Energetic Constraints on H_2-Dependent Terminal Electron Accepting Processes in Anoxic Environments: A Review of Observations and Model Approaches

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Microbially mediated terminal electron accepting processes (TEAPs) to a large extent control the fate of redox reactive elements and associated reactions in anoxic soils, sediments, and aquifers. This review focuses on thermodynamic controls and regulation of H_2-dependent TEAPs, case studies illustrating this concept, and the quantitative description of thermodynamic controls in modeling. Other electron transfer processes are considered where appropriate. The work reviewed shows that thermodynamics and microbial kinetics are connected near thermodynamic equilibrium. Free energy thresholds for terminal respiration are physiologically based and often near −20 kJ mol^{-1}, depending on the mechanism of ATP generation; more positive free energy values have been reported under "starvation conditions" for methanogenesis and lower values for TEAPs that provide more energy. H_2-dependent methanogenesis and sulfate reduction are under direct thermodynamic control in soils and sediments and generally approach theoretical minimum energy thresholds. If H_2 concentrations are lowered by thermodynamically more potent TEAPs, these processes are inhibited. This principle is also valid for TEAPS providing more free energy, such as denitrification and arsenate reduction, but electron donor concentration cannot be lowered so that the processes reach theoretical energy thresholds. Thermodynamics and kinetics have been integrated by combining traditional descriptions of microbial kinetics with the equilibrium constant K and reaction quotient Q of a process, taking into account process-specific threshold energies. This approach is dynamically evolving toward a general concept of microbially driven electron transfer in anoxic environments and has been used successfully in applications ranging from bioreactor regulation to groundwater and sediment biogeochemistry.

1. Terminal Electron Transfer Processes in Anaerobic Decomposition

A large fraction of organic matter buried in soils, sediments, and aquifers is respired with electron acceptors other than oxygen. The occurrence and predominance of such anaerobic terminal electron accepting processes (TEAPs, Table 1) govern the biogeochemistry and microbiology of these environments, as well as the fate of trace elements and contaminants undergoing redox processes (*1*, *2*). On larger spatiotemporal scales, TEAPs influence the release of methane and nitrous oxide to the atmosphere and potentially also the sequestration of carbon in the subsurface. The regulation of TEAPs is thus of importance to almost any biogeochemical process or cycle under investigation and has accordingly received considerable attention. Researchers have recognized early that thermodynamic considerations may provide a framework for analyzing the occurrence of TEAPs under the heading of redox zonation and suboxic diagenesis (e.g., *3*, *4*). From such beginnings much progress has been made with respect to theoretical concepts and environmental applications, and particularly to the critical role molecular hydrogen concentrations play as a control on TEAPs. This review's objective is to outline and evaluate some of this progress and to report on the lessons learned. In particular, we will focus on the following questions: (i) under what circumstances the kinetics of individual H_2-dependent TEAPs may be related to their respective energy gain, (ii) how this relationship can be quantitatively modeled, and (iii) what consequences thermodynamic control on TEAPs has for their spatial and temporal distribution. Reference is given to studies involving other electron donors, as well as fermentation processes, where general concepts can be illustrated or confirmed.

TEAPs are preceded by a network of processes involving the action of extracellular enzymes, fermentation, and syntrophic processes (*5*−*7*) (Figure 1). To simplify the following descriptions, we include methanogenesis under the TEAP heading, being aware that methane is only partly produced from reduction of CO_2. Anaerobic decomposition is initiated by extracellular enzymes, produced by fermenting bacteria, which hydrolyze molecules that cannot pass the outer membrane of bacteria due to their high molecular weight (*8*). Among the many decomposition products, such as simple sugars, amino acids, short-chained organic acids and alcohols, acetate and molecular hydrogen (H_2) are primary substrates for methanogenic *Archaea* and a range of microorganisms mediating TEAPs (*9*). For several reasons, H_2 plays a particularly important role in this respect. The microbial demand for H_2 is strong since the ability to oxidize H_2 through membrane-bound hydrogenases is phylogenetically widespread (*10*). Consequently, H_2 is linked to the metabolism of various electron acceptors such as NO_3^-, SO_4^{2-}, or CO_2, and its concentration controls much of the energy

