H$_2$-driven CO$_2$ Fixation by Extremely Thermoacidophilic Archaea

Michael W.W. Adams, University of Georgia
Robert M. Kelly, North Carolina State University

Electron Source \[ \rightarrow \] Primary Reduced Product \[ \rightarrow \] CO$_2$ \[ \rightarrow \] Biofuel

H$_2$O \[ \rightarrow \] H$_2$ \[ \rightarrow \] CH/O

ARPA-E Workshop on Direct Solar Fuels
October 21, 2009
SOLAR BIOFUEL PRODUCTION

Electron Source → Primary Reduced Product → Biofuel
**SOLAR BIOFUEL PRODUCTION**

- **Electron Source**: Primary Reduced Product → Biofuel

**Green Plants**

- **Electron Source**: 
  - $\text{H}_2\text{O}$
  - Oxidized Electron Source: $\text{O}_2$

- **Visible Light**

- **PS (photosynthesis)**
  - Primary Reduced Product
  - NADPH

- **CC (Calvin cycle)**
  - Secondary Reduced Product: SUGARS
  - Oxidized Substrate: CO$_2$

- **Biomass Conversion**
  - SUGARS

- **Microbial Conversion**
  - Biofuel (of choice*)

$\text{PS = photosynthesis (I+II)}$

$\text{CF = CO}_2\text{ fixation (Calvin cycle)}$

* $\text{C}_x\text{H}_y / \text{C}_a\text{H}_b\text{O}_c$
SOLAR BIOFUEL PRODUCTION

Electron Source → Primary Reduced Product → Biofuel

Electron Source
H₂O

VISIBLE LIGHT

Oxidized Electron Source
O₂

Algae

Primary Reduced Product
NADPH

Secondary Reduced Product
SUGARS

Oxidized Substrate
CO₂

H₂

Biofuel

PS = photosynthesis (I+II)
CF = CO₂ fixation (Calvin cycle)
SOLAR BIOFUEL PRODUCTION

Electron Source \( \rightarrow \) Primary Reduced Product \( \rightarrow \) Biofuel

Algae

Electron Source \( H_2O \)

Oxidized Electron Source \( O_2 \)

Visible Light

PS = photosynthesis (I+II)
CF = CO\(_2\) fixation (Calvin cycle)

Simplified (Biomimetic) Scheme?
SOLAR BIOFUEL PRODUCTION

Electron Source → Primary Reduced Product → Biofuel

Electron Source

Primary Reduced Product

Biofuel

Electron Source

Primary Reduced Product

Biofuel (of choice)

PS Module

CF Module

Oxidized
Electron Source
O₂

VISIBLE LIGHT

Oxidized
Electron Source
H₂O

Primary Reduced Product

H₂

Primary Reduced Product

CO₂

CO₂

PS = photosynthesis (I+II)
CF = CO₂ fixation (Calvin cycle)
SOLAR BIOFUEL PRODUCTION

Electron Source $\rightarrow$ Primary Reduced Product $\rightarrow$ Biofuel

PS Module

Electron Source $\rightarrow$ Primary Reduced Product $\rightarrow$ Biofuel

Primary Reduced Product

Oxidized Substrate $\rightarrow$ Biofuel

CO$_2$

Fuel use with oxidized source (O$_2$) regenerates substrates (CO$_2$, H$_2$O)

O$_2$

CO$_2$ + H$_2$O

PS = photosynthesis (I+II)

CF = CO$_2$ fixation (Calvin cycle)

IDEAL SOLAR ENERGY CONVERSION SYSTEM
SOLAR BIOFUEL PRODUCTION

Electron Source

Oxidized

Substrate

CO$_2$

Biofuel

H$_2$O

Primary Reduced Product

Visible Light

PSM

H$_2$

Primary Reduced Product

CO$_2$

Biofuel

Electron Source

H$_2$O

Visible Light

Oxidized

Electron Source

O$_2$

Primary Reduced Product

H$_2$

Fuel use with oxidized source (O$_2$) regenerates substrates (CO$_2$, H$_2$O)

Biofuel (of choice)

