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

2012 Pacific Rim Summit on Industrial Biotechnology and Bioenergy
Vancouver, Canada October 11th, 2012

Phytonix
The Future of Fuel®

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…

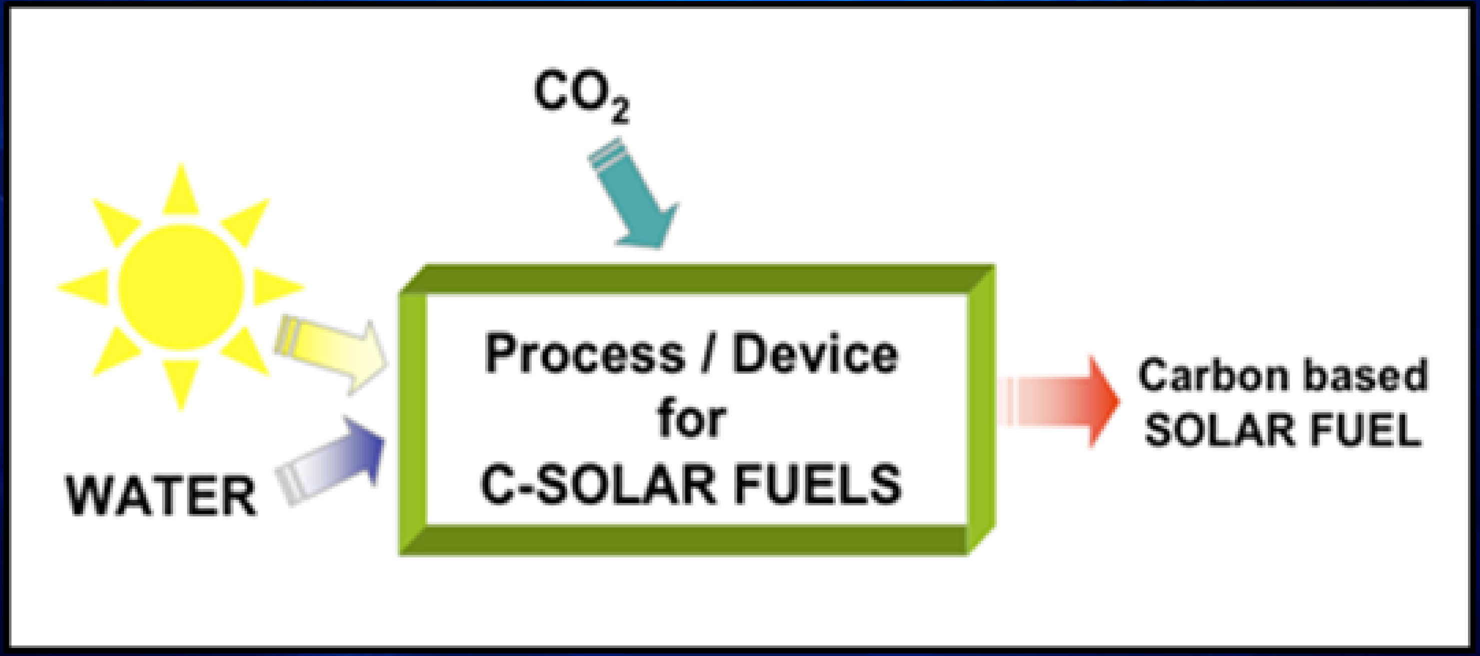
Safely develop and utilize genetically modified cynaobacterial organisms as “fuel production platforms”

- 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.