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TABLE 1. Overview of Important H_2-Consuming TEAPs and Their Gibbs Free Energies under Standard (ΔG^0) and Exemplary Environmental (ΔG_r) Conditions, Respectively

process	reaction stoichiometry	ΔG^0 (kJ mol^{-1})[a]	ΔG_r (kJ mol^{-1})[b]
oxic respiration	$1/2O_2 + H_2 \rightarrow H_2O$	−237.2	−206.7
denitrification	$2/5\ NO_3^- + H_2 + 2/5\ H^+ \rightarrow 1/5\ N_2 + 6/5\ H_2O$	−240.1	−186.6
iron reduction	$2\ FeOOH(a) + H_2 + 4\ H^+ \rightarrow 2\ Fe^{2+} + 4\ H_2O$	−182.5	−39.8
arsenate reduction	$HAsO_4^{2-} + H_2 + 2\ H^+ \rightarrow H_3AsO_3 + H_2O$	−162.4	−53.9
sulfate reduction	$1/4\ SO_4^{2-} + H_2 + 1/4\ H^+ \rightarrow 1/4\ HS^- + H_2O$	−48.0	−9.5
hydrogenotrophic methanogenesis	$1/4\ HCO_3^- + H_2 + 1/4\ H^+ \rightarrow 1/4\ CH_4 + 3/4\ H_2O$	−43.9	−8.2
homoacetogenesis	$1/2\ HCO_3^- + H_2 + 1/4\ H^+ \rightarrow 1/4\ acetate^- + H_2O$	−36.1	+2.4

[a] Calculated from Gibbs free energies of formation (*45, 123, 124*) O_2, H_2, N_2, and CH_4 as gaseous species. [b] At the following conditions: $T = 25$ °C, $[O_2] = 0.21$, $[N_2] = 0.78$, $[CH_4] = [NO_3^-] = [Fe^{2+}] = [HAsO_4^{2-}] = [H_3AsO^3] = [SO_4^{2-}] = [HS^-] = [Acetate^-] = 10^{-4}$, $[HCO_3^-] = 10^{-2}$, $[H^+] = 10^{-7}$, $[H_2] = 10^{-5}$ (corresponding to an aqueous concentration of approximately 8 nmol L^{-1}). Square brackets indicate activities of aqueous species or fugacities of gaseous species. Data have previously been reported in (*28*).

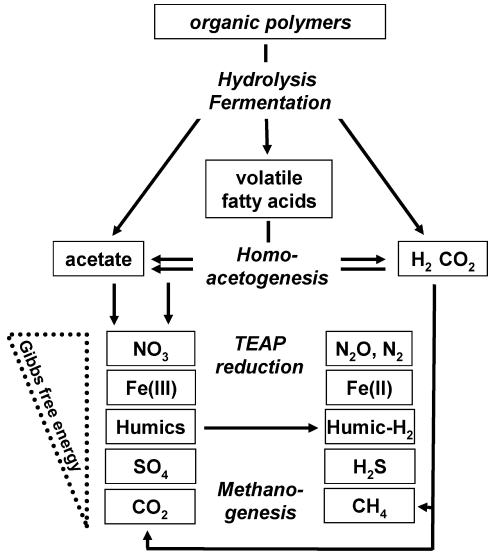

FIGURE 1. Simplified network of processes involved in anaerobic organic matter decomposition.

available to TEA bacteria and the amount of energy that is conserved as ATP in these processes (*11*). H_2 is also a very transient intermediate with a half-life of typically well below a minute (*12*), which allows for almost instantaneous concentration adjustment with changing environmental conditions (*9*). The phenomenon is caused by the relative slowness of the combined kinetics of the sequential hydrolyzation of unreacted organic matter and fermentation, compared to H_2 oxidation by TEAPs. The free energy of oxidation of H_2 is also highly dependent on H_2 activity because it involves only a transfer of 2 electrons. H_2 is further believed to be the most important electron donor for sustaining deep subsurface lithoautotrophic microbial communities (*13*). For such reasons, approaches to analyze and predict patterns of TEAPs have mostly involved H_2 (*14*).

The landmark publications by Lovley and Goodwin (*15*) and Cord-Ruwisch et al. (*16*) pioneered the use of H_2 as a key parameter for studying competition between TEAPs. The concept assumes that steady-state H_2 levels, e.g., in anoxic aquifers, are characteristic of the predominant TEAP. Empirical ranges increase with decreasing energy yield of the TEAP, so that, e.g., Fe(III)-reducers can competitively exclude and inhibit sulfate reducers and methanogens by maintaining low H_2 levels, which is broadly corroborated by empirical data (Table 2) (*17, 18*). This approach has been widely used to investigate the microbiology and redox chemistry of pristine and contaminated sediments (*19*−*24*). H_2 was also suggested as a monitoring parameter for investigating landfill fermentation processes (*25*). Similar observations were made for other low-molecular-weight intermediates, although concentration trends were less clear than for H_2. Fe(III)-reducing sediments had lower concentrations of acetate than methanogenic and sulfate-reducing sediments (*17*), but a later study found no correlation between redox condition and the concentrations of acetate, formate, or propionate. The outlined concept was initially developed by Lovley and Goodwin (*15*), who derived from Michaelis−Menten kinetics that H_2 concentrations under steady-state conditions (i.e., no microbial net growth) should depend only on physiological characteristics of hydrogenotrophic microbes. These characteristics were believed to be the growth yield (Y) and the half-saturation constant (K) for H_2 uptake, which tend to increase and decrease, respectively, with increasing energy yield of a given TEAP. Thus, external factors such as the H_2 supply rate should not influence H_2 concentrations in steady-

