Synthetic Biology for the Calvin-Cycle-Channeled (Photobiological) Synthesis of Butanol & Pentanol Utilizing Carbon Dioxide as the Sole Feedstock

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Technology Reversal

• Phytonix’s biosynthesis technology does not convert biomass into fuels and chemicals, quite the opposite, the biomass converts carbon dioxide directly into the target fuels and chemicals…..

• A “Generation 4” biofuels technology
The Future of Fuel

- Using synthetic biology, metabolic engineering and photobiology, we will...

Safely develop and utilize genetically modified cyanobacterial organisms as “fuel production platforms”

- This will result in low cost and high-yielding biofuels.

- Removing the biomass from the biofuels cost equation can eliminate up to 80% of the production cost.
The Phytonix Advantage

- In 2010, Px purchased the exclusive global license to patent-pending genomic engineering and synthetic biology technologies to develop environmentally safe, high yielding, direct solar liquid transportation fuels.
A More Logical Technology

• The Phytonix process streamlines, simplifies and adds logic to the biofuels production process in terms of inputs, outputs and cost-drivers.

• All fermentative biofuels or chemicals technologies are fundamentally flawed: the biomass feedstock creates many additional cost drivers/production steps and they create about as much waste carbon dioxide as biofuels/chemicals.

• Extractive algal biofuels technologies also require biomass production and multiple, expensive processing steps.

• Px (photobiological) technology converts waste carbon dioxide feedstock directly into biofuels/renewable chemicals, with oxygen as a by-product.
An Industrial Biotechnology Breakthrough

• Photosynthetic organisms that will produce “drop-in” gasoline replacement fuels and renewable chemicals:
  – Px’s scientific development teams are creating photosynthetic organisms (e.g., cyanobacteria) and “static culture” phytoconverters™ that will produce butanol and pentanol utilizing carbon dioxide as the sole feedstock.
  – Butanol and pentanol are promising “drop-in” gasoline replacement fuels that can be used in current pipe-to-pump infrastructure as well as high-value renewable industrial chemicals.
  – These organisms will use efficient, Calvin-Cycle-Channeled engineered biosynthesis pathways to produce butanol and pentanol from carbon dioxide, sunlight, and water.
A sophisticated and elegant solution combining great achievements of natural evolution with cutting-edge scientific ingenuity.....yet allowing a simplified production process that removes many traditional steps and cost-drivers:

- Allows production process to bypass costly steps:
  - Large-scale biomass production or sourcing
  - Biomass collection
  - Transportation/logistics
  - Biomass pre-treatment and lignocellulosic deconstruction
  - Fuel component extraction/production/refining
The Calvin Cycle

Source: Purves et al., 2003
Phytonix’s Synthetic Biology Engineering Strategy

• Don’t “over-engineer” organisms with too many non-native cellular functions – streamline and simplify
• Don’t engineer in competing fuel/chemical pathways
• Save environmental tolerance and parameter control for photobioreactors (Phytoconverters™), do not try to engineer into the organism
• Utilize Phytonix’s proprietary “cell-division-controllable” technology/IP in order to slow/stop cell replication to create “static” cultures
• Static cultures maximize target C4 & C5 molecule yields as the majority of cellular energy is focused on synthesis
• These engineering strategies will help maintain cellular metabolic balance in minimize pathway bottlenecks
Biobutanol Synthesis Pathway

Calvin Cycle → Fructose-6-phosphate → 1,3-Diphosphoglycerate → 2-Phosphoglycerate → Pyruvate → Butyraldehyde → 1-Butanol

Glycogen → 12,44 → 20,22
## Construction of Synthesis Pathways

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Source Organism</th>
<th>GenBank #/Protein ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>NADH-dependent Butanol dehydrogenase (12)</td>
<td>Clostridium carboxidivorans</td>
<td>ADC48983</td>
</tr>
<tr>
<td>Glucose-phosphate isomerase (18)</td>
<td>Saccharomyces cerevisiae</td>
<td>M21696</td>
</tr>
<tr>
<td>Fructose-diphosphate aldolase (20)</td>
<td>Chlamydomonas reinhardtii</td>
<td>JGI Chlre3 protein ID 135202</td>
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</tbody>
</table>
Gasoline-replacing Organisms

- Modular PhytoConverter Systems
  - Designed for direct biobutanol and biopentanol fuel production
  - Continuous, single-step process
  - Will allow Px production facilities to scale from small industrial operations to large-scale commercial facilities
PhytoConvertor Illustration

Planktonix's Cyanobacterial PhytoConvertor
Butanol Basics

• (Bio)butanol (1-butanol) is a four-carbon, straight-chain alcohol.
  – Used to run spark ignition engines (e.g., gasoline-powered vehicles)
  – Can replace gasoline since the energy content of the two fuels is nearly the same.
  – Global industrial chemical market for n-butanol is $6 billion with a 12% CAGR
Fuel Characteristics

