

Hydrogen is, to some degree, the essence of both anabolic biology via carbon and nitrogen fixation (reduction with hydrogen) and catabolic energy metabolism (carbon oxidation and electron transfer of hydrogen equivalents in the form of redox-active components): essential processes of all life that we know. In addition, the prokaryotic world has the ability (by the activity of hydrogenases) to use and/or produce H₂ directly, potentially setting up ecosystems that are powered directly and nearly exclusively by hydrogen, and thus potentially independent of the products of photosynthesis, such as organic carbon and molecular oxygen. It is the existence of such a specific subset of hydrogen-driven systems that we discuss here, in search of the true SLiMEs (subsurface lithoautotrophic microbial ecosystems [1–4]).

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Hydrogen-facilitated ecosystems

For the purposes of discussion we have separated ecosystems that use hydrogen into two types: hydrogen-facilitated (Figure 1) and hydrogen-driven (Figure 2). Hydrogen-facilitated metabolism is a well-known and well-characterized process often called intercellular hydrogen transfer or syntrophism [5–8]. This process is exemplified by the anaerobic degradation of fatty acids, in which different species of anaerobic bacteria oxidize fatty acids or other substrates that would be thermodynamically

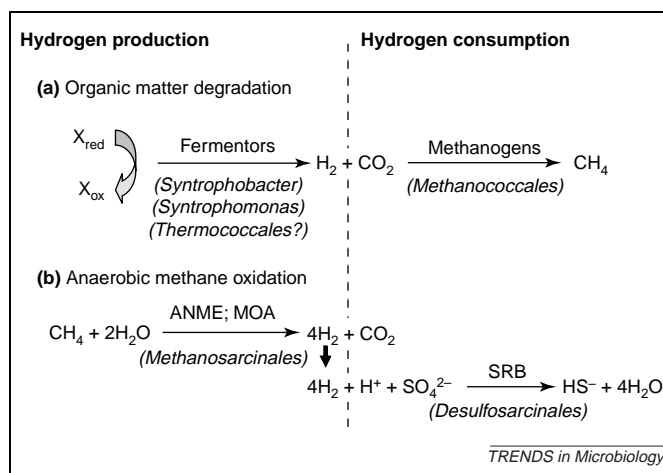


Figure 1. Hydrogen-facilitated ecosystems. This diagram illustrates the way that hydrogen can move between cells to facilitate metabolism of substrates that might otherwise be thermodynamically difficult. **(a)** Organic matter is fermented by syntrophs in general, such as *Syntrophomonas* or *Syntrophobacter* [7,8]. An example of this would be the fermentation of fatty acids to shorter chain fatty acids, plus CO₂ and H₂ (i.e. CH₃CH₂CH₂COO⁻ + 2 H₂O → 2 CH₃COO⁻ + 2 H⁺ + 2 H₂). The thermodynamic barrier to this reaction (ΔG₀ = 48.3 kJ mol⁻¹) can be overcome by coupling of this reaction with that of hydrogen utilization by a methanogenic bacterium, such as those in the genus *Methanococcus*. The effect is twofold: altering the equilibrium of the reaction to the right by product removal, and coupling the reaction to thermodynamically favorable methanogenesis [7,8], thus enabling overall energy to be gained. We have also included the genus *Thermococcus* in this diagram because it is a fermentor that has been identified as a component of a proposed SLiME system [4]. **(b)** A similar process in which a methanogen (e.g. a member of the *Methanosarcinales* spp.), reverses the methanogenic reaction, producing H₂ and CO₂. This reaction is closely coupled to sulfate reduction by sulfate reducing bacteria (SRB) in the group *Desulfosarcinales*, which are hypothesized to remove the hydrogen, facilitating the anaerobic oxidation of methane to CO₂ and produce H₂S as an end-product [9–12].