TABLE 2. Overview of H_2-Consuming TEAPs and Their Threshold and Steady-State H_2 Concentrations, Respectively

process	terminal electron acceptor oxidized/reduced	H_2, ongoing (nM)[a]	source	H_2, threshold (nM)	source
denitrification	nitrate/N_2 or ammonia	<0.05	*15*	0.02−0.5	*16, 125, 126*
reductive dechlorination	chlorinated ethenes/ethene	0.3−2.5	*80, 127, 128*	0.05−0.27	*79, 126, 129*
arsenate reduction	arsenate/arsenite	0.4−0.7	*28*	0.03−0.09	*28*
chromate reduction	Cr(VI)/Cr(III)			<1.0	*130*
iron reduction	Fe(III)/Fe(II)	0.2−1.0	*15, 18*	<0.11−0.31	*126, 131, 132*
sulfate reduction	sulfate/sulfide	1−1.6	*15, 133*	1.8−14	*16, 129*
hydrogenotrophic methanogenesis	CO_2/CH_4	7−13	*15, 133*	19−76	*16, 134*
homoacetogenesis	CO_2/acetate	117−150	*133*	328−724	*16*

[a] Comprises levels that are described as characteristic, steady-state, or compensation concentrations. Data compiled in this table have previously been reported in (*28*).

state systems (*26*). Surprisingly, we found only one study, by Brown et al. (*27*), that actually tested this approach by compiling kinetic data for different TEAPs, using them to calculate theoretical H_2 concentrations. The results showed that steady-state H_2 concentrations predicted by Lovley and Goodwin's (*15*) kinetic expression were 2−3 orders of magnitude above levels found in the field. The same study also suggests that H_2 concentrations, as summarized in Table 2, are not solely related to the physiology of the H_2-consuming microbes, but are also influenced by the presence of other electron donors and the availability of electron acceptors. This was corroborated by changes in steady-state H_2 concentrations during microbial As(V)-respiration in response to different levels of electron donors in incubation experiments (*28*).

While Lovley and Goodwin's (*15*) approach performed well in some systems, an increasing number of studies showed poor agreement with the proposed characteristic H_2 ranges (*29*−*34*). This together with mounting evidence of one or more TEAPs occurring simultaneously (*30, 35*−*39*) challenged the competitive exclusion concept and ultimately led to a shift in perspective in that TEAPs were viewed in the context of their in situ energetics (*29, 39, 40*). The theoretical framework associated with this line of thought is known as the partial equilibrium approach (*39*). The core idea is to separate organic matter degradation conceptually into hydrolyzation and fermentation followed by TEAPs, and thus only into two steps. The important point is that the fermentative production of H_2 is limiting the overall rate, whereas the subsequent TEAP is potentially faster and will therefore lower H_2 concentrations toward a physiologically based thermodynamic threshold. At this point the production and consumption of H_2 become equal. Based on information about the concentration of substrates and process products, thermodynamic calculations can be used to obtain in situ energy yields of a range of TEAPs in the system of interest. These in situ energy yields can be compared to metabolic energy thresholds, or minimum energy requirements, for a given TEAP, allowing conclusions about the energetic feasability of this process under the given conditions. Several studies have so far demonstrated thermodynamic control on H_2 concentrations during methanogenesis or sulfate reduction (*40, 41*).

The partial equilibrium approach described above should not be confused with the partial equilibrium model proposed by Keating and Bahr (*42*) which successively eliminates redox couples from the system equilibrium (described in terms of E_h) as the system gets more reduced. In the context of anaerobic biogeochemistry and microbial processes, the term "partial equilibrium" is generally associated with the above-described concept first presented by Postma and Jakobsen (*39*).

2. Physiological Thermodynamic Constraints on Respiration Processes

As outlined above, an essential element of the analysis of thermodynamic constraints on TEAPs is the comparison of in situ energy yields at steady state with energetic threshold requirements of different TEAPs. The distinction between these two values is important. While the energetic threshold marks the level below which H_2 consumption ceases, steady-state energies are calculated during balanced production and consumption of H_2 (see also Table 2). The energetic threshold, sometimes referred to as the biological energy quantum, for respiratory processes, i.e., oxidative phosphorylation, can be derived from the ATP synthesis energy, the H^+/ATP stoichiometry of the ATP synthase, and the thermodynamic efficiency factor (*43, 44*). The in vivo energy required for synthesis of one mol of ATP is around +50 kJ, depending on intracellular levels of ATP, ADP, phosphate, Mg^{2+}, and H^+ (*6, 45*). While many processes involved in energy conservation are highly efficient and occur close to thermodynamic equilibrium (*45*) the available energy yield cannot exclusively be used for ATP production. Part of the energy is converted to heat providing the critical thermodynamic driving force. This is accounted for by either adding +20 kJ per mol of ATP (*6*) or by including a thermodynamic efficiency factor (*44, 46*). Since the H^+/ATP ratio of the ATP synthase is typically in the range of 3−4 (*47*) the energetic threshold is then equal to the energy released by one proton returning into the cell through the ATP synthase which should be around $^1/_3$ or $^1/_4$ of 70 kJ mol^{-1}, i.e., around −20 kJ mol^{-1} of substrate (*6*). Under starvation conditions, smaller values of −10 to −15 kJ mol^{-1} of substrate may be found (*44, 46*), and for the combined sulfate reduction methane oxidation syntrophy there are only −18 kJ mol^{-1} of substrate to share between the two processes (*48*). Compilations of minimum free energy yields for various TEAPs and fermentation processes can be found in Hoehler (*44*) and Kleerebezem and Stams (*49*). A more holistic energetic analysis including electron flow through the nicotinamide adenine dinucleotide (NAD) redox couple is presented by LaRowe and Helgeson (*50*).

