BIOFUELS

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Bioscience in the 21st Century
November 9, 2011
I. Fossil fuels

II. Microbes and alternative energy (biofuel) production
I. Fossil fuels

II. Microbes and alternative energy (biofuel) production
Deepwater Horizon
Deepwater Horizon

US Coast Guard April 2010

NASA’s Terra satellite May 2010
Deepwater Horizon

A disaster for the Gulf ecosystem, affecting humans and animals alike.
Hydraulic fracturing ("Fracking")
>85% of energy produced in the US comes from burning fossil fuels: coal, petroleum products, and natural gas.

Fossil fuels:
- Non-renewable
- Affect national and global security
- Damage human health
- Cause environmental harm
  - Water, air pollution
  - Oil-spills, damaged ecosystems
  - Increasing atmospheric CO₂ levels → global warming
What are fossil fuels?

~300 million years ago

**BIOMASS**

- Heat
- Pressure

- Coal
- Natural Gas
- Oil (Petroleum)
Fossil fuels = Hydrocarbons

- Molecules made up of hydrogen and carbon
- Contain a lot of energy (the higher the H/C ratio, the better)
- Can be chemically modified or crosslinked to make synthetic rubber, nylon, plastics, other useful chemicals.
- Various lengths and structures → different properties and energy content
Butane

\[
\begin{align*}
\text{CH}_3&-\text{CH}_2-\text{CH}_2-\text{CH}_3 \\
\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_3
\end{align*}
\]
Butane

Isobutane

“isomers”
Octane

3 of 18 possible octane isomers:
What are fossil fuels?

~300 million years ago

BIOMASS

HEAT
PRESSURE

Coal
Natural Gas
Oil (Petroleum)
Natural Gas

methane

$\text{H} - \text{C} - \text{H}$

$\text{H}$

$methane$

$\text{“C}_1\text{”}$

ethane

$\text{“C}_2\text{”}$

propane

$\text{“C}_3\text{”}$
Petroleum

$C_1 - C_{70+}$
Petroleum distillation

- $\text{C}_1$-$\text{C}_4$
- $\text{C}_5$-$\text{C}_9$ (solvents, paint thinners)
- $\text{C}_7$-$\text{C}_11$
- $\text{C}_{12}$-$\text{C}_{15}$
- $\text{C}_{14}$-$\text{C}_{20}$
- $\text{C}_{20}$-$\text{C}_{70}$
- $\text{C}_{70+}$

#C boiling point
Petroleum distillation

Richmond Refinery
(http://crudeoilprice.com/Crude_Oil_Refining.htm)
Coal
Hydrocarbon combustion

\[ C_xH_y + O_2 \rightarrow CO_2 + H_2O + \text{energy} \]

Substrates | Products
---|---
\[ C_xH_y \] | \[ CO_2 \] + \[ H_2O \] + energy

“Exothermic reaction”

The H/C ratio determines the amount of energy released

Incomplete combustion results in toxic byproducts:

- CO (carbon monoxide)
- NO/NO\(_2\) (nitrogen oxides) = SMOG
It’s all about CARBON
## Global reservoirs of carbon

<table>
<thead>
<tr>
<th>Reservoir</th>
<th>Amount ((10^{12} \text{ kg}))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Atmosphere</strong></td>
<td></td>
</tr>
<tr>
<td>(\text{CO}_2)</td>
<td>700</td>
</tr>
<tr>
<td><strong>Ocean</strong></td>
<td></td>
</tr>
<tr>
<td>(\text{CO}_2 (\text{H}_2\text{CO}_3, \text{HCO}_3^-, \text{CO}_3^{2-}))</td>
<td>38,000</td>
</tr>
<tr>
<td>Biomass/organic matter</td>
<td>2,100</td>
</tr>
<tr>
<td><strong>Land</strong></td>
<td></td>
</tr>
<tr>
<td>Crust (carbonate rock)</td>
<td>120,000,000</td>
</tr>
<tr>
<td>Biomass/organic matter</td>
<td>1,700</td>
</tr>
<tr>
<td>Fossil fuel (hydrocarbons)</td>
<td>13,000</td>
</tr>
</tbody>
</table>