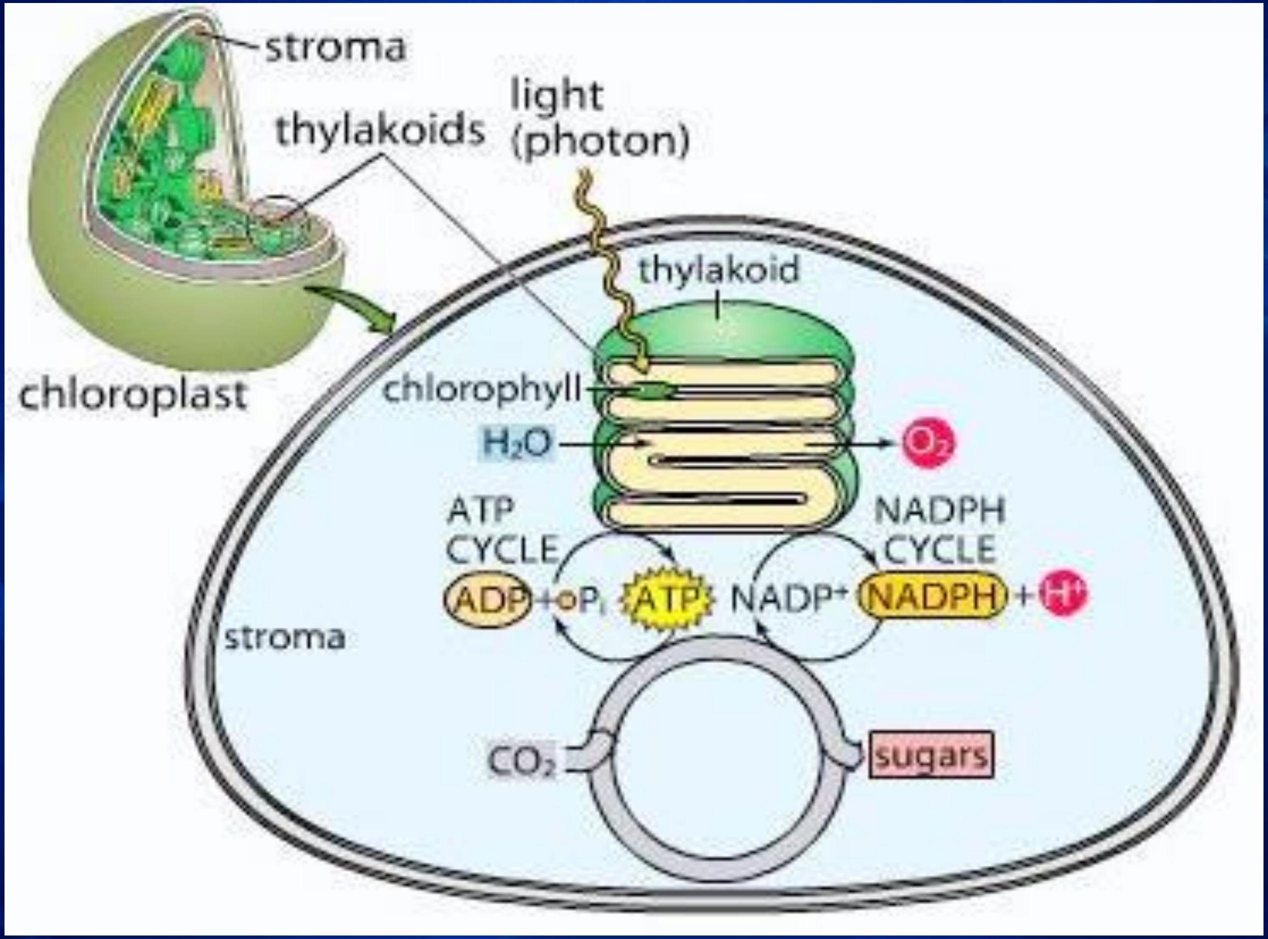
An Industrial Biotechnology Breakthrough

- 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^{TM} 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