While this H^+/ATP-stoichiometry-based derivation is theoretically broadly applicable, several aspects should be kept in mind. (i) In terms of energy conservation, *Archaea* differ from *Bacteria* in some aspects. Methanogenic *Archaea* actively pump sodium ions across their membranes as a central bioenergetic pathway of their metabolism in addition to the classic proton translocation (*51, 52*) and this may account for part of the ATP that is synthesized (*53, 54*). Also, it appears that *Archaea* exhibit a greater variability in H^+/ATP ratios with values that may exceed 4 (*55*). Generally, *Archaea* are considered to be particularly well-adapted to energy stress (*55*). (ii) Energy thresholds may not exclusively have a purely thermodynamic basis but may be caused by

kinetic limitations in a series of enzymatic conversions (*49*). (iii) The reasoning behind energetic thresholds derived from the H^+/ATP stoichiometry of the ATP synthase is not applicable to fermentation processes, which synthesize ATP via substrate level phosphorylation. Since the underlying principle of energy conservation is different it is hardly surprising that different energy thresholds have been found for fermentation processes (*56*) and for acetogenesis utilizing the acetyl-CoA pathway (*57*). In general, minimum energy requirements were found to be smaller than in TEAPs. Homoacetogenesis, for example, is thermodynamically more advantageous compared to TEAPs than it appears based on ΔG_r alone (Table 1) (*57*). This phenomenon may be exacerbated if multiple substrates are used by individual organisms and Gibbs free energy is "pooled", as most recently suggested by Lever et al. (*58*). For a thorough analysis of the bioenergetics of acetogenesis the reader is referred to Drake et al. (*57*). (iv) For the specific case of sulfate reduction coupled to methane oxidation the actual pressure where the process takes place shifts the energy yield and enables the process to occur (*48*).

3. Thermodynamic Constraints on Acetogenesis, Methanogenesis, and Sulfate Reduction

Acetogenesis, methanogenesis, and bacterial sulfate reduction are the best studied processes with respect to thermodynamic regulation and generally adhere to the outlined considerations about the effects of thermodynamic thresholds on respiration (*6, 40, 41, 46, 59*−*63*). Energy thresholds reported by Hoehler range from −20 to −28 kJ mol^{-1}, −9 to −50 kJ mol^{-1}, and −16 to −49 kJ mol^{-1} for hydrogenotrophic acetogenesis, methanogenesis, and sulfate reduction, respectively, when the reaction is written with the lowest possible integer coefficients (*44*). At thermophilic conditions of 60−70 °C, methanogens and sulfate reducers exhibited minimum energy requirements of −50 and −23 to −35 kJ mol^{-1} of H_2, respectively (*59, 61*). Interestingly, only about 10% of this energy is invested in synthesis of biomolecules, with the remainder being lost as heat or metabolic byproduct (*64*). The energy thresholds for these TEAPs control the corresponding H_2 level, in that H_2 concentrations respond to changes in chemistry, e.g., the activity ratio of redox couples, in a way that the ΔG is maintained constant (*40*). Similar observations were made recently for different fermentation processes (*65*).

