Lithosphere holds largest amounts of Sulfur

In terrestrial environments, SOM holds the greatest amounts of S

<table>
<thead>
<tr>
<th>Pool</th>
<th>Reservoir units: teragrams (10^12 g) S</th>
<th>Flux units, teragrams S/yr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atmosphere</td>
<td>2.0 x 10^12</td>
<td></td>
</tr>
<tr>
<td>Biosphere</td>
<td>1.2 x 10^12</td>
<td></td>
</tr>
<tr>
<td>Ocean</td>
<td>1.1 x 10^12</td>
<td></td>
</tr>
<tr>
<td>Sedimentary</td>
<td>5.4 x 10^12</td>
<td></td>
</tr>
<tr>
<td>Volcanic</td>
<td>2.2 x 10^12</td>
<td></td>
</tr>
<tr>
<td>Ice</td>
<td>9.0 x 10^11</td>
<td></td>
</tr>
<tr>
<td>Lithosphere</td>
<td>5.4 x 10^11</td>
<td></td>
</tr>
<tr>
<td>Ignimbrite cones</td>
<td>1.1 x 10^11</td>
<td></td>
</tr>
<tr>
<td>Meteorite ejecta</td>
<td>1.7 x 10^11</td>
<td></td>
</tr>
<tr>
<td>Degradation</td>
<td>5.1 x 10^11</td>
<td></td>
</tr>
<tr>
<td>Phyllosilicates</td>
<td>8.0 x 10^11</td>
<td></td>
</tr>
<tr>
<td>Lignin</td>
<td>5.1 x 10^11</td>
<td></td>
</tr>
<tr>
<td>Sulfides</td>
<td>3.1 x 10^11</td>
<td></td>
</tr>
<tr>
<td>Coal</td>
<td>1.6 x 10^12</td>
<td></td>
</tr>
<tr>
<td>Petroleum</td>
<td>1.4 x 10^12</td>
<td></td>
</tr>
</tbody>
</table>

Physical Weathering: release of sulfides (HS) or sulfates (SO_4) from minerals

Biological transformations: aerobic
- Sulfate-reducing bacteria
  - sulfides are converted to sulfates (SO_4)
  - sulfate is assimilated by plants and microbes

anaerobic
- sulfate-reducing bacteria
  - sulfate converted to sulfides

mineralization of organic S, release as either HS or SO_4

Volatile organic S compounds
- Assimilation of mineral S into biomass

Terrestrial S pools and transformations
**S oxidation states**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Oxidation State</th>
<th>Redox Potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>S0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SO₂</td>
<td>+2</td>
<td>+0.07</td>
</tr>
<tr>
<td>SO₃²⁻</td>
<td>-2</td>
<td>-0.022</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>-4</td>
<td>-0.195</td>
</tr>
</tbody>
</table>

S is highly redox active, used in energy generation as both e⁻ donor and e⁻ acceptor.

**Microbial S transformations**

![Diagram of microbial S transformations]

**S utilization by microbes**

<table>
<thead>
<tr>
<th>Microbial S Utilization</th>
<th>Reaction Equation</th>
<th>Redox Changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiosulfate oxidation</td>
<td>2S₂O₃²⁻ + 2H⁺ → 2SO₄²⁻ + 2H₂O</td>
<td>+0.23V</td>
</tr>
<tr>
<td>Sulfite oxidation</td>
<td>SO₃²⁻ + H₂O → SO₂ + 2H⁺</td>
<td>-0.23V</td>
</tr>
<tr>
<td>Sulfide oxidation</td>
<td>S²⁻ + 2H⁺ → H₂S</td>
<td>+0.00V</td>
</tr>
<tr>
<td>Desulfurization</td>
<td>2H₂S + O₂ → 2H₂O + S</td>
<td>-0.40V</td>
</tr>
<tr>
<td>Sulfite oxidation</td>
<td>SO₃²⁻ + H₂O → SO₂ + 2H⁺</td>
<td>-0.23V</td>
</tr>
<tr>
<td>Sulfide oxidation</td>
<td>S²⁻ + 2H⁺ → H₂S</td>
<td>+0.00V</td>
</tr>
<tr>
<td>Desulfurization</td>
<td>2H₂S + O₂ → 2H₂O + S</td>
<td>-0.40V</td>
</tr>
</tbody>
</table>

![Table of S utilization by microbes]

Source: [Chemical Abstracts, 1995]
5 groups of sulfur-oxidizing bacteria:
- anoxygenic phototrophs (e.g. Green and Purple Sulfur bacteria)
- morphologically conspicuous colorless sulfur bacteria (e.g. Thiospira)
- obligate autotrophic colorless sulfur bacteria (e.g. Thiobacillus)
- facultatively autotrophic colorless sulfur bacteria (e.g. Thiobacillus)
- sulfur dependent archaea (e.g. Thermococcus)