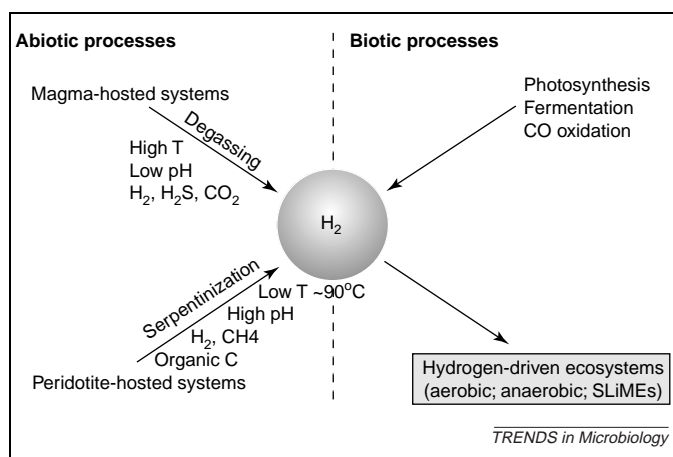


Figure 2. Hydrogen-driven ecosystems. This diagram illustrates the role of hydrogen and the connection that it forms between the geological world and the biological world. On the left side are the two major abiotic pathways for hydrogen production, by outgassing of magma-hosted systems and by serpentinization of ultramafic rock systems. The latter systems produce hydrogen primarily as a function of the interaction of anaerobic water with hot, highly reduced, iron-containing rocks. The ferrous iron can reduce the water to hydrogen, forming altered minerals and abundant molecular hydrogen. (i.e. the major reaction is simply the reduction of water to hydrogen by interaction with ferrous iron at high temperature).

difficult or impossible without the syntrophic interactions (Figure 1). The thermodynamics of the individual reactions are such that even small amounts of accumulated end-products (in this case, hydrogen) can result in inhibition of the reaction – one of the roles of the syntrophic partner is thus to remove hydrogen, facilitating the total reaction. This theme is an oft-repeated one in anaerobic metabolism, involving a wide variety of end-products in addition to hydrogen [7,8]. One example of syntrophism is the anaerobic oxidation of methane [9–12] (Figure 1), in which methanogens in the group *Methanosarcinales* (and perhaps others [11]) can make a living running the reaction backwards as long as a suitable hydrogen utilizing organism is present. Thus, these anaerobic methane-oxidizing Archaea, referred to as anaerobic methane oxidizers (ANMO; [9]) or methane oxidizing Archaea (MOA; [11]) have been found in stable consortia with sulfate-reducing bacteria, working together to oxidize methane anaerobically. Of note in Figure 1 is the unusual role of methanogens in the catalysis of such pathways. On one hand they are the hydrogen consumers that remove end-product hydrogen and facilitate the oxidation of fatty acids – on the other hand, they catalyze the production of hydrogen at the expense of methane oxidation: hydrogen that must be removed for the reaction to proceed (Box 1). Although it is difficult to say precisely when such interactive metabolisms arose, it has been speculated recently that these interactions are ancient, perhaps thousands of millions of years in age [13], consistent with the hypothesis that the atmosphere of the early Earth was hydrogen rich [14].

Hydrogen-driven ecosystems

Hydrogen-driven ecosystems can be viewed as two similar but easily distinguishable types; one using either oxygen or an oxygen-derived oxidant, such as nitrate or sulfate, to metabolize the hydrogen and the other using geologically