## Global reservoirs of carbon

<table>
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<tr>
<th>Reservoir</th>
<th>Amount ($10^{12}$ kg)</th>
<th>Rate of cycling</th>
</tr>
</thead>
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<td><strong>Atmosphere</strong></td>
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Atmosphere

Ocean

Biomass
(organic carbon compounds)

Fossil Fuels
(hydrocarbons)

Dissolved

Released

Photosynthesis

Respiration, combustion

Combustion

The carbon cycle

✓

✗

[Diagram showing the carbon cycle with the atmosphere containing CO₂, ocean with CO₂, H₂CO₃, HCO⁻³, CO₂⁻³, fossil fuels with hydrocarbons, and biomass with organic carbon compounds.]
What are fossil fuels?

~300 million years ago

**BIOMASS**

- **HEAT**
- **PRESSURE**

- **Coal**
- **Natural Gas**
- **Oil (Petroleum)**
The carbon cycle

Atmosphere

(CO₂)

Ocean

(CO₂, H₂CO₃, HCO⁻³, CO₂⁻³)

Biomass

(organic carbon compounds)

Fossil Fuels

(hydrocarbons)

~300 million years ago!!!
The carbon cycle

Atmosphere

\( \text{CO}_2 \)

Ocean

\( \text{CO}_2, \text{H}_2\text{CO}_3, \text{HCO}^{-3}, \text{CO}_2^{-3} \)

Biomass

(organic carbon compounds)

Fossil Fuels

(hydrocarbons)

Photosynthesis

Respiration, combustion

Released

Dissolved

Combustion

~300 million years ago!!!
Atmospheric CO$_2$ levels

From nasa.gov
The carbon cycle

**Time history of atmospheric CO$_2$**

http://www.youtube.com/user/CarbonTracker
I. Fossil fuels

II. Microbes and alternative energy (biofuel) production
Alternatives to fossil fuels

Nuclear energy

Geothermal energy

Hydrogen

Wind energy

Tidal

Solar energy
Biofuels
**Biofuel:** A fuel derived from biomass

“carbon neutral”

\[
\text{CO}_2 \xrightarrow{\text{production}} \text{Biofuel} \xrightarrow{\text{combustion}} \text{CO}_2
\]

“\(\text{CO}_2\) in = \(\text{CO}_2\) out”

What is the biofuel?
What is the source of carbon/energy?
The carbon cycle

Atmosphere (CO$_2$)

Photosynthesis

Respiration, combustion

Dissolved

Released

Biomass (organic carbon compounds)

Fossil Fuels (hydrocarbons)

Ocean (CO$_2$, H$_2$CO$_3$, HCO$^{-3}$, CO$_2^{-3}$)

~300 million years ago!!!
Biomass

PHOTOSYNTHESIS

$\text{CO}_2$

“energy in”

BIOMASS

sugar

“energy out”

ATP

RESPIRATION/FERMENTATION

Small carbon molecules (acids, alcohols)

cellulose (sugar storage)

BIOSYNTHESIS

carbohydrates

lipids

proteins

nucleic acids
BIOMASS

Carbon-based compounds generated by living or recently-living organisms

Contain ENERGY in the form of chemical bonds

Originally from the sun!
BIOMASS

PHOTOSYNTHESIS

CO₂

"energy in"

sugar

"energy out"

ATP

BIOSYNTHESIS

cellulose (sugar storage)

carbohydrates

lipids

proteins

nucleic acids

Small carbon molecules (acids, alcohols)

RESPIRATION/FERMENTATION
BIOMASS

PHOTOSYNTHESIS

CO₂

sugar

“energy in”

Small carbon molecules (acids, alcohols)

“energy out”

ATP

BIOSYNTHESIS

cellulose (sugar storage)

carbohydrates

lipids

proteins

nucleic acids

RESPIRATION/FERMENTATION

“energy out”
Wood (cellulose) is a biofuel
Cellulose

Firewood: 18-22 GJ/t
Crude oil: 42-45 GJ/t

http://bioenergy.ornl.gov/papers/misc/energy_conv.html
BIOMASS

PHOTOSYNTHESIS

CO₂

“energy in”

“energy out”