CO$_2$

O$_2$

CH/O

Fuel use with oxidized source (O$_2$) regenerates substrates (CO$_2$, H$_2$O)

CO$_2$ + H$_2$O

IDEAL SOLAR ENERGY CONVERSION SYSTEM

PS = photosynthesis (I+II)

CF = CO$_2$ fixation (Calvin cycle)
**SOLAR BIOFUEL PRODUCTION**

**Electron Source**
- H₂O
- CH/O
- PS
- PSM
- DARK
- CFM
- H₂
- PSM
- Visible Light
- PS
- Oxidized Electron Source O₂
- Primary Reduced Product H₂
- H₂

**Primary Reduced Product**
- CO₂
- Biofuel

**Biofuel** (of choice)

**KEY RESEARCH AREAS**

**PS = photosynthesis (I+II)**

**CF = CO₂ fixation**
PS = photosynthesis (I+II)
CF = CO₂ fixation
**Key Biological Reactions**

**PSM:**
1. **Water photolysis** (visible light):
   \[
   H_2O \rightarrow O_2 + 2H^+ + 2e^- 
   \]
2. **Hydrogen production**
   \[
   2H^+ + 2e^- \rightarrow H_2 
   \]

**CFM:**
1. **Hydrogen activation**
   \[
   H_2 \rightarrow 2H^+ + 2e^- 
   \]
2. **CO₂ to biofuel conversion** (reduced carbon/CH/CHO)
   \[
   CO_2 + 2H^+ + 2e^- \rightarrow C_xH_y / C_aH_bO_c 
   \]

Much higher energy yield using H₂ as reductant for CO₂ fixation to biofuel compared to sugar to biofuel
Structure/Function Studies of NiFe-hydrogenase

- Hydrogenase is not a ‘commodity enzyme’
- Very complex maturation process *in vivo* to assemble complex, O₂-sensitive, NiFe-catalytic site (CO, CN ligands, etc.)
- No genetically-tractable organism that produces hydrogenase available for detailed structure/function studies
- **Recent breakthrough** – production of recombinant form of the NADPH-dependent *Pyrococcus* hydrogenase in *E. coli*

**Fundamental structure/function studies to design:**

- ‘Minimal’ hydrogenases (NiFe-site plus peptides)
- H₂ production and H₂ activation catalysts
- Decreased O₂-sensitivity
- Electron carrier specificity
- Pathways of electron transfer
Hydrogen Production from Polyglucose
(13 enzymes, Pi, NADP – catalytic)

\[(C_6H_{10}O_5)_n + 7 H_2O \rightarrow 12 H_2 + 6 CO_2\]

#1: 6 H_2O + 6 Pi \rightarrow 6 G1P

#2: 6 G1P \rightarrow 6 G6P

#3: 6 G6P \rightarrow 6 6PG

#4: 6 6PG \rightarrow 5 G6P

#5-12: 5 G6P \rightarrow 6 Ru5P

#13: NADPH-dependent H_2-evolving Pyrococcus Hydrogenase

Microbial CO₂ Fixation Pathways

**Calvin Cycle**
- plants, cyanobacteria, purple and green bacteria

**Reverse Tricarboxylic Acid Cycle**
- Chlorobium limicola
- Desulfobacter hydrogenophilus
- Hydrogenobacter thermophilus

**Reductive Acetyl-CoA Pathway**
- Acetobacterium woodii
- Defulfbacterium autotrophicum

Adapted from https://lecturer.ukdw.ac.id/dhira/Metabolism/CarbonAssim.html
Microbial CO₂ Fixation Pathways (Thermophiles)

Dicarboxylate/4-Hydroxybutyrate Cycle

- *Igncoccus hospitalis*
- Ramos-Vera, W. H. et al. (2009)

3-Hydroxypropionate/4-Hydroxybutyrate Cycle

- *Chloroflexus aurantiacus*
- Alber, B. et al. (2006)

3-Hydroxypropionate Cycle

- *Metallosphaera sedula*
- Berg, I. A. et al. (2007)
The Extremely Thermoacidophilic Archaeon *Metallosphaera sedula*

