Engineering microbes for production of biofuels and chemicals

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Forces of change
What has changed drastically during the past 25-30 years?

- Continuous increase of the cost of fuels and raw materials
Oil supply and cost curve

Available oil in billion barrels

- Already produced
- OPEC ME
- Other conv. oil
- EOR
- Heavy oil Bitumen
- Super deep
- Oil shales

Source: IEA (2005)
What has changed drastically during the past 25-30 years?

• Continuous increase of the cost of fuels and raw materials

• Strategic challenges in securing the required amounts of fuels and raw materials

• Grave consequences for climate change
Global CO2 emissions
Atmospheric Carbon Dioxide

Measured at Mauna Loa, Hawaii

Carbon dioxide concentration (ppmv)

Annual Cycle
Jan Apr Jul Oct Jan
What has changed drastically during the past 25-30 years?

• Continuous increase of the cost of fuels and raw materials
• Strategic challenges in securing the required amounts of fuels and raw materials
• Serious concerns about climate change

• Development of Biotechnology and Metabolic Engineering: Core technologies for converting renewable resources to fuels and chemicals
Technology advances:

Engineering the metabolism of microbes to convert them to chemical factories for the production of biofuels and chemicals

Biotechnology beyond medicine
Cells: Little chemical factories with thousands of chemical compounds interconverted through thousands of chemical reactions

Main substrate: Sugars

Products: Virtually infinite
Microorganisms
They are found everywhere, from the human gut to the hot springs of Yellowstone Park
Engineering microbes to produce any product

- Substrates
- Rxn 1
- Rxn 2
- Rxn 3
- Rxn 4
- Rxn 5
- Rxn 6

P 2

P 4

- CoA activator
- CoA remover

Pentanol

HPLC analysis

Engineering microbes to produce any product
Types of biofuels and biofuel feedstocks

- Ethanol from corn
- Biodiesel from plant seeds and vegetable oils
- Ethanol from sugarcane
- Other feedstocks (not competing with food): cellulosics, algae
- Other biofuels than ethanol (butanol, lipids, hydrocarbons)
Contributions from my lab

1. Improving ethanol tolerance of yeast

Extensions: Improving microbial tolerance to toxicity


Product **toxicity** is a major problem in engineering microbes for production of biofuels and biochemical products.

It is important that studies aiming at improving tolerance are conducted under bioprocess-relevant conditions.
EtOH increased upon K-Pi supplementation

K-Pi supplementation and pump engineering enhances:

I. Growth despite accumulating EtOH
II. Tolerance despite accumulating EtOH
Despite accumulating EtOH, KCl+KOH boosts:

I. Cell growth

High KCl+KOH enhance population viability

- OD\textsubscript{600} at 24 h: ~11%
- Population viability:
  - 98% live
  - 70% live
High KCl+KOH enhance population viability

Despite accumulating EtOH, KCl+KOH boosts:

I. Cell growth
II. Tolerance
Tolerance and titer highly correlated

Product **toxicity** is a major problem in engineering microbes for production of biofuels and biochemical products. It is important that studies aiming at improving tolerance are conducted under bioprocess-relevant conditions.
2. Engineering xenobiotic pathways to prevent contamination

Extensions:
Eliminating the need for the use of antibiotics

3. Engineering yeast to metabolize all sugars from biomass hydrolysis

Extensions: Use of vast amounts of lingo-cellulosics for biofuels

Biofuel (ethanol) from renewables

- Fuel ethanol from corn starch or sugar
  - Used as such or blended with gasoline

- Ethanol from plant biomass
  - Readily fermentable starch and sucrose
  - Resistant lignocellulosic fractions
## Performance of the *S. cerevisiae* strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Description</th>
<th>Conditions</th>
<th>Ethanol yields g/g</th>
<th>Xylitol yields g/g</th>
<th>Ethanol production g·g⁻¹·h⁻¹</th>
<th>Xylose consumption g·g⁻¹·h⁻¹</th>
<th>µ&lt;sub&gt;max&lt;/sub&gt; h⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>H131-A31</td>
<td><em>XylA, PsXyl3, PsTal1, TKL1, RPE1, RKI1</em></td>
<td>Aerobic batch, SDX</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>0.031 ± 0.022</td>
</tr>
<tr>
<td>H131E1-A31</td>
<td>Selection of H131-A31, aerobic sequential batch</td>
<td>Aerobic batch, SDX</td>
<td>0.200</td>
<td>&lt;0.01</td>
<td>0.034</td>
<td>0.169</td>
<td>0.197 ± 0.006</td>
</tr>
<tr>
<td>H131E3-A31</td>
<td>Selection of H131E1-A31, micro-aerobic sequential batch</td>
<td>Anaerobic batch, 2×YNB, 4% xylose</td>
<td>0.440</td>
<td>&lt;0.01</td>
<td>0.120</td>
<td>0.273</td>
<td>0.061 ± 0.002</td>
</tr>
<tr>
<td>H131E5-A31</td>
<td>Selection of H131E5-A31, anaerobic chemostat</td>
<td>Anaerobic batch, 2×YNB, 4% xylose</td>
<td>0.410</td>
<td>&lt;0.01</td>
<td>0.233</td>
<td>0.568</td>
<td>0.073 ± 0.002</td>
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<td>H131E8-A31</td>
<td>Selection of H131E8-A31, anaerobic chemostat</td>
<td>Anaerobic chemostat, YNBX</td>
<td>0.438</td>
<td>&lt;0.01</td>
<td>0.641</td>
<td>1.464</td>
<td>0.148</td>
</tr>
<tr>
<td>RWB 217</td>
<td><em>XylA, XKS1, TAL1, TKL1, RPE1, RKI1, gre3Δ</em></td>
<td>Anaerobic batch, synthetic medium</td>
<td>0.43</td>
<td>0.003</td>
<td>0.46</td>
<td>1.06</td>
<td>0.09</td>
</tr>
<tr>
<td>RWB 218</td>
<td>Selection of RWB 217</td>
<td>Anaerobic batch, synthetic medium</td>
<td>0.41</td>
<td>0.001</td>
<td>0.49</td>
<td>1.2</td>
<td>0.12</td>
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</tbody>
</table>
Towards *integrated and complete* processes for biofuel and chemical production from renewable feedstocks
http://bioenergy.ornl.gov/papers/misc/bioenergy_cycle.html
Carbon cycle

