Widespread Thermodynamic Limitations to Microbial Processes in U.S. Aquifers

by

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A THESIS

Presented to the Department of Environmental Studies
and the Robert D. Clark Honors College
in partial fulfillment of the requirements for the degree of
Bachelor of Science

May 2023

An Abstract of the Thesis of

Gabriel Harris for the degree of Bachelor of Science
in the Department of Environmental Studies to be taken June 2023

Title: Widespread Thermodynamic Limitations to Microbial Processes in U.S. Aquifers

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With the climate crisis threatening the potability of surface waters, studying groundwater issues is more important than ever. One important aspect of groundwater environments which impact water chemistry are microbes that dwell in aquifers and catalyze oxidation-reduction electron transfer reactions. These reactions can both introduce new contaminants to water and detoxify contaminants in groundwater. In aquifers, microbial processes are limited by the laws of thermodynamics, which dictate how much energy is available for microbial respiration.

This study aimed to quantify the energy available to microbial life in U.S. aquifers using publicly available data from around the country obtained from the National Water Information System (NWIS) database. To do this, I simplified the microbial food chain into a model and calculated Gibbs free energy for each step in the food chain. First, I used species concentration data from the NWIS database to calculate the redox potential for each reaction with the Nernst equation. Then, I used a modified version of the Gibbs free energy equation to calculate free energy from the redox potential. Additionally, I calculated the Thermodynamic Potential Factor (TPF) for each reaction, which further describes the energy availability.

While no broad-scale thermodynamic limitations were identified, this study concluded that the energy available to microbial life is highly dependent on pH. This is significant, as it indicates that pH could potentially be used as an indicator for microbial activity. I also concluded that the rate-determining step in the microbial food chain is likely the production of acetate and H_2 from dissolved organic matter. This could have implications for groundwater chemistry in the future, as dissolved organic carbon levels in groundwater are expected to change because of climate change.

Acknowledgements

I would like to thank my primary thesis advisor Professor Qusheng Jin for supporting my interests in studying microbial processes and assisting me with my research along the way. Without his advice I would not have been able to develop this research project into a thesis, and I am grateful that I got to work with him. I would also like to thank my parents, my partner, and the rest of my friends and family who supported me throughout the thesis process and the many ups and downs of last four years. Finally, I would like to thank Professor Lindsay Hinkle along with everyone else who helped to proofread and provide feedback on my thesis.

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Introduction

Climate Change, Groundwater, and Microbes

Amid an ongoing climate crisis, water issues are more important than ever. With climate change expected to worsen as the 21st century progresses, many vulnerable populations will be forced to relocate and find new sources of drinking water. In addition, experts expect that the increasing global temperatures associated with climate change could cause a decline in water quality in surface waters such as lakes and rivers due to increases in algal growth and eutrophication (Bartlett and Dedekorkut-Howes 2022). Because of this, more communities will have to turn toward groundwater as a primary source of drinking water. Currently, water stored in underground aquifers makes up roughly thirty percent of earth's fresh water. While more recently researchers have been studying groundwater quality in the context of global warming, the full effects of climate change on groundwater quality are still unknown (Aladejana et al. 2020; Tesoriero, Stratton, and Miller 2021). An aspect of groundwater environments with potential for further research is microbial processes, which facilitate the breakdown of organic matter in aquifers. By studying these processes, I hope to gain a greater understanding of how contaminants are introduced and transported through groundwater, and how that affects drinking water quality.

Microorganisms that dwell in groundwater environments play an important role in determining groundwater quality. When organic material from decomposing plants or animals infiltrates into a groundwater environment, microbes catalyze reactions which degrade the organic matter into acetate and H_2. These compounds then act as electron donors for further microbial redox reactions (Grundl et al. 2011 et al. 2011; Bradley et al. 2020). By acting as a catalyst and speeding up redox reactions, microbes can store the excess energy produced from

the reaction as ATP to use for their metabolism. This is important to groundwater quality, as

redox reactions catalyzed by microbial life can alter the concentrations of different contaminants

in aquifers.

Arsenic Contamination
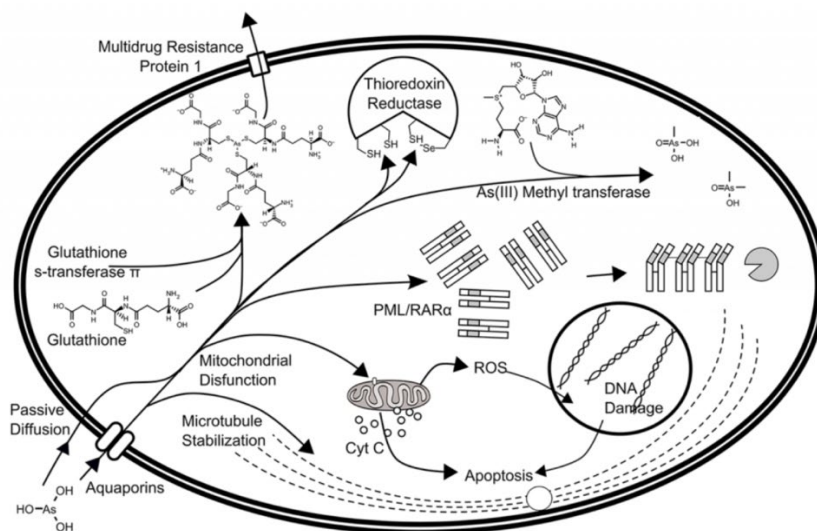
Figure 1: Mechanisms for arsenite (As(OH)$_3$) toxicity in the human cell
(Swindell et al. 2013). Arsenite enters the cell through passive diffusion or
aquaporins transporters, then binds to multiple different proteins. Once inside the
cell, arsenite can cause oxidative stress, interfere with microtubules, produce
reactive oxygen species, mimic phosphorus in DNA production, and halt the cell
cycle completely.