Box 1. Outstanding questions

- In a recent article hypothesizing the existence of a SLiME community called HyperSLiME [4], *Thermococcus* was identified as a major group. These are Archaea that are known to ferment, thus raising the possibility that a stable ecosystem exists that includes hydrogen production from decaying bacterial biomass, perhaps with the production of hydrogen to fuel further SLiME metabolism. Understanding the evolution of anaerobic ecosystems in the absence of photosynthetic influences will be important for the construction of models of early Earth metabolism, and for establishing reasonable hypotheses concerning the possibility of non-photosynthetic ecosystems elsewhere.
- The issue of growth rates and efficiencies of natural SLiMEs, should they exist, will be important to resolve because it is not at all clear that rapid growth was an important part of ecosystems in the early days of life. In the absence of predation, there might have been little need for rapid growth and in ecosystems in which predators are not present the same could still be true. Thus, one of the keys to recognizing SLiMEs might be to suppose that maintenance energy is the key to long term survival and that growth could be the thing that is done occasionally when conditions become compatible with population expansion. In general, it is hard, if not impossible, to study maintenance energy under anything but defined conditions, therefore, environmental estimates are not available. However, it is tempting to speculate in energy limited environments, that cell maintenance (e.g. transport, repair, membrane integrity) could dominate the energetics of the cell, while growth takes a back seat.
- Throughout this opinion, we have focused on hydrogen as the driving force for SLiMEs. Are there other potential players that we might have missed? Perhaps the one factor that has not appeared in the current thinking about SLiMEs is the possibility that CO could have a role. Weiss *et al.* [46] noted that the most abundant energy sources available to life on Mars were H₂ and CO, both of which are used by a wide number of different organisms on Earth. In fact, the recent work with CO-oxidizing anaerobic bacteria has demonstrated several Bacteria and Archaea that grow at the expense of CO, producing molecular hydrogen as an end-product [22–26]. If such life existed as part of a SLiME, then some of the energy used in the community could come from a biological source. However, it should be noted that the CO on Mars is of photochemical origin, and the lack of a flux into the subsurface was used by these authors to argue that if there was subsurface life on Mars, it was of no consequence to the atmospheric energy sources – in other words, if subsurface life exists on Mars, it leaves no global signal in terms of hydrogen and/or carbon monoxide.

supplied oxidants, primarily carbon dioxide (Figure 2). In both cases, the hydrogen supply is through geological sources but in the case of the SLiMEs the system operates in the absence of any influences from the photosynthetic surface. This distinction poses the question that is crucial to this opinion: ‘Is it possible for communities to exist and persist for long periods on geologically supplied energy sources independent of photosynthesis and primary production at the surface of the Earth?’ Such ecosystems were first suggested in 1992 both by Tommy Gold [15], who postulated the existence of the ‘deep hot biosphere’, and by Boston *et al.* [16], who entertained the possibility of chemosynthetic ecosystems in the subsurface of Mars. Both papers hypothesized that viable communities might be operating by geochemical energy input alone. In the case of Gold’s hypothesis, a subsurface community that could use geological oxidants and reductants and prosper in the subsurface of Earth, and perhaps elsewhere [15]; in the case of Boston *et al.*, a subsurface community powered by the chemoautotrophic hydrogen–carbon dioxide couple (and perhaps other redox couples [16]).

The appearance and definition of SLiME

A few years later, Stevens and McKinley [1] posited that a subsurface community in the Columbia River Basalt (CRB) Basin was driven by hydrogen that was generated by the interaction of basaltic rocks with anaerobic water; these researchers proposed the acronym SLiME to describe the community [1].

As discussed elsewhere in this article, there is no disagreement as to whether hydrogen-driven ecosystems exist but there is great disagreement as to what constitutes a true SLiME in the tradition of Gold [15], and especially as to whether true SLiMEs have been found.

A true SLiME should be energetically powered by the geosphere: both the energy sources (electron donors) and the oxidants (electron acceptors) should be supplied entirely by geological processes. The system should be also free of those electron donors and acceptors generated by photosynthesis or its products. Supplying abiotic energy sources should be no problem because there are adequate ways to supply geological fuels in the form of reduced gases [17], such as hydrogen, methane and hydrogen sulfide, amongst others (Figure 2). Hydrogen [18], along with methane and other gases, can be produced geologically by one of two mechanisms: (i) degassing: the outgassing of mantle-based rocks, releasing volatiles such as CO₂, H₂ and H₂S in neutral or slightly acidic fluids [17], or (ii) serpentinization: a process involving the reaction between water and highly reduced ultramafics (magnesium and/or iron rich minerals referred to as peridotites), releasing high pH fluids containing H₂ and CH₄ but little CO₂ because of the elevated pH (Figure 2). At the high temperatures and pressures of the deep sea hydrothermal environments, serpentinization systems are thought to produce substantial amounts of methane and hydrogen [19], and perhaps even organic compounds by carbon reduction via Fischer-Tropsch reactions, in which metal-catalyzed reduction of CO₂ results in the formation of methane and other short-chain hydrocarbons [20].