An important consequence of microbial life near thermodynamic thresholds is the potential for slowdown of metabolism by accumulation of metabolic products. Such phenomena are known from anaerobic bioreactors (*66*). Several incubation-type studies have suggested that anaerobic respiration slows down or ceases when energy thresholds are approached (*46, 56, 67*−*69*). The finding that fermentation processes and methanogenesis may occur at very small ΔG_r has also been interpreted as an adaptation to survival under "substrate starvation" in depositional environments in which a given microbial community is exposed to residual and increasingly recalcitrant organic matter (*46*). Turning this argument around, Beer et al. (*70*) proposed that small increases in the ΔG_r of methanogenesis by accumulation of respiration endproducts, i.e., CO_2 and/or CH_4, may contribute to a slowdown of organic matter decomposition under such conditions. The authors estimated CO_2 and CH_4 production in a diffusion dominated and "quasi-closed" organic-rich peat aquifer and related production rates to the ΔG_r of respiration processes. Deeper into the peat, respiration nearly ceased despite little change in organic matter quality, and in situ energy yields of acetoclastic methanogenesis ($CH_3COOH \Rightarrow CO_2 + CH_4$) reached −20 to −25 kJ mol^{-1} CH_4, i.e., when the reaction is written with the lowest possible integer stoichiometric coefficients. The process thus approached the lower range of observed ΔG_r values (−23 to −35 kJ mol^{-1} CH_4) for ongoing methanogenesis reported from experimental studies with rice paddy soil (*71*). Fermentative degradation of acetate, propionate, and butyrate attained Gibbs free energies close to 0 kJ mol^{-1} using the same convention as above. In contrast, hydrogenotrophic methanogenesis ($4\,H_2 + CO_2 \Rightarrow CH_4 + 2\,H_2O$), which had produced most of the CH_4 pool according to ^{13}C isotope data, still provided Gibbs free energies of −35 to −40 kJ mol^{-1} CH_4. The obtained ΔG_r for hydrogenotrophic methanogenesis coincided with values of about −35 kJ mol^{-1} determined for several anaerobic freshwater systems during ongoing methanogenesis (*71, 72*) and surpassed the theoretical energy threshold of −20 to −25 kJ mol^{-1} for the process (*6*). More experimental work has to be undertaken to clarify if individual respiration processes in methanogenic aquifers and soils may indeed slow down in response to endproduct accumulation and to identify the effects on the network of electron transfer processes.

4. Thermodynamic Constraints on Intermediate and "High Energy" TEAPs

While thermodynamic constraints clearly affect sulfate reduction, acetogenesis, and methanogenesis, this situation is likely different for TEAPs that yield far more energy than the above-mentioned, as has been argued before (*40*). Since the energy yield is so far from metabolic energy thresholds, kinetic limitations, such as enzyme kinetics and limits on diffusive flux, probably control H_2 and thus also ΔG_r levels of TEAPs in these systems. For TEAPs falling into the intermediate range of energy yields, the situation is less clear, mostly because a universal energy threshold that applies to all types of anaerobic respiration does not exist. Energetic thresholds may vary even within a single microbial species depending on the choice of electron acceptor (*73*). Many of the TEAPs that play an important role in groundwater contamination fall into this intermediate energetic range. Microbial respiration of iron oxides, chlorinated organics, Cr(VI), and As(V) represent examples of these TEAPs.

A substantial amount of work has been done on the energetics and H_2 levels during dechlorination of chlorinated organics (*74*−*80*). For the frequently encountered class of chlorinated ethenes it has become clear that thermodynamics does not impose a control on H_2 levels, in the sense that values near thermodynamic equilibrium are reached (*78, 80*). Its energetic threshold is almost certainly much lower than any in situ energy yield encountered under field or laboratory conditions. Another TEAP that has received much attention in the past decade is microbial reduction of As(V) to As(III) (*81*−*83*). However, there is at present only one experimental study that specifically looked at energetic limits of As(V)-reduction with H_2 as electron donor (*28*). So far, the data point to a similar conclusion as for chlorinated ethenes: H_2 levels do not appear to be adjusted by thermodynamic constraints. This is in agreement with the finding that arsenate reduction under relevant in situ geochemical conditions still provided energy yields of −45 to −60 kJ mol^{-1} H_2 (*84*).

Bacterial iron oxide reduction is a particularly interesting process with respect to thermodynamic control. Owing to a wide range of metastable phases and surface properties, the bacterial reduction of iron oxides proceeds under a broad spectrum of energetic conditions, influencing the sequence of TEAPs (*84, 85*). It may, in theory, be possible to identify the boundary between energetic and kinetic control on H_2 levels just within this subgroup of TEAPs. Liu et al. (*86*) found that microbial reduction of goethite by *Shewanella putrefaciens*, strain CN32, consistently ceased at around ΔG values of −23 kJ mol^{-1}. In contrast, Roden (*87*) observed ΔG values

more positive than −20 kJ mol^{-1} during active hematite and goethite reduction in enrichment cultures from wetland sediments. Dominik and Kaupenjohann (*88*) investigated the energetic end point of goethite-, lepidocrocite-, and ferrihydrite-reduction by *Geobacter metallireducens* growing on acetate. The final ΔG values varied considerably (−50 to −200 kJ mol^{-1}) among different minerals indicating that iron oxide reduction did not cease due to a common thermodynamic constraint; it has to be noted though that acetate concentrations were in part very far from natural conditions in this study. An inherent difficulty in studies on iron oxide reduction is the structural integrity of the mineral phase, which is compromised mainly by (i) sorption and surface precipitation of Fe(II) (*89*) and (ii) fast transformation of iron oxide surfaces catalyzed by dissolved Fe(II) (*90*). A recent field observation suggests that iron reducers in a landfill leachate plume may be restricted to a fixed energetic minimum (*91*). Decreasing H_2 concentrations possibly in response to decreasing pH values fixed the free energy gain from iron reduction at relatively constant values of $\Delta G_r = -20$ to -26 kJ mol^{-1} substrate for lepidocrocite reduction. The opposite pH effect was observed in alkaline environments causing less available energy for iron reducers (*92*).