Looking at the example of arsenic helps to illustrate how some microbial redox reactions

can introduce contaminants to groundwater while others remediate them. When ingested, arsenic

is extremely deadly. This is because arsenic species can infiltrate cells and interfere with cellular

respiration through multiple mechanisms, as illustrated in figure 1. In aquifers, arsenic species

can form a weak bond with charged mineral surfaces through a process called adsorption. When

arsenic is adsorbed by a mineral surface, it is effectively removed from the solution, negating its

negative health impacts. However, microbes that reduce ferric and silicate minerals destroy these

mineral surfaces, and release arsenic back into groundwater (Alam, Wu, and Cheng 2014; Dai et

al. 2018). Conversely, microbes that use arsenic as an electron donor can help to detoxify arsenic in groundwater.

In most groundwater environments, arsenic is found as arsenite (As(III)) and arsenate (As(V)). While both have the potential to be toxic, the dominant groundwater arsenite species at neutral pH has no charge, as shown in figure 2. This means that it can diffuse through human cell membranes and inhibit their respiration, as the pH of the human body remains between 7.35 to 7.45. On the other hand, arsenate remains charged at most pH values, making cell infiltration more difficult due to the fact that cell membranes are hydrophobic, and resist charged particles.
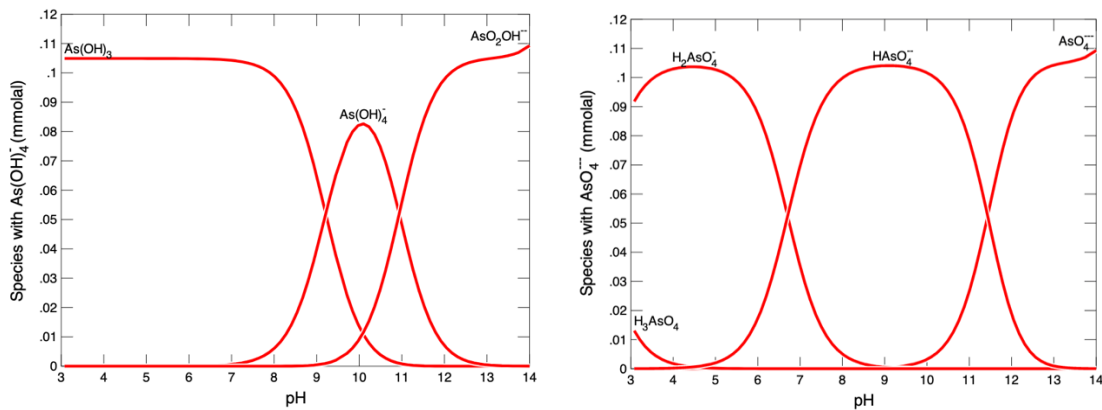
Figure 2: Speciation of arsenite (left) and arsenate (right) by pH. Arsenite has no charge at low to neutral pH, while arsenate remains charged at pH values above 4. Uncharged arsenite, aka As(OH)$_3$, can infiltrate into cells and interfere with respiration, while arsenate is stopped by the hydrophobic cell membrane. Concentrations calculated with Geochemist's Workbench.

When microbes oxidize arsenite to arsenate, they detoxify groundwater by replacing neutral arsenite molecules with charged arsenate molecules that cannot infiltrate human cells. Because redox reactions catalyzed by microbes can both introduce and remediate contaminants in groundwater, studying microbial processes can inform us about the complexities of groundwater chemistry, especially in the added context of the climate crisis.

Thermodynamic Limitations

In natural environments such as groundwater aquifers, microbial metabolism is limited by thermodynamics (Bradley et al. 2020). The laws of thermodynamics describe the transfer of energy between systems and their surroundings. In the case of microbial life, thermodynamic laws dictate the amount of energy available for microbial redox processes based on the physical and chemical nature of their surroundings. These limitations vary across different environments and largely depend on the chemical composition of the groundwater solution. The amount of energy available for microbial metabolism determines the rate at which microbes can catalyze redox reactions, and thus the rate at which these reactions proceed, assuming that the microbes fully utilize all the energy available to them. The rate at which microbes degrade and reduce organic material in turn impacts water quality by determining how fast contaminants can be introduced or removed from aquifers, as shown previously through the example of arsenic. The laws of thermodynamics thus affect water quality though microbe facilitated redox reactions. While the factors that affect thermodynamic limitations are well known, they have yet to be studied extensively in groundwater microbes.

One way to identify thermodynamic limitations is by calculating the Gibbs free energy for different microbial processes using species concentration measurements. Gibbs free energy is a thermodynamic value that describes the available energy in a system in units of kJ/mol. If the Gibbs free energy value for a reaction is negative then the reaction proceeds forward, while positive values mean external energy input is needed for the reaction to proceed. In the context of microbial reactions, free energy values that are negative and far from zero indicate that the reaction is far from equilibrium, and microbes have sufficient energy available. Gibbs free energy values that approach zero indicate limitations in energy available for microbial

respiration. Using the Gibbs free energy, I can identify thermodynamic limitations to microbial life.

The United States has already started to experience the effects of climate change, so understanding groundwater chemistry in the context of the climate crisis is important. This, along with the wide availability of groundwater data from the U.S. Geological Survey, makes the United States an ideal setting for a comprehensive study of groundwater dynamics. This study seeks to identify potential thermodynamic limitations to microbial redox processes in US aquifers using publicly available groundwater data from across the country. Past research has explored thermodynamic limitations to microbial processes in other contexts, but there has yet to be an extensive study into thermodynamic limitations in groundwater environments (Bradley et al. 2020). By calculating the energy available to microbes across a wide scope of conditions, I hope to be able to identify environmental conditions which may impact energy availability, and in turn determine how these conditions could impact groundwater quality. To do this, the complexities of microbial processes need to be simplified into a framework so the redox potential and free energy can be calculated for each step individually. Comparing the energy of each step can also give insight into the limiting factors at play in microbial processes. This study simplifies microbe facilitated reactions to study them at the thermodynamic level and better understand the underlying processes that impact groundwater chemistry. In doing this I hope to expand upon existing knowledge surrounding microbial life in US aquifers and provide a useful broad scale framework with which to investigate the nuances of microbial metabolism further.