However, in these ecosystems, the availability of electron acceptors is a potential problem because the production of oxidants is difficult to achieve in such highly reduced settings: almost certainly these environments are electron acceptor limited. Add to this the fact that, even in the subsurface environment, one is continuously impacted by the organic carbon produced by primary production, and by oxygen or oxygen-derived oxidants that are produced during photosynthesis, and it is not surprising that some controversy surrounds the definition and existence of SLiMEs.

Evidence against and for SLiME communities

Controversy first arose with the publication of Anderson *et al.* [2] who used several lines of evidence to argue that the original report of Stevens and McKinley [1] did not contain sufficient evidence for a hydrogen-driven community. The first dealt with energy supply: it was argued that there was little evidence to support the proposed production of hydrogen by the basalt–water system. Stevens and McKinley had suggested that the basalt–water system could support the community they observed in the Columbia River Basalts, and based their contention

on the production of hydrogen in the laboratory with crushed basaltic rocks [1]. However, Anderson *et al.* argued that basaltic rocks were probably not capable of sustaining hydrogen production at the level needed to support this community, even for short times [2]. Their laboratory experiments with basaltic rocks under various conditions supported these contentions. Anderson *et al.* also noted that the levels of dissolved organic matter were potentially enough to support a heterotrophic community [2].

Of major importance in this discussion was the work of Fry *et al.* [21] (cited by Anderson *et al.* [2]), in which the CRB community analysis revealed a group of microbes more consistent with a heterotrophic metabolism rather than a methanogenic one. Anderson *et al.* also pointed out that the isotopic fractionation of carbon was more compatible with that of a heterotrophic community, and concluded – rightfully so – that this community was probably not a good example of a ‘true SLiME’.

The next report of a SLiME-like community was by Chapelle *et al.* [3], who reported a subsurface *Archaea*-dominated microbial community in the groundwater system beneath the Lidy Hot Springs in Idaho. It was proposed that this community constituted a hydrogen-driven ecosystem consisting primarily of methanogens. Although it was demonstrated that archaeal 16S rRNA genes were present and that the predominant groups were closely related to methanogens, there was no evidence that methane was being produced by them (either by physiological studies designed to demonstrate methane production, or by an isotopic analysis of the methane to indicate its biological origin). This apparently small point looms larger as many groups of methanogens are now clearly capable of both consuming (oxidizing) and producing methane [9–11], and several groups of *Archaea*, including both methanogens and members of the genus *Thermococcus*, are known to grow autotrophically on carbon monoxide [22–26], producing H₂ as a product (Box 1). Given that dissolved inorganic carbon was in great excess, fractionation during methane production would have been expected. Overall, the Lidy Springs community would appear to be consistent with its designation as a SLiME, however, some important unknowns need to be established before it can be regarded as certain.

A final point as regards the Lidy Hot Springs and the criteria for SLiME communities: the waters of Lidy contained ample levels (millimolar) of sulfate, an oxygen-derived oxidant, which might have been expected to compete to some degree with CO₂ as an electron acceptor, or even to be involved with anaerobic methane oxidation.