### Species of Thiobacillus and substrates

<table>
<thead>
<tr>
<th>Species</th>
<th>Chemolithotrophic</th>
<th>Chemosynthetic</th>
<th>Oxidation Mediated by Thiobacillus Thiosulfates is:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfur oxidation</td>
<td>S oxidation</td>
<td></td>
<td>HS⁻ + O₂ → SO₄²⁻ + H⁺ + SO₄²⁻ = -46 kJ</td>
</tr>
<tr>
<td>Microbes' environment may be pH &lt; 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HS⁻ oxidation can occur under anaerobic conditions.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thiobacillus denitrificans facultative anaerobe and couple S oxidation to respiratory denitrification.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HS⁻ oxidation is not mediated by oxygenases as is CH₄ and NH₄ oxidation.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**S oxidation pathway**

Oxygen is not directly involved in S oxidation pathway

Two pathways exist for oxidation of sulfite to sulfate

APS = adenosine 5-phosphosulfate

AMP = adenosine 5-monophosphate

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**Acidification accompanying S oxidation**

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**Environmental effects of S oxidation: Acid Mine Drainage**

Mine spoils, material stockpiled as wastes from mine excavation

Minerals in spoil piles often contain pyrite (FeS₂)

Exposure to air accelerates S-oxidation; chemical and biological.

Rain water leaching through piles is acidified (pH to <1), solubilizes metals
Use of S-oxidizers in biocontrol

Streptomycetes scabices causative agent of potato scab disease

S. scabices prefers neutral to slightly alkaline conditions,

acid produced (e.g., pH 5) from S oxidation inhibits growth of S. scabices but not the potato plants

---

Disimilatory Sulfate & S reduction

Obligate anaerobes that use either H₂ or organics as e⁻ donors and S oxyanions or S as e⁻ acceptors produce sulfides.

Catabolism (energy metabolism) is shown in black; anabolism (cell synthesis) is shown in red

---

S oxidation states

Reduction of sulfate or sulfide occurs via a number of intermediates.

Unlike nitrate-reducing bacteria, S-reducers usually do not release intermediate oxidation states, but only the final product sulfide.
Diversity of Sulfate & S reducers

Sulfate- or sulfur-reducing microorganisms are long-established functional groups. They are not necessarily coherent from the viewpoint of modern molecular systematics.

Phylogenetic trees reflecting the relationships of groups of sulfate-reducing bacteria to other organisms on the basis of 16S rRNA sequences. (A) Overview showing the three domains of life: (1), Eubacteria; (2), Archaebacteria; (3), Eukaryotes.

Characteristics of sulfate-reducing bacteria (SRB)

Bacteria and Archaea
Differ in use of $\text{SO}_4^-$, $\text{S}_2\text{O}_3^-$ as TEA e-donors coupled to S reduct.
Bacterial genera identified by the prefix "Desulfo".

SRB-Methanogen Competition/Syntrophism
SRBs use the same e-donors as methanogens ($\text{H}_2$, acetate) and by coupling these to sulfate reduction obtain higher energy yields than methanogens.

- **Chemolithotrophic growth**
  
  $\text{H}_2 + 2\text{SO}_4^- \rightarrow \text{H}_2\text{O} + 2\text{HS}^- + \text{H}^+$

- **Chemooorganotrophic growth**
  
  lactate + $\text{SO}_4^- \rightarrow \text{acetate} + \text{CO}_2 + \text{H}_2\text{O} + \text{HS}^-$

High sulfate environments: SRB compete with may dominant over methanogens.

Low or no sulfate environments, SRB grow syntrophically with methanogens as may proton-reducers (producing $\text{H}_2$ for interspecies $\text{H}_2$ transfer.)
SRB may grow as "proton-reducers" that are physiologically linked to methanogens in hydrogen transfer.

High positive ΔG' makes this reaction unfavorable for supporting growth.

H₂ produced is rapidly consumed at rates at a very low level, the energetics become favorable.

Methanogens consume H₂ and making the reaction energetically favorable. This is an example of "syntrophy".