Microbial Reaction Network

In aqueous environments, microbes facilitate a series of chemical reactions which break down organic material. For the purposes of this study, the complex microbial food chain can be

simplified into a model made up of a series of reactions illustrated by figure 3. In this simplified

model, organic matter (DOC/CH$_2$O) is degraded into acetate and H$_2$. From here, acetate and H$_2$

act as electron donors for multiple redox reactions including Fe(III), SO$_4$$^{2-}$, and Mn(IV). Acetate

and H$_2$ also serve as the electron donors for microbes called methanogens which produce

methane (CH$_4$) through a process known as methanogenesis. In addition, organic matter can be

oxidized to CO$_2$ using both O$_2$ and NO$_3$$^-$ as electron acceptors. This reaction network simplifies

the microbial food chain to its base reactions and assumes that microbial reactions will proceed

unless impeded by thermodynamics. Using this model, I derived equations for each step of the

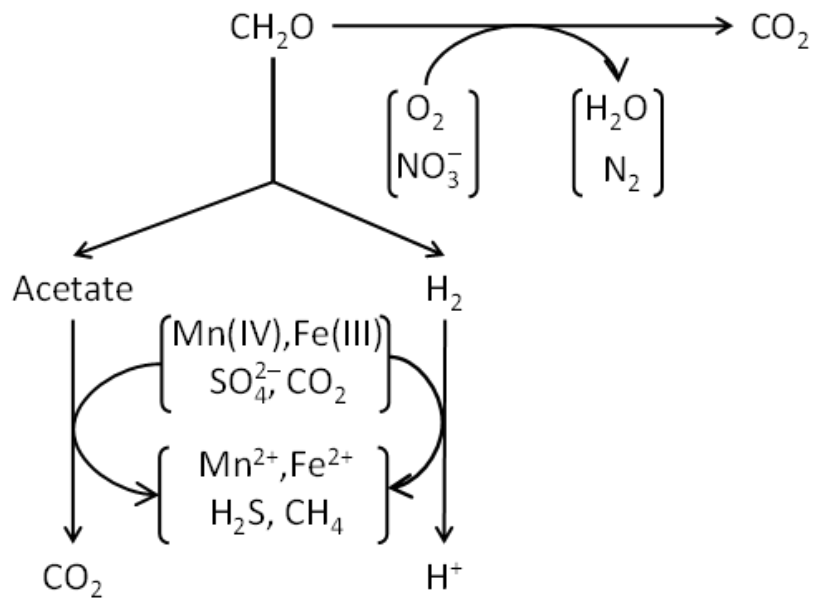microbial food chain and calculated available energy.

Figure 3: Simplified microbial reaction network in aquifers. Dissolved organic carbon (DOC/CH$_2$O) is directly oxidized to CO$_2$ by aerobic respires and nitrate reducers or degraded to acetate and H$_2$ by fermenters. Acetate and H$_2$ then serve as the electron donors for microbes that reduce manganese and ferric iron oxyhydroxides, sulfate, and CO$_2$.

<u>Methods</u>

1. Data Source

For the purposes of this study, I utilized the U.S. Geological Survey's National Water Information System (NWIS) online database. The NWIS database is a free to use collection of water quality data from over 1.9 million sites across the United States. Using the data retrieval function, a dataset containing water quality samples for selected parameters can be downloaded, along with supplemental data such as site number, sample date, and geographic coordinates. The database assigns each parameter a specific five-digit code which can be entered in combination to download all samples containing the corresponding data. Some parameters have multiple codes associated with them depending on the sampling method and other factors such as reported units and filtration. In these cases, I adjusted the units, and combined multiple parameter codes to maximize available data under the assumption that any variations caused by different sampling methods are negligible. As the database is updated frequently, for the purposes of this study I only used samples taken before August 2022. To combine data, I used the programing language R, matching samples with the site number and sample date parameters. Aquifer data was obtained using a geospatial dataset of U.S. principal aquifers (U.S. Geological Survey 2003). Using the coordinates associated with each sample site number, I plotted the samples and grouped into aquifers using ArcGIS pro. I removed outliers from data using the interquartile range (IQR), with outliers defined as falling below Q1 - 1.5*IQR and above Q3 + 1.5*IQR.

2. Redox Classifications

One important aspect in understanding microbial metabolism are the redox conditions of the water. Using criteria defined by Francis Chapelle and Peter McMahon, groundwater samples can be classified by redox conditions using the concentration of corresponding indicator species

(McMahon and Chapelle 2008). Using this method, I classified each data point into oxic,

suboxic, anoxic, and mixed redox categories, as shown in table 1.

Table 1: Redox classification scheme developed in McMahon and Chapelle 2008, and Chapelle et al. 2009.