A more recent report of a SLiME candidate comes from Takai *et al.* [4], with the report of a hydrogen-driven community, which was called Hyper-SLiME, in the subvent region of the Central Indian Ridge (CIR), previously discovered and chemically characterized by Gamo *et al.* [27]. Geochemical analyses showed that the hydrothermal fluids emanating from two different vents had low Mg concentrations, indicating little or no mixing with ambient seawater: accordingly, there were low (micromolar) levels of sulfate. Gas analysis showed millimolar amounts of hydrogen and CO₂, and micromolar levels of methane, the

isotopic fractionation of which suggested that it was partially of biological origin. Microbiological analyses included cultivation dependent and independent methods, both of which pointed to the abundance of Archaea in the groups *Methanococcales* and *Thermococcales* [4]. As sampling sites moved away from the central chimneys to more seawater-diluted sites, the nature of the populations shifted to much more bacteria-rich communities. Finally, hybridization probes were constructed to each of the major groups detected and it was found that the *Methanococcales* were in fact dominant types in the samples that were collected. Unfortunately, the types and levels of dissolved organic carbon were not determined for these samples.

Criteria for the existence of SLiMEs

One thing that is clear from the papers discussed previously is the obvious need for more specific criteria that can be used to establish whether there is in fact such a thing as a 'true SLiME'. To this end, we have put together a list of proposed criteria (Box 2). These criteria, if all met, would establish the existence of a SLiME and might serve as signposts for finding such environments on our planet and perhaps elsewhere. The short answer to this question would appear to be that there are as yet no unequivocal demonstrations of a true SLiME community. In all cases, one or more pieces of data are either missing or are even contradictory to the inclusion of the true SLiME site. Even with this first attempt at criteria establishment, it is clear from the discussion that it is extremely difficult to unequivocally establish that an environment is truly a SLiME. If we add to this the criterion that the environment should persist indefinitely in the absence of photosynthesis, the designation as a SLiME becomes even more problematic.

Other possible SLiME sites

Several other potential SLiME communities have been proposed but for now the paucity of data precludes a serious assessment. For example, the availability of abundant reduced gases is consistent with the proposals

of SLiME-like communities in the subsurface vent areas of the mid-oceanic ridge (MOR) areas [28–36]: these proposals are based primarily on the observations of abundant biomass exiting from hydrothermal vents, the detection of biomarker molecules in the vent fluids and the identification of microbes in the hydrothermal emissions and inside the walls of black smokers. However, these intriguing proposals have been difficult to substantiate because, although the MORs are often located in areas of low sedimentation and organic input, they are nevertheless subject to dilution by seawater, which contains many components (i.e. dissolved organic carbon and/or oxygen and sulfate) from the surface environment.

Serpentinization environments (Figure 2) could be other candidates for SLiMEs. These sites can be deep-sea [37–39] or terrestrial [40,41] and tend to be ultrabasic (pH ~ 11.5 or higher), leading to the formation of massive carbonate precipitates. The extremely low numbers of microbial cells ($\sim 10^3 \text{ ml}^{-1}$) in samples from terrestrial sites [40,41] suggest that this high pH regime is extremely harsh. Deep-sea serpentinization sites have been found in both the Atlantic ocean (the so-called Lost City carbonate deposits [38,39]) and in the Pacific [37], where carbonate deposits tend to be less massive but more widely distributed.

Thus, we see that finding the true SLiME is difficult: serpentinization can provide ample energy in the form of hydrogen, methane and even reduced carbon, but the conditions are harsh: degassing environments offer a lower pH regime but are almost always mixed to some degree with entrained seawater, making it difficult or impossible to establish a photosynthesis-independent system. On the surface of the Earth, these systems provide ample energy that can be used rapidly and efficiently by any of several microbial types over a wide range of pH values. Indeed, Spear *et al.* reported recently a hydrogen-driven community in Yellowstone Park, even in the presence of abundant hydrogen sulfide [42]: a community consisting primarily of hydrogen using micro-aerophiles – not a SLiME, but a hydrogen-driven system of great interest.

The broader picture: why should we care?