ATP

RESPIRATION/FERMENTATION

Small carbon molecules (acids, alcohols)

sugar

cellulose (sugar storage)

carbohydrates, lipids, proteins, nucleic acids

BIOSYNTHESIS

ethanol
BIOMASS

PHOTOSYNTHESIS

CO₂

“energy in”

“energy out”

ATP

RESPIRATION/ FERMENTATION

BIOSYNTHESIS

sugar

cellulose (sugar storage)

carbohydrates

lipids → biodiesel

proteins

nucleic acids

Small carbon molecules (acids, alcohols)

ethanol

“energy out”
“Generations” of biofuel

ethanol = 1\textsuperscript{st} and 2\textsuperscript{nd} generation

lipids $\rightarrow$ biodiesel = 3\textsuperscript{rd}+ generations

Microbes play a critical role in producing the biofuel!
- Remarkable metabolic capabilities
- Can grow quickly and in large amounts
- Can be manipulated and optimized in the laboratory
Ethanol: 1\textsuperscript{st} and 2\textsuperscript{nd} generation biofuel

\begin{center}
\begin{tikzpicture}
\draw [thick] (-0.5,0) -- (0.5,0);
\draw [thick] (0,0) -- (0,0.5);
\draw [thick] (-0.5,0) -- (0,-0.5);
\draw [thick] (0.5,0) -- (0,-0.5);
\draw [thick] (-0.5,0) -- (-0.5,0.5);
\draw [thick] (0.5,0) -- (0.5,0.5);
\end{tikzpicture}
\end{center}

- Ethanol: 26.7 GJ/t
- Crude oil: 42-45 GJ/t

http://bioenergy.ornl.gov/papers/misc/energy_conv.html
Generation 1: Ethanol from corn (sugar)

Generation 1 biofuels: Corn (shown at left) and yeast can be mixed in a bioreactor for fermentation, which produces ethanol for use as fuel, along with water and carbon dioxide by-products (above).

BIOMASS

PHOTOSYNTHESIS

CO₂ → sugar → cellulose (sugar storage)

“energy in”

Small carbon molecules (acids, alcohols) → ATP → carbohydrates, lipids, proteins, nucleic acids

“energy out”

RESPIRATION/FERMENTATION

ethanol
Saccharomyces cerevisiae

- Unicellular yeast (eukaryote)
- Can ferment sugar $\rightarrow$ ethanol
- Also a model organism for many cell biological studies
Problems with corn-based ethanol biofuels

Waste
   Corn husks/stalks not used
   To make 1 gallon of ethanol need:
      21 pounds of corn (fresh water + ½ gallon of fossil fuel!)

Food-shortages
   Corn grown for ethanol production vs. food
   Corn prices driven up by demand

Inefficiency of ethanol as a biofuel
   Can’t produce enough ethanol to meet energy demands
“Generation 2” biofuel: Ethanol from cellulose

**Generation 2 biofuels:** Cellulose from plant sources such as sawgrass (shown below) is mixed in a bioreactor with either yeast or bacteria for fermentation, which produces ethanol, butanol or other compounds for use as fuel, along with water and carbon dioxide by-products (above).

BIOMASS

**PHOTOSYNTHESIS**

- **CO₂**
- **“energy in”**
- **sugar**
- **cellulose** (sugar storage)

**RESPIRATION/FERMENTATION**

- **ATP**
- **“energy out”**
- **Small carbon molecules** (acids, alcohols)
- **carbohydrates**
- **lipids**
- **proteins**
- **nucleic acids**

**BIOSYNTHESIS**

- **ethanol**
“Generation 2” biofuel: Ethanol from cellulose

Reduced waste

Entire plant (grass) is utilized.
Grasses like sawgrass grow quickly, without fertilizer, and with minimal/no need for pesticides.

Decreased competition with food crops

BUT:

Still need a lot of land

Inefficiency of cellulose digestion
Cellulose is difficult to convert back to sugar!

Inefficiency of ethanol as a biofuel
“Generation 2” biofuels: Room for improvement

**Strategy:** Find or design a “super-microbe” that can digest cellulose and/or make other alcohols!
Q-Microbe

Isolated from the Quabbin Reservoir in western Massachusetts

Bacterium called *Clostridium phytofermentans*

Digests cellulose with ease (how??!!) to make ethanol!
Synthetic biology

A new combination of biology and engineering aimed toward designing and constructing biological systems and pathways not found in nature.