Threshold Concentrations for Identifying Redox Processes in Water (modified from McMahon and Chapelle, 2008)									
General Redox Category	Predominant Redox Process	Distinguishing Fe(III)- from SO_4-reduction	Water Chemistry Criteria (mg/L)						
			O_2	NO_3^-	Mn^{2+}	Fe^{2+}	SO_4^{2-}	Fe^{2+}/H_2S ratio	Comments
Oxic									
	O_2 reduction		≥0.5	—	<0.05	<0.1	—		—
Suboxic									
	—		<0.5	<0.5	<0.05	<0.1	—		Further definition of redox processes not feasible
Anoxic									
	NO_3^- reduction		<0.5	≥0.5	<0.05	<0.1	—		—
	Mn(IV) reduction		<0.5	<0.5	≥0.05	<0.1	—		—
	$Fe(III)/SO_4^{2-}$ reduction		<0.5	<0.5	—	≥0.1	≥0.5		—
		Fe(III) reduction	<0.5	<0.5	—	≥0.1	≥0.5	>10	
		Mix - $Fe(III)/SO_4^{2-}$ reduction	<0.5	<0.5	—	≥0.1	≥0.5	≥3≤10	
		SO_4^{2-} reduction	<0.5	<0.5	—	≥0.1	≥0.5	<3	
	Methanogenesis		<0.5	<0.5	—	≥0.1	<0.5		—
Mixed									
									Criteria for more than one redox process are met

Data points are classified into Oxic, Suboxic, and Anoxic categories based on species concentration data in mg/L for 6 indicator species. To distinguish between iron and sulfate reduction zones, the ratio of iron to sulfide concentrations in mg/L is used.

These categories describe the predominant redox process that is expected at each data point.

Oxic zones represent O_2 reduction. Anoxic zones are separated into NO_3^- reduction, Mn(IV)

reduction, $Fe(III)/SO_4^{2-}$ reduction, and methanogenesis. Data points containing high

concentrations of iron and sulfate can then be separated into Fe(III) reduction, SO_4^{2-} reduction

and a $Fe(III)/SO_4^{2-}$ mixed zone (Chapelle et al. 2009). Using species concentration data obtained

from NWIS for O_2, NO_3^-, Mn^{2+}, Fe^{2+}, SO_4^{2-}, H_2S, and CH_4 levels, I was able to classify the

points into their corresponding redox conditions. Due to the specific criteria of the redox

classification scheme, 63,911 points with data available were unable to be classified. These

redox classifications provide a useful summary of redox conditions and are important in filling in missing data needed for free energy calculations.

3.1 Redox Potential

Before calculating Gibbs free energy, I first had to calculate redox potential. Oxidation/reduction, or redox potential (Eh) describes the tendency of an aqueous solution to undergo a redox reaction and is useful in determining the energy available to a system. The NWIS database has data entries for redox potential measured using a Pt-electrode probe, but past research has shown that there are large discrepancies between field measured Eh and Eh computed for redox couples using species concentration (Lindberg and Runnells 1984). Due to these discrepancies, I have chosen to calculate the redox potential individually by redox couple to ensure accuracy.

Using the microbial reaction network outlined previously, redox couples for each reaction can be defined, as shown in table 2. Each redox couple consists of one reactant species and one product species that make up half of a redox reaction. The redox potential for two half reactions can be used in combination to determine the Gibbs free energy for the full reaction. For DOC oxidation, the corresponding redox couples are HCO_3^-/DOC for the first half of the reaction, and O_2/H_2O or NO_3^-/N_2 for the second half, depending on the electron acceptor. Here bicarbonate (HCO_3^-) is used in place of carbon dioxide as the dominant carbonate species in groundwater environments (Andersen 2002). For acetate and H_2 oxidation the redox couples are HCO_3^-/acetate, H^+/H_2, SO_4^{2-}/H_2S, HCO_3^-/CH_4, Mn^{2+}/birnessite, Fe^{2+}/goethite, and Fe^{2+}/ferrihydrite. In place of Fe(III), which occurs primarily as minerals in neutral or near-neutral pH groundwater, I have used the ferric minerals ferrihydrite and goethite (Appelo and Postma

2013). Similarly, the mineral birnessite is used in place of Mn(IV). Total iron and total

manganese concentrations are used for Fe^{2+} and Mn^{2+} respectively.

Table 2: Redox couples for reactions in the microbial reaction network.

Reaction	Redox Couple (Electron Donor)	Redox Couple (Electron Acceptor)
DOC Oxidation	HCO_3^-/DOC	O_2/H_2O
		NO_3^-/N_2
Acetate and H_2 Oxidation	HCO_3^-/acetate & HCO_3^-/H_2	SO_4^{2-}/H_2S
		HCO_3^-/CH_4
		Mn^{2+}/birnessite
		Fe^{2+}/goethite & Fe^{2+}/ferrihydrite

Each redox couple consists of a reactant species and a product species, separated
by electron donors (oxidation) and electron acceptors (reduction). Redox couples
represent half reactions which in combination form the reactions that make up
microbial breakdown of organic matter (Figure 3).

To calculate redox potential for each redox couple, a dataset containing species

concentration for both species, along with pH, temperature, and electrical conductivity was

downloaded from the NWIS database. With datasets obtained, redox potential was calculated

with the geochemical modeling program, Geochemist's Workbench (GWB) using the

spreadsheet function (GSS). The thermodynamic datasets which GWB uses do not have an entry

for DOC, so for redox couples containing DOC (CH_2O) the entry for glucose ($C_6H_{12}O_6$) was

used as a proxy, with energy values adjusted to account for the differences in carbon. Redox

potential (Eh) was calculated in GSS using the Nernst equation,

$$Eh = E^O - \frac{RT}{nF}\ln(Q).$$

Here E^O is the standard reduction potential, T is the temperature in Kelvin, Q is the reaction quotient and n is the number of electrons transferred in the reaction. R and F are the gas and Faraday's constant, respectively.