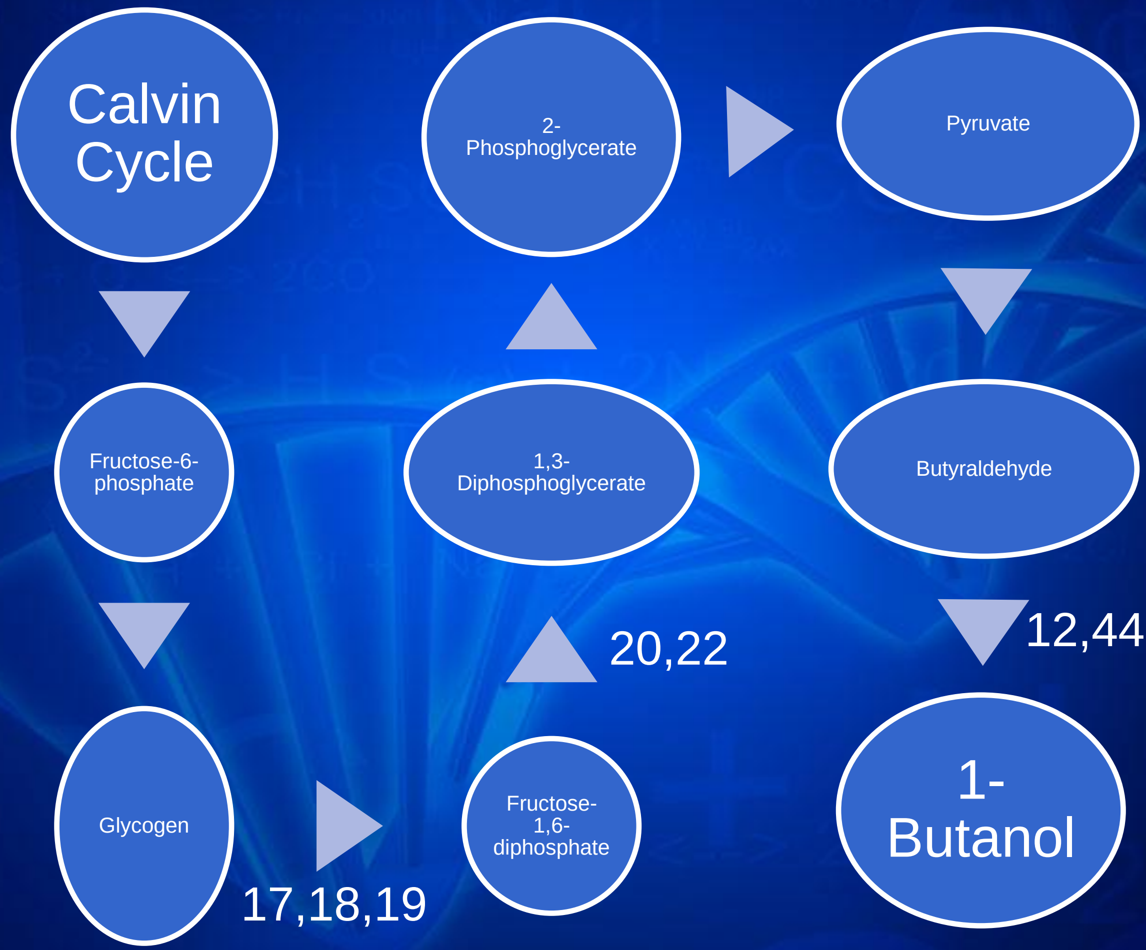


Source: Purves et al., 2003

Phytonix’s Synthetic Biology Engineering Strategy

- Don’t “over-engineer” organisms with too may non-native cellular functions – streamline and simplify
- Don’t engineer in competing fuel/chemical pathways
- Save environmental tolerance and parameter control for photobioreactors (Phytoconverters$_{TM}$), 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