I want to see how much we can tweak cells and probe the limits of nature.... We’re at a point in biology where we don’t have to accept what nature has given us.

—JAY KEASLING
CEO, Joint BioEnergy Institute
Synthetic biology

Artemisia annua (annual wormwood)

= anti-malaria drug
Synthetic biology

Saccharomyces cerevisiae (yeast)
Synthetic biology

Saccharomyces cerevisiae (yeast)

Artemisia annua (annual wormwood)

= anti-malaria drug
Microbial Cell Factories

Research

Metabolic engineering of *Saccharomyces cerevisiae* for the production of n-butanol

Eric J Steen¹,², Rossana Chan¹,³, Nilu Prasad¹,³, Samuel Myers¹,³, Christopher J Petzold¹,³, Alyssa Redding¹,³, Mario Ouellet¹,³ and Jay D Keasling*¹,²,³,⁴

Abstract

**Background**: Increasing energy costs and environmental concerns have motivated engineering microbes for the production of "second generation" biofuels that have better properties than ethanol.

**Results and conclusion**: *Saccharomyces cerevisiae* was engineered with an n-butanol biosynthetic pathway, in which isozymes from a number of different organisms (*S. cerevisiae*, *Escherichia coli*, *Clostridium beijerinckii*, and *Ralstonia eutropha*) were substituted for the Clostridial enzymes and their effect on n-butanol production was compared. By choosing the appropriate isozymes, we were able to improve production of n-butanol ten-fold to 2.5 mg/L. The most productive strains harbored the *C. beijerinckii* 3-hydroxybutyryl-CoA dehydrogenase, which uses NADH as a co-factor, rather than the *R. eutropha* isozyme, which uses NADPH, and the acetoacetyl-CoA transferase from *S. cerevisiae* or *E. coli* rather than that from *R. eutropha*. Surprisingly, expression of the genes encoding the butyryl-CoA dehydrogenase from *C. beijerinckii* (*bcd* and *etfAB*) did not improve butanol production significantly as previously reported in *E. coli*. Using metabolite analysis, we were able to determine which steps in the n-butanol biosynthetic pathway were the most problematic and ripe for future improvement.
BIOMASS

PHOTOSYNTHESIS

CO₂

“energy in”

BIOSYNTHESIS

sugar

“energy out”

cellulose (sugar storage)

Small carbon molecules (acids, alcohols)

ATP

lipids → biodiesel

carbohydrates

proteins

nucleic acids

RESPIRATION/FERMENTATION

“energy out”
Lipids

Free fatty acid

Triglyceride = Main constituent of vegetable oil and animal fats

Phospholipid

Cholesterol
Triglycerides → Biodiesel

diagram of triglyceride and transesterification reactions leading to glycerol and biodiesel.
Biodiesel is a biofuel

Biodiesel: 38 GJ/t
Crude oil: 42-45 GJ/t

http://bioenergy.ornl.gov/papers/misc/energy_conv.html
Problem with biodiesel

Soybean as the source of the oil

- Not enough land
- Takes energy to farm, harvest, and extract oil
“Generation 3” biofuel: Biodiesel from CO$_2$

**Generation 3 biofuels:** When photosynthetic algae or cyanobacteria (shown growing inside cylinders at right) are exposed to sunlight and carbon dioxide, they produce and stockpile fats inside their cells. Exposing the cells to a chemical solvent frees these fat molecules, which can be refined into biodiesel (above).
BIOMASS

CO$_2$ PHOTOSYNTHESE

"energy in"

CELLULOSE (sugar storage)

BIOSYNTHESIS

biodiesel

lipids

proteins

nucleic acids

"energy out"

ATP

RESPIRATION/FERMENTATION

sugar

Small carbon molecules (acids, alcohols)
Algae as a source of triglycerides for biodiesel
Summary

Renewable and carbon-neutral forms of energy, including biofuels, are desperately needed to replace fossil fuels.

Microbes are poised to play an increasingly pivotal role in the generation of biofuels, especially through synthetic biology.