- Sunlight 
  - Photosynthesis
  - Biomass: τ ≈ 1-100 years

- CO₂
  - Combustion
  - Industrial biotechnology

- Carbohydrates
  - Fuels & chemicals

Industrial Biotechnology
First and foremost, biofuels are a feedstock story
Feedstocks must be cheap and aggregated

Examples:
1. Lignocellulosic biomass
2. Waste solids
3. Waste gases
4. Algae (cheap?)
Potential of biofuels (USA)

- 70-100 gallons ethanol/dry ton of biomass
- 42-60 B gallons Ethanol/year or 28-40 B gallons of gasoline equivalent
  20-30% of gasoline used

(1 ton of ethanol = 333 gallons, or 1 Gallon = 3 kgs, or 1 B Gallons = 3 M tons)
NEW Contributions from my lab

4. Engineering oleaginous yeast for overproduction of lipids

Extensions:
Open up the potential for Green Diesel or Renewable Diesel from biomass or waste


Rising global diesel demand

- High demands in diesel consumption in both industries
- Diesel consumption in the U.S. projected to grow at 400-500 million gallons per year

US energy information administration, 2016.
Benefits of green diesel

Economical

- Current aviation diesel costs
- Projected aviation diesel costs
- Jet fuel costs
- Jet fuel + carbon costs

USD per gallon

2000 2010 2020 2030 2040 2050

Projected aviation diesel costs

Environmental

- Air transport action group, 2011
- US energy information administration, 2016
- Alternative energy news, 2016

Ideal physical and chemical properties

- Green diesel
- Jet fuel
- Gasoline
- Biodiesel
- Bioethanol

Energy density (MJ/kg)

-10 10 30 50
(a) Graph showing the lipids titer vs. time for different conditions:
- AD DCW
- AD Lipid titer
- ADgy DCW
- ADgy Lipid titer
- ADgm DCW
- ADgm Lipid titer

(b) Graph showing the lipid titer vs. time for different conditions:
- ADgm DCW
- ADgm Lipid titer
- ADgm-hi DCW
- ADgm-hi Lipid titer

(c) Graph showing the lipid yield vs. genotype:
- AD
- ADgapC
- ADgy
- Adme
- ADgm

* indicates significant difference.
Importance in advancing Renewable Diesel

Base case: Sugars at $200/ton (~9c/lb)

Feedstock cost of lipids produced from sugars from renewable biomass:

1. At a yield of 0.18g/g (state of the art): $1,100/ton
2. At a yield of 0.30g/g (our work): $660/ton
3. Oil selling price range: $700-1,100
Alternative feed stocks

- Glucose is expensive
- Acetic acid is interesting alternative. Can be supplied at large volumes from
  - Anaerobic digestion
  - Fixation of CO2 with CO or Hydrogen using anaerobic acetogenic bacteria

Trash → VFA → Yarrowia Fermentation

Gases → VFA → Lipids, Food, Biodiesel
4. Optimization of Nitrogen feed based on RQ/CTR feedback control

- Working volume 1.5 L
- Maintain carbon at zero
Importance of waste utilization

1. Waste generation: 1 ton/person (US, 2011)
2. Fermentable fraction: 25% (US)-50% (China) (use 35%)
3. Potential for 3-5B gallons diesel/year (USA)
4. Cost of waste: can be negative at $100/ton
5. Potential depends on capacity to aggregate waste economically
### Algae

<table>
<thead>
<tr>
<th></th>
<th>Gallons GE/acre/year</th>
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<tbody>
<tr>
<td>Soybeans</td>
<td>48</td>
</tr>
<tr>
<td>Sesame</td>
<td>74</td>
</tr>
<tr>
<td>Jatropha</td>
<td>202</td>
</tr>
<tr>
<td>Cellulosic (for ethanol production)</td>
<td>533</td>
</tr>
<tr>
<td>Sugarcane (for ethanol)</td>
<td>566</td>
</tr>
<tr>
<td>Algae</td>
<td>~6,000</td>
</tr>
</tbody>
</table>
Biofuel production by direct photosynthesis

Sun → Algae → Oil-alkane production

Metabolic Engineering; Secretion? Oil recovery

Just growth

Algae

Productivities are high but cultures very dilute Key challenge: Cost-effective dewatering

Biomass

Other biofuels (ethanol)

G. Stephanopoulos, MIT

Fuel Choices Summit 2016
3 November 2016
**A final word about chemicals**

It is now possible to produce commodity chemicals (as well as, of course, specialty chemicals):

1. With cost-effective processes
2. Using renewable feedstocks
3. Small, efficient specialized units
4. Examples: Ethylene glycol, glycolic acid, biopolymers, organic acids (diacids), others