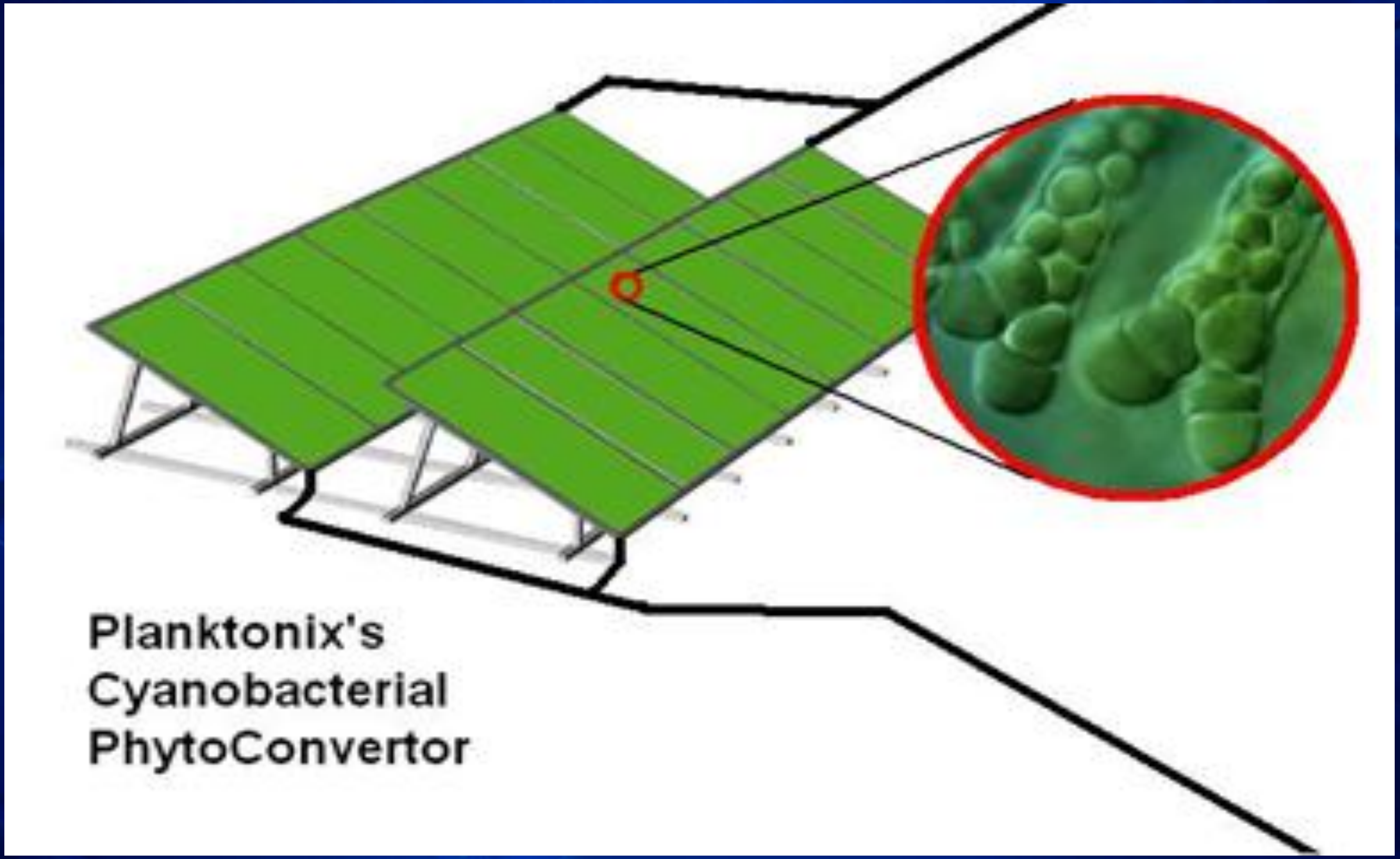
Construction of Synthesis Pathways

Enzyme	Source Organism	GenBank #/Protein ID
NADH-dependent Butanol dehydrogenase (12)	Clostridium carboxidivorans	ADC48983
Glucose-phosphate isomerase (18)	Saccharomyces cerivisiae	M21696
Fructose-diphosphate aldolase (20)	Chlamydomonas reinhardtii	JGI Chlre3 protein ID 135202

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



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

Quality	Methanol	Ethanol	Butanol	Gasoline
Chemistry	CH_3OH	C_2H_5OH	$C_4H_{10}OH$	Many
Energy Content (per gallon)	63,000 Btu	78,000 Btu	110,000 Btu	115,000 Btu
Vapor Pressure @ 100F (Reid V.P.)	4.6 PSI	2.0 PSI	0.33 PSI	4.5 PSI
Motor Octane	91	92	94	96
Air-to-Fuel Ratio	6.6	9	11.1	12-15

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

- **In 2011, the Advanced Research Projects Agency of the US Department of Energy and scientists at Arizona State University reviewed the solar conversion efficiency and mass balance of Synechocystis sp. PCC 6803.** **<u>Source: "Cyanobacteria as biocatalysts for solar-driven biofuel production", Vim Vermass, 2011, ASU/ARPA-E</u>**