Such differences in the effectiveness of thermodynamic control on TEAPs have also been identified in mesocosm and field investigations. Washington et al. (*93*) calculated redox potentials for a range of subsurface redox couples at different sites. They found a large gap between redox potentials calculated from measured H_2 concentrations and from oxidized iron or nitrogen, respectively. This indicates that the hydrogenotrophic reduction of Fe(III) or NO_3^- did not approach thermodynamic equilibrium. In contrast, redox potentials based on the sulfate and CO_2 redox couples, respectively, clustered close to the H_2-based redox potential, in agreement with the low energetic thresholds for sulfate reduction and methanogenesis. Watson et al. (*34*) set up an aquifer-derived microcosm from a phenolics-contaminated site. They found that energy thresholds did not sufficiently predict observed patterns of TEAPs, in that TEAPs did not proceed despite adequate in situ energy levels. Similarly, field data from the well-studied Middendorf aquifer, South Carolina, showed no competitive exclusion or microbial zonation based on in situ energy yields (*94*). Rather, the energy yield was sufficient for all TEAPs, except hydrogenotrophic methanogenesis, along the entire flow path. In contrast, other field studies have demonstrated the usefulness of looking at in situ energy yields, e.g., showing energetic advantages of sulfate reduction versus methanogenesis (*92, 95*).

Some contradictory findings may have been caused by other constraints on terminal respiration. To mention are, for example, stoichiometric constraints on metabolism by major and minor nutrients, toxic inhibitions, and lack of steady state. Microbial metabolism not only requires energy but also macro- and micronutrients, necessities that can constrain terminal respiration in the subsurface. This has, for example, been described with respect to bacterial carbon and nitrogen mineralization in modeling studies (*96, 97*), though these studies did not focus on TEAPs. For micronutrients, an example is the specific requirements of methanogens for certain trace metals (*98*), and low methane production rates in wetland soils have been attributed to a lack of these (*99*). Differences in such nutrient requirements can hence influence the competition between TEAP mediating bacteria and methanogenic A*rchaea*. Toxic inhibition of anaerobic bacterial metabolism may also occur. A well-known example is the inhibition of methanogenesis by accumulation of acetic acid in organic rich soils (*100*) and sludge digesters (*66*), which can be a consequence of nonsteady-state conditions of respiration processes (*101, 102*) and thermodynamic inhibition of terminal respiration.

A further problem arises from local heterogeneity, i.e., an inadequate scale of empirical observation with respect to TEAPs and thermodynamic controls. In most instances, the scale of observation of solute concentrations and solid phase analyses is much larger than the scale of microbial consortia and aggregates in which respiration occurs. Measured H_2 concentrations and calculated ΔG_r values thus do not reflect the conditions faced by individual microorganisms even in macroscopically homogeneous environments, as was pointed out by Hoehler et al. (*46*). As a result, respiration processes can occur despite being—apparently—thermodynamically inferior to others, or even endergonic at the scale of observation. This has been shown in an exemplary way with respect to propionate degradation in anaerobic slurry incubations of soil samples (*69*). The authors attributed this apparent endergonic propionate degradation to a "shielding" of propionate degraders by hydrogen-consuming methanogens in microbial aggregates. Similarly, hydrogenotrophic methanogenesis occurred under apparently endergonic conditions in wetland soils dominated by sulfate and iron reduction and H_2 concentrations insufficient to support methanogenesis (*103*). A model study showing how the presence of microniches can explain this if the organic matter is reactive, e.g., in a wetland soil, is described in Jakobsen (*95*). The analysis of TEAP patterns in subsurface environments is thus only as accurate as the scale of observation and sampling devices are appropriate.

5. Modeling Thermodynamic Control on TEAP Kinetics

The traditional way of modeling microbial processes such as TEAPs or preceding fermentation steps is based on Michealis−Menten and Monod kinetic approaches (*104*). A shortcoming of these models is that they do not account for complete competitive exclusion and thermodynamic feasibility of the process; i.e., positive rates can be predicted for endergonic processes (*105*). Competitive exclusion and sequential use of TEAs based on differences in their redox potential can be incorporated by using empirical inhibition factors, indirectly via biomass growth, or by defining threshold concentrations of electron acceptors below which the model shifts to the next available electron acceptor (*34, 106−108*).

The past decade has also seen the emergence of a great number of models that couple kinetic expressions with the outlined thermodynamic constraints either in the fermentation step, the TEAP, or both (*42, 49, 66, 104, 105, 109−114*). One of the earlier attempts is the so-called "partial redox disequilibrium" model by McNab and Narasimhan (*115*), which essentially treats organic matter degradation and chemical reactions using first-order kinetics and equilibrium thermodynamics, respectively. However, this is done without specification of a certain intermediate such as H_2 or any energetic threshold requirements.