3.2 Acetate and H₂ Assumptions

As acetate and H_2 act as electron donors for multiple redox reactions in the microbial food chain, their associated redox potentials are important in calculating available energy. However, the NWIS database is limited in the number of entries for the two parameters. Only using the values from the NWIS database would result in very few redox potential calculations and even fewer available energy values. To maximize the amount of usable data, I have substituted values for acetate and H_2 in my redox calculations. For acetate I fixed the concentration at 10 µmol/L, which is consistent with common assumptions of groundwater chemistry and the limited data that is available (P. B. McMahon and Chapelle 1991). This allows me to calculate the redox potential for HCO_3^-/acetate using the pH, temperature, and electrical conductivity measurements from the corresponding half reaction during energy calculations. The assumed concentrations for H_2 depend on the redox zone each point lies in. These values are derived from a study linking hydrogen concentrations to redox reactions in aquifers (Lovley and Goodwin 1988). Using the redox classification from section 2, I can approximate H_2 concentration in each of the primary redox zones and calculate the redox potential for H^+/H_2 using these fixed values similarly to acetate.

3.3 Available Energy

Using the microbial model outlined in the introduction, I broke the microbial food chain into a series of redox reactions. For each reaction, the calculated redox potential for the two

respective half reactions can be used to calculate the free energy. This is done using a modified version of the Gibbs free energy equation,

$$\Delta G = -nF\ (Eh(A) - Eh(D)),$$

with $Eh(A)$ and $Eh(D)$ being the calculated redox potential for the electron acceptor and electron donor, respectively. Here, n represents the number of electrons transferred in the reaction and F represents Faraday's constant. For the redox reaction of acetate $+ H_2O \rightarrow HCO_3^- + CH_4$ (acetoclastic methanogenesis) I calculated free energy directly from species activity using the equation,

$$\Delta G = RT * \ln(Q/K).$$

This is because there is no redox potential for acetoclastic methanogenesis. In this equation Q is the reaction quotient while K is the equilibrium constant for the equation.

3.4 Organic Matter Degradation

I also calculated free energy for the degradation of organic matter into acetate and H_2. The reactions for organic matter degradation are,

$$H_2O + CH_2O \rightarrow CO_2 + 2H_2$$

and,

$$2CH_2O \rightarrow CH_3COO^- + H^+.$$
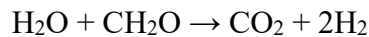
By combining these into one reaction (Lovely and Klug, 1986), the full reaction becomes,

$$3CH_2O + H_2O \rightarrow CH_3COO^- + 2H_2 + H^+ + CO_2.$$

In this reaction, 3 molecules of organic matter (CH_2O) are degraded, producing 2 molecules of H_2 and one molecule of acetate (CH_3COO^-). To calculate the Gibbs free energy for organic matter degradation, I broke the larger process into individual reactions for each step. These steps

are organic matter breakdown, acetate production, and H_2 production. I then calculated the

standard Gibbs free energy (ΔG^O) for each reaction:

DOC Breakdown (ΔG^O1): $CH_2O + O_2 \rightarrow CO_2 + H_2O$
Acetate Production (ΔG^O2): $CH_3COO^- + 2O_2 + H^+ \rightarrow 2CO_2 + 2H_2O$
H_2 Production (ΔG^O3): $H_2 + \frac{1}{2}O_2 \rightarrow H_2O$.
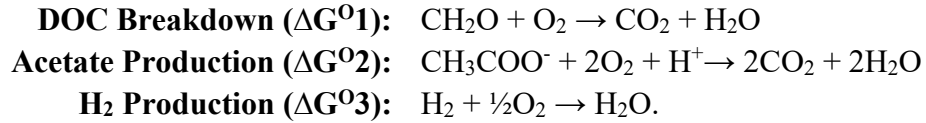
I calculated ΔG^O using ΔG^O = -RT lnK, with K values from GWB along with temperature from

the NWIS database. For ΔG^O1, I am once again using glucose as a proxy for CH_2O and

accounting for the difference in energy. Combining the three ΔG^O values gives an overall ΔG^O

value for organic matter degradation, which can be used to calculate free energy. I did this by

taking $3*\Delta G^O1 - 1*\Delta G^O2 - 2*\Delta G^O3$, adjusting ΔG^O to account for the differences in acetate and

H_2 concentrations mentioned previously. I can now calculate ΔG for organic matter degradation

using the Gibbs free energy equation, $\Delta G = \Delta G^O + RT * lnQ$.

4. Thermodynamic Potential Factor

      To assess the thermodynamic limitations to each step of the microbial food chain, I

calculated the thermodynamic potential factor (TPF). The TPF is a dimensionless factor ranging

from 0 to 1 and describes how microbial metabolism varies with the energy available in the

environment, accounting for energy conserved in the form of ATP (Jin and Bethke 2005). To

calculate TPF, I first used an ATP yield optimization model developed by Qusheng Jin (Jin and

Bethke 2003). This model assumes microbes optimize their energy conservation to maximize the

flux of ATP production and gives TPF values for available energy values ranging from 0 to 100

kJ/mol. This is calculated using the equation,

$$Ft = 1 - exp\left(-\frac{f}{\chi RT}\right)$$

where Ft is the TPF and f is the thermodynamic drive. R is the gas constant and T is temperature in Kelvin. The model assumes a χ value of 1, where the χ value is equal to the average stoichiometric number of an equation. The stoichiometric number depends on the number of electrons transferred in the reaction (Jin and Bethke 2005). Because of this, I adjusted the calculated energy values for reactions in which more electrons are transferred to be consistent with the model. After adjusting the values, I used the minimum and maximum energy values for each reaction to fit a curve to the TPF values from the model. Each fitted equation describes the relationship between TPF and available energy values from the model. Using the fitted equation, I then calculated the TPF for each available energy value.