This discussion has been focused on extreme subsurface environments: places that allow escape from the over-riding effects of photosynthesis and its products. However, the interest in SLiMEs extends to both ancient ecosystems on Earth and similar ecosystems that might exist on other planetary bodies. In both cases, knowing the properties of hydrogen-driven systems that can function in the absence of oxygen or other photosynthetic products would be of potential interest.

Stepping back in time, it is easy to imagine organic-poor, anaerobic seawater being entrained by circulating hydrothermal waters or interacting with ultramafic minerals to produce hydrogen, leading to ecosystems with similar characteristics to the subsurface environments of today: similar thoughts prevail for potential non-photosynthetic extraterrestrial worlds. For example, if extremely hot water, containing H_2 , CO_2 and methane, were to be vented into a dark anoxic ocean, then the

Box 2. Criteria for designation as a 'true SLiME'

- (i) • A geologically produced energy source should be present and a reasonable scenario provided for its continuous production.
 - Energy sources from photosynthesis should not be present.
- (ii) • A geologically produced supply of appropriate electron acceptor(s) should be present and a reasonable scenario provided for its continuous renewal.
 - Electron acceptors from photosynthesis (or dependent upon photosynthesis) for their regeneration should not be present.
- (iii) • Appropriate types of organisms, consistent with the proposed metabolism of the environment should be present.
 - Quantitative estimates using cultivation-independent rRNA sequence analyses (or equivalent methods) should be used.
 - Fluorescent probe analysis of samples, with relevant probes should be used.
- (iv) • Substrate and product analysis should be provided to support the hypothesis of SLiME metabolism.
 - Isotope fractionation of reactants and products should be used.
 - Rate(s) of processes (measured or estimated) should be used.

choices available to any metabolic system might be extremely limited. Assuming that a recycling system could be achieved for nitrogen and other key elements, then life could potentially exist but it would have limited options as to metabolic pathways and a limited future in terms of the heights it could achieve [43]. As products accumulate, the energetics of making a living would get worse [7,8] unless those products (methane, acetate and biomass) could be disposed of. Of special interest in this scenario is the level of sulfate in the ancient ocean and the resupply of this oxidant once it is converted to sulfide. In the absence of photosynthesis (to either oxidize the sulfide or to produce oxygen for its oxidation), it is not obvious how to contend with this problem.

One issue of great importance in understanding the subsurface community is that of growth rate. It has been proposed that growth rates in the deep subsurface are extremely slow [1], in the order of thousands of years. If so, then the amount of energy that might be needed to sustain such a community would be much less than is commonly imagined and it would be difficult to study experimentally. However, in a recent article, the microorganisms in the deep subsurface were reported to be active or alive, with carbon turnover rates comparable to those seen in surface environments [44]. Although one cannot equate carbon turnover rates to growth without more information, if it is true that SLiMEs are in fact growing rapidly, then the key will be to understand how they manage such a feat under such energy limiting conditions (Box 1).

Concluding remarks

If long-lived SLiMEs are indeed possible, then, as several authors have noted, the search for life in the universe takes on a new dimension. One in which hidden microbial communities powered by geochemically supplied reductants and oxidants could exist in many other worlds. Many authors have commented on these possibilities, with the general ideas of deep subsurface metabolism [15,43] or with more specific thoughts on the potential for hydrogen and/or CO₂ metabolism that could power ecosystems comprising methanogens and/or acetogens [16,45]. With regard to potential subglacial life on Europa, Gaidos *et al.* [43] have pointed out that, although a hydrogen-based methanogenic ecosystem is possible, in the absence of light and accompanying photosynthetic resupply of oxidants that have been depleted by the lithotrophic metabolism, the metabolic levels to which life can aspire will be severely limited. Thus, the real question with regard to SLiMEs might be: are SLiMEs capable of metabolic achievements on long time scales, and what kind of microbiological (e.g. syntrophic interactions) or geological (e.g. burial of reductants) 'tricks' might they have invented to do so? (Box 1). The answer to this question could contain insights into the earliest life on our own planet and perhaps to the nature of life in other, non-photosynthetic worlds.

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