**SRB transformations of metals and chloroaromatics**

**Metals**

Reduce:
- Fe³⁺ (no growth)
- U⁶⁺ (no growth)
- Cr⁶⁺ (no growth)
- As⁵⁺ (growth)

Methylate Hg

**Chloroaromatics**

Reductive dehalogenation of chlorobenzoates (growth)

**Trace S gases in the atmosphere**

Low levels of S in atmosphere

Most sulfur gases are rapidly returned (within days) to the land in rain and dry deposition

<table>
<thead>
<tr>
<th>Gas</th>
<th>Concentration</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂S</td>
<td>0.5 - 1 ppmv</td>
<td>Reference and Reference (1973)</td>
</tr>
<tr>
<td>CH₃SH</td>
<td>0.1 - 0.5 ppmv</td>
<td>Reference and Reference (1973)</td>
</tr>
<tr>
<td>COS</td>
<td>3 - 12 ppbv</td>
<td>Reference and Reference (1973)</td>
</tr>
<tr>
<td>CS₂</td>
<td>0.1 - 0.3 ppbv</td>
<td>Reference and Reference (1973)</td>
</tr>
<tr>
<td>H₂S</td>
<td>0.1 - 0.5 ppmv</td>
<td>Reference and Reference (1973)</td>
</tr>
<tr>
<td>CH₃SH</td>
<td>0.1 - 0.3 ppbv</td>
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<td>CS₂</td>
<td>0.1 - 0.3 ppbv</td>
<td>Reference and Reference (1973)</td>
</tr>
</tbody>
</table>

*Note: units are parts per billion (ppbv), except for H₂S which is parts per million (ppmv).*
Types and sources of S gases

<table>
<thead>
<tr>
<th>Volatile</th>
<th>Mechanical properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>MM</td>
<td>Hydrogen sulfide (H₂S)</td>
</tr>
<tr>
<td>DMS</td>
<td>Methane sulfide (CH₃S)</td>
</tr>
<tr>
<td>DMDS</td>
<td>Methyl mercaptan (CH₃SH)</td>
</tr>
</tbody>
</table>

Volatile S: terrestrial sources and sinks

Volatile S compounds produced by heterotrophic microbes during aerobic or anaerobic decomposition of S-containing compounds

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Aerobic conditions</th>
<th>Anaerobic conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methionine</td>
<td>1.6-8.8</td>
<td>1.6-2.9</td>
</tr>
<tr>
<td>Methionine</td>
<td>1.6-8.8</td>
<td>1.6-2.9</td>
</tr>
<tr>
<td>Cystine</td>
<td>0.6-1.6</td>
<td>0.6-1.6</td>
</tr>
<tr>
<td>Cysteine</td>
<td>0.6-1.6</td>
<td>0.6-1.6</td>
</tr>
</tbody>
</table>

| Zein: a mixture of water insoluble proteins that constitute about half of the protein in corn or 4-5 weight % of the corn.

Gluten is composed of storage proteins, the prolamins, comprising monomeric gliadins and polymeric glutenins.

Major products: MM, DMS, DMDS
Phosphorus: Pools and cycles
Phosphorus cycle. Reservoir units, teragrams (10^{12} g); flux units, teragrams P/yr

P in Terrestrial Environments

P released into the mineral pool as phosphate
Phosphate assimilated into/released from biomass without reduction or oxidation.
Like nitrogen and sulfur, SOM contains the greatest amount of P (30-50% of the total)

Mineral forms of P
All known phosphate minerals are orthophosphates [anionic group is PO_4^{3-}]
Over 150 species of phosphate minerals:
Small amounts of mineral P in soil (ca. 1% of total)
Organic forms of P

SOM contains the greatest amount of P (30-50% of the total).
The chemical nature of much of the organic P is unknown.
Up to 50% may be inositol hexaphosphate (phytic acid).

Reduced forms of phosphorus:
Phosphite, hypophosphite occur in bacteria; functions unknown.
Phosphine is produced in natural environments, reacts rapidly in atmosphere, short half life (ca. 5-24 h).
Trace amounts of P in atmosphere, predominant movement is from terrestrial environments to streams, lakes and oceans.

Fe and Mn Cycles

Cycling revolves around the transition from oxidized insoluble forms (Fe$^{3+}$/Mn$^{4+}$) to reduced, soluble oxidation states (Fe$^{2+}$/Mn$^{2+}$).
Fe Oxidation

Ferrous iron (Fe²⁺) used as an electron donor linked with oxygen reduction
High levels of Fe²⁺ are needed
But: aerobic conditions, neutral pH iron is essentially all solid Fe³⁺ oxides
Two adaptations for use of Fe²⁺: Low pH and/or low O₂

Fe Oxidation at low pH

The pH effect on Fe³⁺ concentrations is reflected in the energy yield:

\[ \text{Fe}^{2+} + \text{O}_2 + \text{H}^+ \rightarrow \text{Fe}^{3+} + \text{H}_2\text{O} \]
\[ \Delta G' (\text{pH 7}) = -0.25 \text{ kJ} \]
\[ \Delta G' (\text{pH 0}) = -2.54 \text{ kJ} \]

Acidithiobacillus ferrooxidans, an acidophilic iron-oxidizer, pH optimum for growth of 2 to 3
Contribute to formation of acid mine drainage

Thiobacillus-type [rods] in yellow floc from acid water

Fe Oxidation at Neutral-Alkaline pH, Low Oxygen Levels

Neutral-alkaline pH, Fe²⁺ concentrations increase with decreasing oxygen concentration.