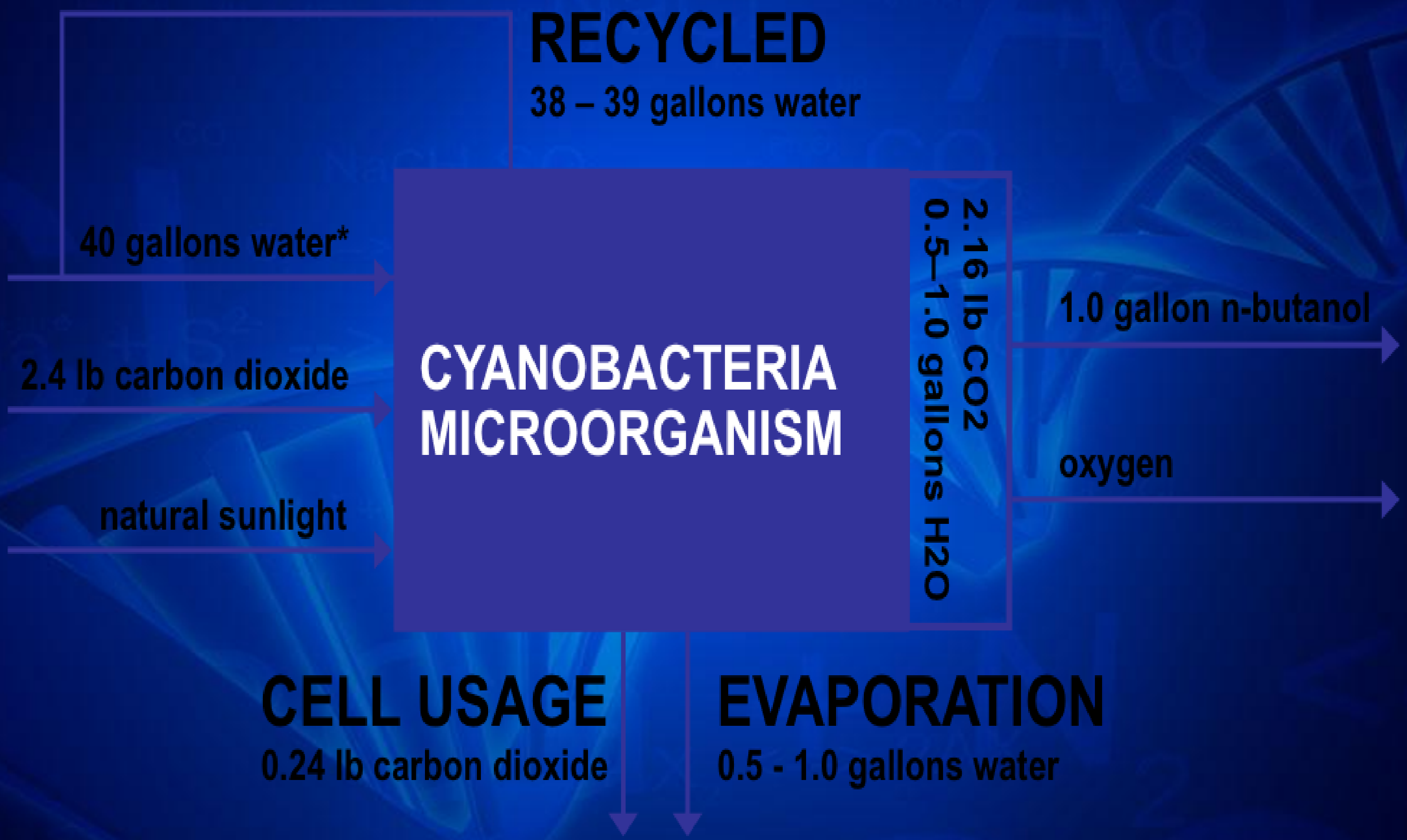
<u>The study determined characteristics of Synechocystis sp. PCC 6803:</u>

- **fresh water organism but was salt tolerant (0-1.2 M NaCl)**
- **temperature tolerant (up to 45ºC)**
- **Efficient light (photon-electron) harvesting of 28% (calculation below)**

Computation of efficient light harvesting - NADP Light reactions (linear electron flow)

- **48 H2O + 48 NADP + 192 hn+ 64 ADP/Pi-> 48 NADPH + 24 O2 + 64 ATP**
  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 x 100% / (177 x 192) = *<u>28% solar utilization efficiency/energy conservation</u>***
- **higher plant photosynthesis (including green algae) is approximately 20% as efficient at photosynthesis as cyanobacteria.**

Mass Balance (inputs & outputs)