<table>
<thead>
<tr>
<th>Quality</th>
<th>Methanol</th>
<th>Ethanol</th>
<th>Butanol</th>
<th>Gasoline</th>
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</thead>
<tbody>
<tr>
<td>Chemistry</td>
<td>CH$_3$OH</td>
<td>C$_2$H$_5$OH</td>
<td>C$<em>4$H$</em>{10}$OH</td>
<td>Many</td>
</tr>
<tr>
<td>Energy Content (per gallon)</td>
<td>63,000 Btu</td>
<td>78,000 Btu</td>
<td>110,000 Btu</td>
<td>115,000 Btu</td>
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<tr>
<td>Vapor Pressure @ 100F (Reid V.P.)</td>
<td>4.6 PSI</td>
<td>2.0 PSI</td>
<td>0.33 PSI</td>
<td>4.5 PSI</td>
</tr>
<tr>
<td>Motor Octane</td>
<td>91</td>
<td>92</td>
<td>94</td>
<td>96</td>
</tr>
<tr>
<td>Air-to-Fuel Ratio</td>
<td>6.6</td>
<td>9</td>
<td>11.1</td>
<td>12-15</td>
</tr>
</tbody>
</table>

Higher energy content, less evaporative (safer), no seasonal blends required, can be used as a “drop-in” gasoline replacement fuel, large n-butanol industrial chemical market.
SOLAR CONVERSION EFFICIENCY: A PHYTONIX TARGET HOST ORGANISM


The study determined characteristics of Synechocystis sp. PCC 6803:
- fresh water organism but was salt tolerant (0-1.2 M NaCl)
- temperature tolerant (up to 45°C)
- Efficient light (photon-electron) harvesting of 28% (calculation below)

Computation of efficient light harvesting - NADP Light reactions (linear electron flow)
- \[48 \text{H}_2\text{O} + 48 \text{NADP} + 192 \text{hn}^+ \rightarrow 48 \text{NADPH} + 24 \text{O}_2 + 64 \text{ADP/Pi}\]
  A mole of quanta of 680 nm light: 177 kJ
  A mole of C16 fatty acid: 9800 kJ Maximum energy efficiency (680 nm illumination):
- \[9800 \times 100\% / (177 \times 192) = \text{28\% solar utilization efficiency/energy conservation}\]
- higher plant photosynthesis (including green algae) is approximately 20% as efficient at photosynthesis as cyanobacteria.
Mass Balance (inputs & outputs)

**Cyanobacteria Microorganism**

- **Inputs:**
  - 40 gallons water
  - 2.4 lb carbon dioxide
  - Natural sunlight

- **Outputs:**
  - 38 – 39 gallons water
  - 2.16 lb CO2
  - 0.5 – 1.0 gallons H2O
  - 1.0 gallon n-butanol
  - Oxygen

**Cell Usage**
- 0.24 lb carbon dioxide

**Evaporation**
- 0.5 - 1.0 gallons water
Carbon-negative Technology

• Phytonix’s biobutanol & biopentanol production are significantly carbon-negative
  – Since CO$_2$ is used as the sole, direct feedstock

• Our butanol & pentanol fuel lifecycles through combustion are carbon-neutral

• Butanol and pentanol combust more cleanly than gasoline and other fossil-based fuels.
  • Complete combustion of n-butanol emits only CO$_2$ and water
  • No emissions of carbon monoxide, NO$_x$, or SO$_x$
Px’s Technology Is Efficient

- **Cost Effective:** Px’s technology will be far more cost-effective than biomass and petroleum-based fuels: < $1.00/gallon

- **High Yields:** Our technology provides the potential to make ~20,000-40,000 gallons fuel/chemicals per acre/year.
Butanol Separation

- Separation technology
  - Will use <energy than other approaches in use now
  - Will achieve >8% n-butanol concentration
    - At which point it will "phase separate" into 100% n-butanol
  - Will occur through state-of-the-art membrane filtration technology and pervaporation
Biosafety Focus

- The Phytonix biosafety guarded genomics/molecular biology technology is
  - Appears to be unique and novel in the biofuels industry

- Our biosafety-guarded technology can be used for other transgenic and modified organisms
  - Not just for biofuels production
  - Phytonix owns the exclusive rights to sublicense this technology… and will do so at a reasonable cost to encourage its use.
Land Required Per Biofuels Technology Generation to Displace All U.S. Gasoline:

Each biofuel generation uses less land to produce a given volume of biofuel

Algal/CB photobioreactor–based biofuels have by far the highest productivity yield per acre.
Global Application

- We expect to develop highly adaptive organisms that proliferate in a variety of environments and media
  - Direct sunlight, ambient sunlight, cool to warm temperatures, and fresh, salt, and brackish water
  - This adaptability will enhance the global adoption of Px’s photobiological fuel production technology.
& Answers

QUESTIONS