Results & Discussion

Available Energy and pH

Upon examining the free energy for microbial reactions in groundwater, a few trends are visible. First, most data points in each reaction remain far from thermodynamic equilibrium and are not limited with regards to energy. This is because the majority of Gibbs free energy values are negative and remain far from zero, and thus, the energy available for microbial respiration is high. This fact is reinforced by the thermodynamic potential factor values, which remain close to 1 for most points, showing they are far from equilibrium. For some reactions, there are observable energy limitations present at high or low pH values. In general, energy available to microbes is highly dependent on pH. For acetate and H_2 oxidation reactions (Figures 4, 5, & 6), pH and Gibbs free energy were positively correlated, with lower pH environments having the lowest free energy values.
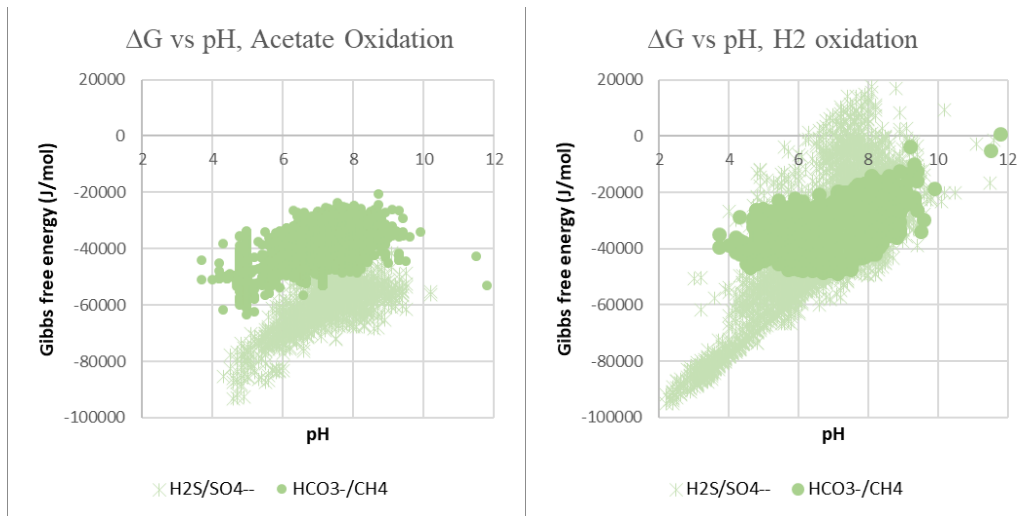
Figure 4: Scatterplots showing the Gibbs free energy for microbial redox reactions in US aquifers plotted vs pH. Acetate (left) and H_2 (right) are oxidized to CO_2 and H^+ respectively, reducing CO_2 to CH_4 and SO_4^{2-} to H_2S.

Figure 5: Scatterplots showing the Gibbs free energy for microbial redox reactions in US aquifers plotted vs pH. Acetate (left) and H_2 (right) are oxidized to CO_2 and H^+ respectively, reducing ferric minerals goethite and ferrihydrite to Fe^{2+}.
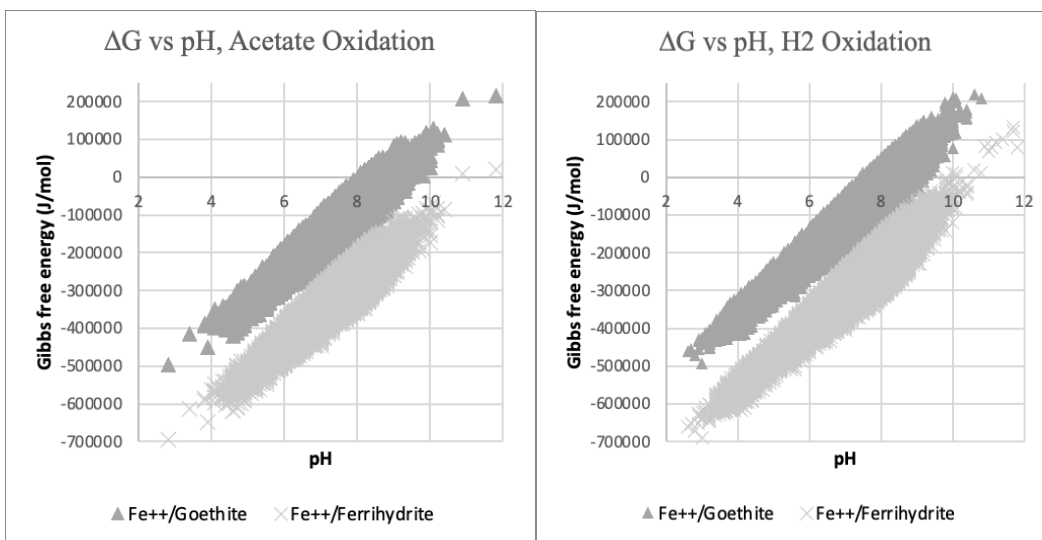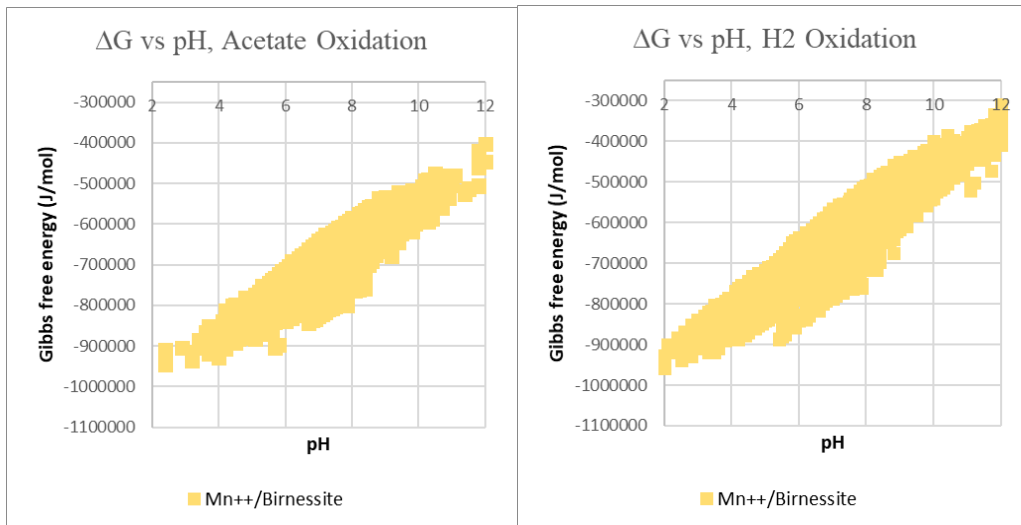
Figure 6: Scatterplots showing the Gibbs free energy for microbial redox reactions in US aquifers plotted vs pH. Acetate (left) and H_2 (right) are oxidized to CO_2 and H^+ respectively, reducing birnessite to Mn^{2+}.