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 CO2 and water
    - No emissions of carbon monoxide, NOx, or SOx

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

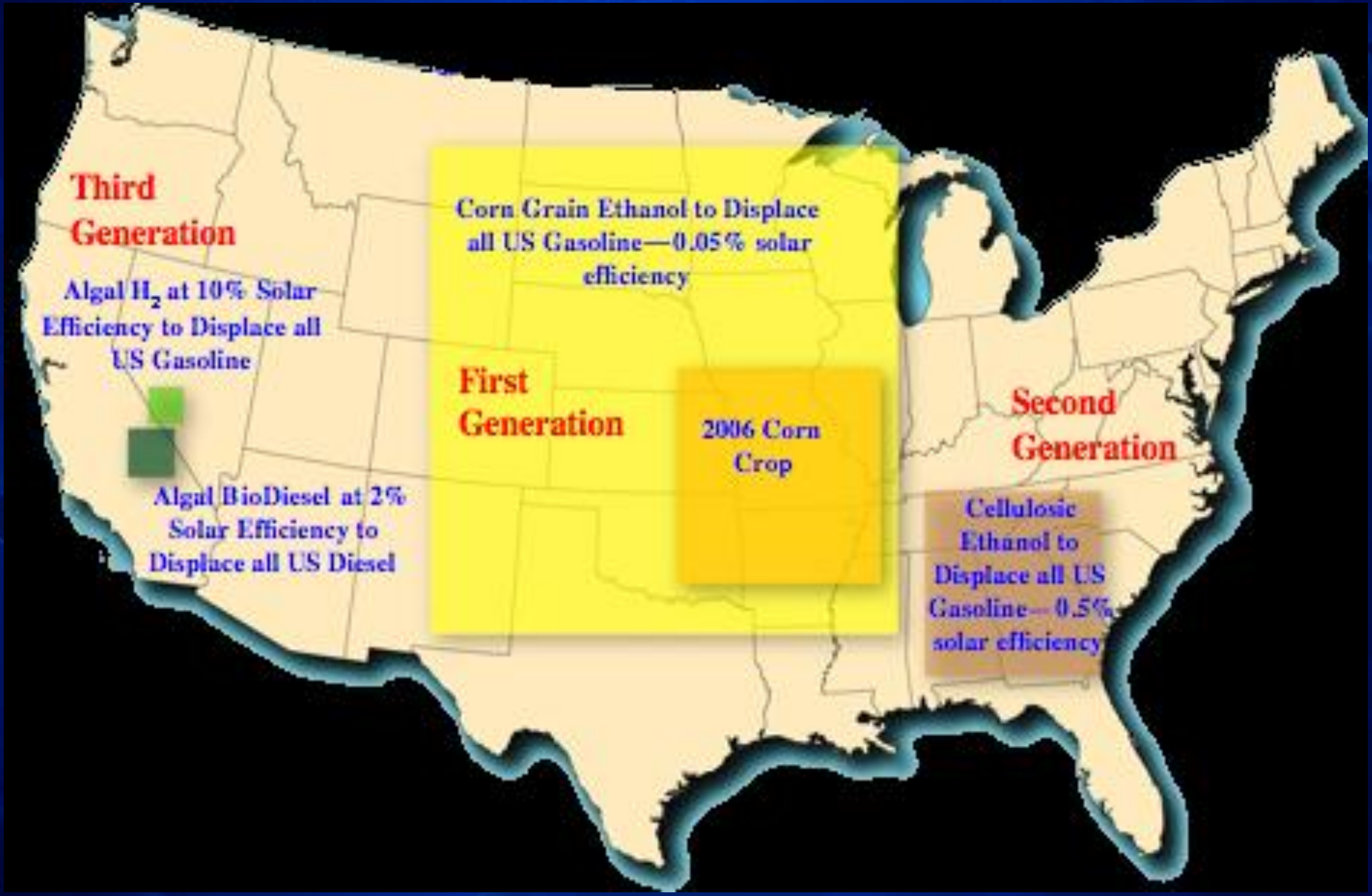
- Separation technology
  - Will use <energy than other approaches in use now
  - Will achieve >8% n-butanol concentration
    - At which point it will "phase separate" into 100% n-butanol
  - Will occur through state-of-the-art membrane filtration technology and pervaporation

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