The "iron bacteria" (e.g., Gallionella, Leptothrix, Siderocapsa) have adapted to grow by oxidizing Fe²⁺ at low O₂ concentrations (0.1 - 0.2 mg L⁻¹).

Low energy yields, microbes must oxidize large amounts of Fe²⁺ to sustain growth.

Small populations of iron bacteria generate a lot of Fe³⁺.

Problem for the well water industry as the resulting FeOOH (hydroxydes) precipitates may clog wells.
**Light Micrographs of Iron bacteria**

*Gallionella ferruginea* [braid-like] in red floc from neutral water

*Leptothrix cholodnii* [sausage-like] in red floc from neutral water

*Leptothrix discophora* thick rounded holdfasts [doughnut-like], which are parts of the bacteria that attach to rocks or microscope slides, like these here on microscope slide left in neutral water riffle

---

**TEM Micrographs of Iron bacteria & Iron deposits**

Low magnification image of Gallionella and Leptothrix stalks and sheaths

Gallionella stalk coated with nanometer-scale Fe(OH)3 and FeOOH aggregates

---

**Light Micrographs: Iron bacteria and Iron deposits**

*Gallionella*  
Note the twisted strands of iron oxide characteristic of this organism  
Wet mount, 400 X

*Gallionella*  
Stained with crystal violet, 1000 X
Fe(III) and Mn(IV) reduction affects cycling of iron and manganese fate of a variety of other trace metals and nutrients degradation of organic matter

Fe(III)-reducers can outcompete sulfate-reducing and methanogenic microorganisms for electron donors can limit production of sulfides and methane in submerged soils, aquatic sediments, and the subsurface

RB may be useful agents for the bioremediation of environments contaminated with organic and/or metal pollutants

**Fe/Mn-reducing microbes (FMRM)**

A wide phylogenetic diversity of microorganisms, (archaea and bacteria), are capable of dissimilatory Fe(III) reduction.

Most microorganisms that reduce Fe(III) also can transfer electrons to Mn(IV), reducing it to Mn(II).

Two major groups, those that support growth by conserving energy from electron transfer to Fe(III) and Mn(IV) and those that do not.

**FMRM that Conserve Energy to Support Growth from Fe(III) and Mn(IV) Reduction**

Phylogenetically diverse

Most cultured FMR are in the family Geobacteraceae in the delta -Proteobacteria:

- Geobacter
- Desulfuromonas
- Desulfuromusa
- Pelobacter

Acetate is a primary electron donor

Most Geobacteraceae also can use hydrogen
Phylogenetic Diversity of Fe/Mn reducers

Phylogenetic tree, based on 16S rDNA sequences, of microorganisms known to conserve energy to support growth from Fe(III) reduction.

Microbes that conserve energy from Fe/Mn reduction

Micrographs of Fe/Mn-reducers

Phase contrast micrographs of various organisms that conserve energy to support growth from Fe(III) reduction. Bar equals 5 mm, all micrographs at equivalent magnification.
Mechanisms for Electron Transfer to Fe(III) and Mn(IV)

Mechanism(s) of electron transfer to insoluble Fe(III) and Mn(IV) are poorly understood.

Possibilities include:
- Direct contact with and reduction of Fe(III) and Mn(IV) oxides
- Solubilization of Fe(III) and Mn(IV) oxides by chelators, reduction of solubilized species
- Indirect reduction mediated by extracellular electron shuttles (quinone groups in humics)

Mechanisms for Electron Transfer to Fe(III) and Mn(IV)

Identification of AQDS/humate-reduction mutants:

a. Genetic screen used to isolate mutants defective in AQDS reduction.
   Because AQDS is clear when oxidized and orange when reduced to AHDS, mutants that remained clear were identified.

b. The AQDS-reduction defective mutants were tested for their ability to grow on and reduce humate substrates with measured activity by Fe(III) reduction, and did so with reduced humate with Fe(III). The wild-type (open circles) rapidly reduced humate, whereas the mutant (open squares) did not. Data shown are triplicate determinations of Fe(III) reduction by the mutants with and without inoculation controls.