As mentioned previously, energy available to microbes is highest when Gibbs free energy is far from zero. This means that low pH environments had less limitations on energy with higher pH environments being more limited. For organic matter oxidation and degradation (Figures 7 & 8), the opposite was true, with lower pH environments being more limited in energy availability.
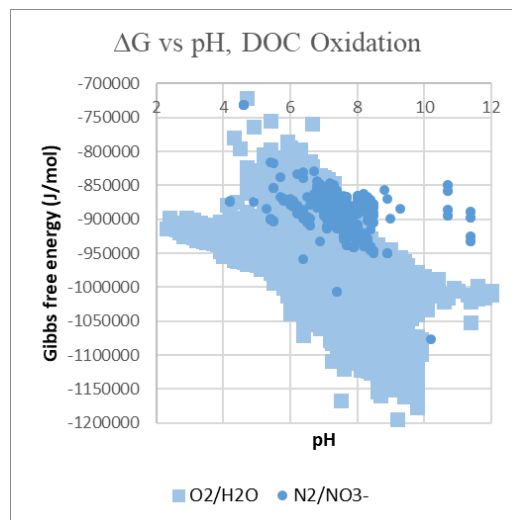

Figure 7 Scatterplot showing the Gibbs free energy for microbial redox reactions in US aquifers plotted vs pH. Organic carbon (DOC) is oxidized to CO_2, reducing O_2 to H_2O and NO_3^- to N_2.

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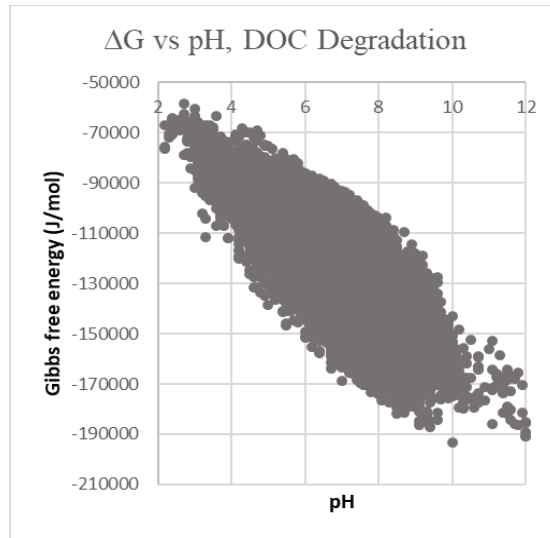
Figure 8: Scatterplot showing the Gibbs free energy for microbial reactions in US aquifers plotted vs pH. Organic carbon (DOC) is degraded to acetate and H_2 by microbial fermenters.

To demonstrate this correlation, I calculated Spearman's correlation coefficients for Gibbs free energy and pH for each reaction, with results shown in Table 3. For both acetate and H_2 oxidation, ferrihydrite, goethite, and birnessite reduction all showed a strong positive correlation between pH and ΔG, while SO_4^{2-} and HCO_3^- reduction showed a weak to moderate positive correlation. O_2 and NO_3 reduction had a weak negative correlation between pH and ΔG and organic matter degradation saw a moderate negative correlation. In addition, the calculated Eh values for every redox couple showed a strong negative correlation with pH.

Table 3: Results from Spearman's rank correlation tests between Gibbs free energy and pH for reactions in the microbial food chain.

Electron Acceptor	Spearman's ρ	
DOC Degradation	-0.598	
O_2/H_2O	-0.227	
N_2/NO_3^-	-0.223	
	Acetate Oxidation	*H_2 Oxidation*
Ferrihydrite/Fe^{2+}	0.843	0.853
Goethite/Fe^{2+}	0.839	0.83
Birnessite/Mn^{2+}	0.829	0.837
SO_4^{2-}/H_2S	0.584	0.642
HCO_3^-/CH_4	0.319	0.293

Spearman's ρ ranges from 1 to -1, with 1 representing perfect positive correlation, and -1 representing perfect negative correlation. The closer to 1 or -1 a ρ value is, the more correlated the two variables are.

Possible Rate Determining Steps

Analyzing the results from the thermodynamic potential factor calculations can help give

further insight into the dynamics at play in groundwater. TPF ranges from 0 to 1, with values

approaching 1 representing high energy availability, and 0 representing thermodynamic

equilibrium (Jin and Bethke 2003). The results from the TPF calculations reinforce my previous

observation on available energy's pH dependance. In the example of ferrihydrite and goethite

reduction, which showed the highest pH dependence, TPF remains close to 1 at low pH and only

begins to drop off around a pH of 8, as shown in Figure 9. This once again shows that lower pH

environments have fewer energy limitations, with limitations being found in neutral to high pH
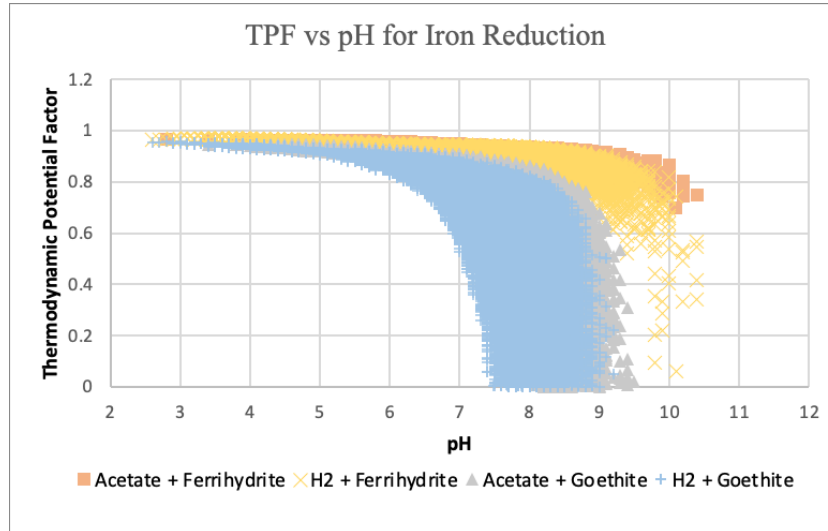
environments.