Hoh and Cord-Ruwisch (*66*) introduced a modified Michaelis−Menten kinetic expression for modeling H_2-producing fermentation processes, such as fermentation of propionate. In this approach the substrate concentration is multiplied by the factor $(1 - Q/K)$ or $(1 + Q/K)$, where Q and K are the reaction quotient and the equilibrium constant, respectively. As a result, the rate approaches zero when Q approaches K, i.e., when thermodynamic equilbrium is approached. Far from equilibrium, i.e., at very negative Gibbs free energy yields, the model does not deviate significantly from the Michealis−Menten model.

Fennell and Gossett (*113*) used a similar approach, however, they introduced a critical minimum energy, ΔG_{crit}, which is subtracted from the situ energy yield, ΔG_r, of electron donor fermentation before entering the thermodynamic factor, Φ that takes on the form

$$\Phi = 1 - \exp\left(\frac{\Delta G_r - \Delta G_{crit}}{RT}\right) \quad (1)$$

Without the critical energy yield this concept is identical to Hoh and Cord-Ruwisch's model (*66*) since

$$\frac{Q}{K} = \exp\left(\frac{\Delta G_r}{RT}\right) \quad (2)$$

This thermodynamic factor Φ is then multiplied with the classic Michealis−Menten expression which more realistically predicts that fermentation ceases when the energy yield equals ΔG_{crit}. In contrast, Hoh and Cord-Ruwisch's model predicts zero rates only when the process reaches true thermodynamic equilibrium, i.e., $\Delta G_r = 0$. The same approach as in Fennell and Gossett (*113*) was chosen by Kleerebezem and Stams (*49*) who investigated butyrate fermentation.

As for modeling of the second step of the overall process, i.e., the TEAP, which was dechlorination in this case, Fennell and Gosset (*113*) again used a Michealis−Menten approach involving terms for the electron acceptor (PCE) and donor (H_2). In contrast to the fermentation step, here Gibbs free energies were not introduced directly. Instead, the H_2 concentration was corrected by a threshold H_2 level characteristic of dechlorination ($c_{H_2} - c_{H_2,thr}$). Thereby the rate approaches zero when the hydrogen level reaches the H_2 threshold. Although this has been an improvement relative to the plain Michaelis−Menten expression, the main shortcoming is that H_2 thresholds may not be constant for a given TEAP. Rather, they may depend on the in situ energetics of the system (*40*). A similar approach was presented by Hunter et al. (*104*) who treat TEAPs with a modified Monod kinetic expression with predefined "limiting" electron acceptor concentrations. These determine the kinetic reaction order, e.g., zero- vs first-order, and competition, i.e., inhibition vs simultaneous occurrence, between different TEAPs. Again, this approach does not at all account for in situ energetic requirements or minimum energy yields.

It was not until a few years later that Liu et al. (*86*) used Fennell and Gossett's fermentation model (*113*) for describing the kinetics of bacterial goethite reduction. The critical energy idea was introduced into the TEAP step by coupling a kinetic Monod expression giving a process rate (v) with a free energy term $f(\Delta G_r)$ which takes on the familiar form of eq 1:

$$\frac{dC(\text{Goethite})}{dt} = -v \cdot \left[1 - \exp\left(\frac{\Delta G_r - \Delta G_{crit}}{RT}\right)\right] \quad (3)$$

where C(Goethite) is the total goethite concentration and ΔG_{crit} was determined experimentally. Yet another slight variation of this is the "energy limited kinetics" model by Curtis (*105*), which allows TEAPs to occur using the free energy function

$$f(\Delta G_r) = \left\{\begin{array}{l} 1 - \exp\left(\frac{\Delta G_r - \Delta G_{crit}}{RT}\right), \Delta G_r \le \Delta G_{crit} \\ 0, \Delta G_r > \Delta G_{crit} \end{array}\right\} \quad (4)$$

as long as ΔG_r is equal to or more negative than a minimum threshold energy limit. For ΔG_r values that are more positive than the threshold energy the entire term becomes zero. The expression $f(\Delta G_r)$ is then coupled to a kinetic rate expression.

Jin and Bethke (*116, 117*) and Bethke et al. (*118*) present general and physiologically based expressions with a thermodynamic potential factor, F_T, representing the energetic drive of the process.

$$F_T = \left[1 - \exp\left(\frac{\Delta G_r - m\Delta G_p}{\chi RT}\right)\right] \quad (5)$$

Again, from the energy yield of the process, ΔG_r, a critical energy yield is subtracted. This is, however, not an experimental value as in Liu et al. (*86*), but a theoretical number based on the energy requirement, ΔG_P, needed to synthesize a number of m ATP in a specific TEAP (*116−118*). Also the energetic drive is divided by a stoichiometric number, χ, which represents the number of times the rate-limiting step in the respiratory chain occurs, typically associated with proton translocation across the cell membrane. Using sulfate reduction with H_2 as electron donor as an example and reaction stoichiometry based on one H_2, $m = {}^1/_3$ and $\chi = 2$; a derivation and compilation of values for a variety of TEAPs can be found in ref *118*. Dale et al. (*119*) use essentially the same F_T in their bioenergetic analysis of anaerobic oxidation of methane.