**Genome:** 2.2 Mb (46.2% G + C) 2304 ORFs

**Growth:** Mixotrophic aerobe; metal mobilizer

$T_{opt} = 73°C; \ pH_{opt} = 2.0$

*Huber et al. 1989*

## Comparative genomics: Extreme thermoacidophiles

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Ss (Sulfolobus solfataricus) St (Sulfolobus tokodaii) Sa (Sulfolobus acidocaldarius)
Ms (Metallosphaera sedula) Cm (Caldivirga maquilingensis) Si (Sulfolobus islandicus, strains L,M,U,YG & YN)
Ab (Aciduliprofundum boonei) Aa (Acidianus ambivalens) Sm (Sulfolobus metallicus) Pt (Picrophilus torridus)
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**Species**:  
- **Ss** (Sulfolobus solfataricus)  
- **Ms** (Metallosphaera sedula)  
- **Ab** (Aciduliprofundum boonei)  
- **St** (Sulfolobus tokodaii)  
- **Cm** (Caldivirga maquilingensis)  
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- **Si** (Sulfolobus islandicus, strains L,M,U,YG & YN)  
- **Aa** (Acidianus ambivalens)  
- **Sm** (Sulfolobus metallicus)  
- **Pt** (Picrophilus torridus)
Growing *E. coli*
Metal mobilization by *M. sedula*

Chalcopryte, $t = 0$ days with *M. sedula*

- CuFeS$_2$ solids
- 10g/L
- pH 2

Chalcopryte, $t = 21$ days with *M. sedula*

- $\text{Cu}^{2+}$

Iron oxidation
Sulfur oxidation
(Inorganic) Carbon fixation
Heavy metal tolerance
Adhesion to solids

Metal and RISC oxidation components on Fe$^{2+}$, S°
Metal and RISC oxidation components on CuFeS$_2$

Auernik, KS, and RM Kelly. Molecular hydrogen impacts chalcopyrite bioleaching efficiency for the extremely thermoacidophilic archaeon *Metallosphaera sedula*. (submitted)
Versatile growth physiology of *M. sedula*

Auernik, KS, and RM Kelly. Physiological versatility of the extremely thermoacidophilic archaeon Metallosphaera sedula supported by heterotrophy, autotrophy and mixotrophy transcriptomes (submitted)
Auernik, KS, and RM Kelly. Physiological versatility of the extremely thermoacidophilic archaeon Metallosphaera sedula supported by heterotrophy, autotrophy and mixotrophy transcriptomes (submitted)
Overall:

$$2\text{CO}_2 + 4\text{ATP} + 5\text{NADPH} + \text{CoA} + \text{NAD} \rightarrow \text{Acetyl CoA} + 3\text{ADP} + \text{AMP} + 3\text{Pi} + \text{PPi} + 5\text{NADP} + \text{NADH}$$

M. sedula 3-HP/4-HB CO$_2$ fixation pathway

4-hydroxybutyryl-CoA dehydratase

M. sedula 3-HP/4-HB CO₂ fixation pathway

Auernik, KS, and RM Kelly. Physiological versatility of the extremely thermoacidophilic archaeon Metallosphaera sedula supported by heterotrophy, autotrophy and mixotrophy transcriptomes (submitted)
# 3-HP/4-HB CO₂ Fixation Pathway in *Metallosphaera sedula*

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<th>#</th>
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<td>14</td>
<td>Acetoacetyl-CoA β-ketothiolase</td>
<td>Candidates: Msed_0656, 1647, 1290, 0396, 0386, 0271, 0270</td>
<td>N/A</td>
<td>NAD-requiring</td>
</tr>
</tbody>
</table>

R = recombinant version characterized; N = native version characterized; * = obtained from *Sulfolobus tokodai*

**note that enzymes 1 and 2 are bi-functional and each catalyze two steps in the cycle**
Fundamental Studies Needed:

1. Photosystems I and II
2. Hydrogenases
3. CO$_2$ fixation pathways

Key issues:

1. Mechanisms of catalysis and design of biomimetic systems?
2. Electron transfer pathways *between* the three systems?