Figure 9: Thermodynamic potential factor (TPF) plotted against pH for ferrihydrite and goethite reduction in U.S. aquifers. Both acetate and H_2 serve as electron donors for ferric mineral reduction to Fe^{2+}.

Part of the goal in calculating the TPF was to identify the rate-determining step for

microbial metabolism. The rate-determining step is defined as the step in a larger reaction which

dictates the speed at which the entire process can operate. Normally, if reaction steps are rate

determining they should be far from equilibrium. However, the calculations reveal that most

reactions have TPF values close to 1 and are therefore far from equilibrium. Because of this,

identifying the rate-determining step is more difficult. Looking at the species concentration data

used in redox potential calculations can help identify the rate-determining step. The two most

likely limiting steps in our model for the microbial food chain are particulate organic matter

dissolving to DOC, and DOC degradation producing acetate and H_2, as they have the most

potential to impact the remaining reactions. If DOC production was the limiting step, then DOC

levels should remain low across all redox zones as DOC consumption would outpace DOC

production. The data for DOC does not display this trend and varies by redox zone. On the other hand, data for H_2 and acetate is limited, and concentrations appear low across all redox zones. The abundance of DOC data that varies across all redox zones leads me to believe that the production of acetate and H_2 is the rate-determining step in the microbial food chain. This would explain the missing acetate and H_2 values as well, as many of the data points were reported as undetected or less than measurable values for both acetate and H_2. This result is further reinforced by the TPF calculations for DOC degradation, which, unlike other reactions, remains close to 1 at all pH values (0.914 +/- 0.008 SD), indicating that the reaction is far from equilibrium throughout most pH conditions.

Conclusion

With the ongoing climate crisis, the United States' groundwater resources are more vulnerable than ever. Studying the dynamics at play in groundwater can help inform us of how best to protect freshwater in the future. This study performed a wide scale analysis of groundwater chemistry in U.S. aquifers in an attempt to better understand energy availability to groundwater dwelling microbes. These microbes facilitate redox reactions which alter groundwater chemistry, so understanding their energy availability can help us better understand groundwater contamination. This study concluded that the energy available to microbial metabolism is highly dependent on the environmental conditions of the groundwater, specifically pH. This result is consistent with past research into microbial iron and sulfate reduction and provides a wider view into the influence of pH on additional microbial processes (Kirk et al. 2016). While I did not identify any widespread thermodynamic limitations for a specific microbial process, this pH dependence is still valuable information in identifying more specific limitations to microbial life. As pH is an easy groundwater parameter to measure, it could be

used as a possible indicator for redox potential and energy availability without needing to sample and analyze a large number of different species concentrations. As mentioned previously, electrode probes commonly used to measure redox potential have proved to be unreliable in some circumstances. Since pH can be calculated directly from hydrogen ion (H^+) activity, which can be measured in multiple ways, pH could be an important indicator for Eh (Lindberg and Runnells 1984). These results also suggest that if energy availability is limited by pH, then pH could potentially be used to facilitate or inhibit microbial redox processes. However, this would require further research to determine if the relationship between pH and free energy goes further than the correlation I observed. With the possibility that climate change will alter groundwater chemistry, the pH dependence of microbial processes could be a helpful tool for building models of groundwater chemistry in the future.

While TPF values could not conclusively identify the rate-determining step for microbial processes, species concentration data indicates that the limiting step is the production of acetate and H_2. This conclusion is consistent with common assumptions made by geochemists about groundwater conditions. The rate-determining step is important to understand and could have implications for groundwater chemistry in the future. A 2020 study predicted changes in groundwater DOC concentrations because of climate change, and found that, especially in rapidly urbanizing areas, DOC infiltration into groundwater was likely to increase in the future (McDonough et al. 2020). With microbial processes being limited by DOC degradation, DOC could accumulate in groundwater and not be degraded to acetate and H_2 fast enough to remediate. High rates of DOC accumulation would pose a threat to groundwater potability and require increased water treatment.

Overall, this study uncovered broad trends regarding the energy available to microbial redox processes in groundwater aquifers of the U.S. With climate change putting strain on groundwater resources, understanding the dynamics at play in microbial communities is important. My results can help provide a wide scale picture of microbial processes in the United States and the forces that drive them. While recent research has expanded understanding on microbial life in groundwater, microbial processes are complex and microbes' full impacts on groundwater quality is still not known. This study helped to confirm some common assumptions made about groundwater quality on a larger scale, but further research is needed to fully understand how thermodynamic limitations might affect groundwater contaminants. Future research could analyze concentrations of contaminants like arsenic and how they relate to energy availability to better understand the connections between thermodynamics, microbes, and groundwater contamination. The NWIS database contains entries for thousands of chemical species, so there is room for further research into microbial processes beyond organic matter degradation as well. As the climate crisis progresses, properly managing our limited groundwater resources will be extremely vital and understanding the complex microbial ecosystems that reside in aquifers will be important in preventing contamination and ensuring access to drinking water in the future.

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