Thermodynamic constraints have also been incorporated into reactive transport models. In the "compartmentalized approach" suggested by Abrams and Loague (*111*) thermodynamic equilibrium calculations are performed to prevent the kinetic rate expression from violating thermodynamic constraints, but only on a subset of the dominant species. Yet another way of incorporating thermodynamic constraints in biogeochemical models is to check processes leading to biomass formation for their thermodynamic feasibility using values observed at in situ conditions (*34*).

Brun and Engesgaard (*120*) and Brun et al. (*121*) explicitly separated degradation of organic carbon into a two-step process in which the degradation step is simulated by some kinetic expression, and all other geochemical reactions, including the TEAP, are modeled by equilibrium thermodynamics. The same general approach is the basis for the 3-D partial equilibrium model by Phanikumar and McGuire (*114*).

A recent study by Jakobsen and Cold (*95*) incorporated metabolic energy thresholds directly into a partial equilibrium framework. Here, *effective* equilibrium constants, i.e., log$K$ values, for several TEAPs were calculated by adding an energy threshold to the ΔG of a reaction. Such modified log$K$ values can be easily incorporated into a thermodynamic equilibrium code such as PHREEQC. If the database used contains the appropriate thermodynamic constants, any effects from speciation, complexation, precipitation, ion-exchange, etc. affecting reactant and product activities and thereby the ΔG of the process are included. In Jakobsen (*122*) this was extended further to describe a system with concomitant localized methanogenesis and methane oxidation by implementing an "energy-gap" in which the energy available for either process is too low for any of the two processes to occur.

7. Conclusions

The kinetics of H_2-dependent TEAPs, methanogenesis, and syntrophic fermentation processes is under thermodynamic control when these processes operate near metabolic minimum energy requirements, which appear to be smaller for processes that provide little free energy, such as methanogenesis. As was shown based on hydrogen as a substrate, this control can consist of a slowdown of a TEAP and its exclusion by utilization of substrates by energetically more potent TEAPs. The "tightness" of thermodynamic control appears to be variable for a number of reasons. With free energy being abundant, hydrogen concentrations are lowered to levels that exclude energetically less favorable TEAPs, as is illustrated by the data compiled in Table 2, but theoretical energy thresholds of microbial metabolism are not reached because of minute concentrations limiting diffusion and enzyme kinetics. Such is the case for denitrification and very likely also for arsenate reduction and dechlorination of chlorinated organics. Thermodynamic control on the reduction of iron and manganese hydroxides does not adhere to

simple generalizations because of the phase transfer involved and variable surface properties and mineralogies. Further limits to thermodynamic control stem from a lack of steady state, toxic and stoichiometric constraints on microbial mediation, and the spatial organization and heterogeneity on scales ranging from microbial consortia to soil horizons and geologic strata. Despite such shortcomings, thermodynamic considerations have proven to provide a useful fundamental and increasingly quantitative concept for the analysis of TEAP kinetics and distribution.

A number of research directions should be mentioned. We need more quantitative information about the nature and limits of thermodynamic control on individual TEAP kinetics near thermodynamic equilibrium. Functional relationships between the free energy available and the rate of individual TEAPs have to be established experimentally, so that modeling approaches can be more accurately parameterized. This could be accomplished by controlled reactor or column experiments, in which partial pressures and concentrations of substrates and metabolites are manipulated, while the rates of TEAPs and syntrophic processes are monitored, using mass balances and isotopic tracers. In terms of individual TEAPs, particular attention needs to be directed toward a better representation of thermodynamic properties of hydroxide surfaces and the mechanisms of metal hydroxide reduction, i.e., by considering the transfer of electrons by electron shuttles and mobilization of metals by organic ligands. In these efforts more systematic information about thermodynamic controls in systems of increasing functional complexity is needed. Currently it is poorly understood to what extent and under what conditions our reductionistic knowledge obtained from in vitro experiments can be extrapolated to the "real world" where a multitude of biogeochemical processes occur simultaneously. Part of this effort needs to be directed to the consequences of chemical heterogeneity on scales of microbial consortia, microaggregates, pore space to the soil horizon, and geologic strata, and on ways to represent this heterogeneity effectively in modeling. From an integrative perspective, more information is required on thermodynamic constraints in entire anaerobic decomposition networks. In this respect it is an important question to what extent the overall rate of organic matter decomposition may be affected by changes in the dissipation of free energy within the decomposition network.

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