"Bounded Opportunities: Physical, Biological, Material and Economic Constraints on Photobioreactor Design"

Peter Lammers
New Mexico State University

“Our mistake is not that we take our theories too seriously, but that we do not take them seriously enough”

-Steven Weinberg, Nobel Prize-winning Particle Physicist
A Very Short Story About Fuel Scales

Acres of algal production required to supply feedstock requirements for California refineries @ 3,500 gallons/acre/year

*We have a long way to go.....*

<table>
<thead>
<tr>
<th>California Refineries and Crude Oil Capacities</th>
<th>Barrels/day</th>
<th>barrels/year</th>
<th>gallons/year</th>
<th>acres require</th>
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<tbody>
<tr>
<td>BP West Coast Products LLC, Carson Refinery</td>
<td>240,000</td>
<td>87,600,000</td>
<td>3,579,200,000</td>
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<td>Chevron U.S.A. Inc., El Segundo Refinery</td>
<td>276,000</td>
<td>100,740,000</td>
<td>4,231,080,000</td>
<td>1,208,880</td>
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Roles for PBRs at all Scales

• We have an incomplete understanding of photosynthetic regulation in response to light, temperature and chemical variations

• We have only the vaguest understanding of aquatic ecology

Hence...

• Focus on PBR designs that enable discovery

• *Reject the idea that the opportunity for innovation is past...*
Reviews on PBRs

Background - 1

- Well designed PBRs allow >5-10 fold higher cell densities at harvest relative to open raceway systems.

- Cell density at harvest is a major factor for techno-economics and life cycle assessment
  - Lower harvest energy requirements are offset by capital expenses and scale-up limitations

- With good mixing, dense cultures in full sunlight have minimal photo-inhibition

- We do not fully understand why...PBR systems that mimic natural conditions are essential data systems to refine theory
Background - 2

- Commercial product specifications must determine cultivation system design specifications (*One size does not fit all*)
  - Fuel, wastewater treatment, renewable chemical feedstocks, high-value biochemicals, fish-meal replacements, single-cell protein
  - Obviously different economics will drive different choices

- PBR systems provide physical protection to stabilize cultures
  - Inoculum scale up is critical at large scales

Background - 3

- **PBR design requires interdisciplinary biology, chemistry & engineering teams**

**Three Examples from Current Collaborators**

- PBR-based raceway simulators to study emergent photosynthetic properties *(Huesemann, PNNL; Kramer, MSU)*

- Encapsulated Algae as Micro-PBRs to study ecological interactions *(Starkenburg, LANL)*

- Scalable closed PBR design driven by water efficiency concerns *(Lammers NMSU, Algenol, Sapphire)*

Track 1: Progress in Algal Biology 3:30-5:00 session
Early Exponential Growth (Inset) Gives Way to Linear Growth Due to Self Shading

Photosynthesis saturates at 300 µmole/m²-sec at low cell densities (~0.1 gAFDW/L)

A recent effort: What do all the chloroplast-associated genes do? Study system: Arabidopsis

What are the functions of chloroplast genes?

>3,500 genes (proteins) (5000 separate lines)

“Industrial phenotyping”

The astonishing result:

Lab phenotyping

>3,500 genes (proteins)

2%

Reproducible phenotypes

Photosynthesis, growth, amino acid composition, etc.

98%

No obvious phenotypes

Genes of Unknown Function (GUFs)

Courtesy of David Kramer, Michigan State U
What are the functions of genes (or residues) of unknown function?

Often called “Genetic redundancy”
A Darwinian Paradox:
If something is redundant, it is redundant;
If it is redundant, it will be lost over time.

Hypothesis: These genes are not redundant.
Prediction: They must be important under conditions that we don’t measure in the lab.

…and this is important for bioenergy because it is these functions which determine robustness, fitness and productivity in the field.

E.g. Nowak et al., 1997; Kafri, Springer & Pilpel. 2009
The Dynamics Problem

Plants and algae have evolved to cope with unpredictable, fluctuating environmental conditions, but we study them under ‘static’ controlled conditions

- OK for ‘reductionist’ experiments
- But, miss important regulatory factors

Kulheim et al., (2002) Science

Courtesy of David Kramer, Michigan State U
• If we do not get this right, we could spend huge time and money obtaining **precisely the wrong characteristics**.

Mutate something
Introduce a gene
Breed
Isolate...

**Beautiful!**

**Sucks.**

Different environments

Courtesy of David Kramer, Michigan State U
Approach: Next Generation Phenotyping (Phenometrics)

REP: Ramped Environmental Perturbations for photosynthetic organisms

High throughput, high density, non-invasive measurements of energy transduction, growth, etc.
Can we reveal distinct, emergent phenotypes?
>3,500 genes (proteins)

- Reproducible phenotypes
- No obvious phenotypes

2% 98%

Novel (emergent) More severe Similar Emergent No visible phenotype

Can we reveal Emergent Functions?
Can we use this approach to develop more efficient and robust algae and crops?
Light (µmol m⁻² s⁻¹)

Day 1  Day 2  Day 3

REP

Day 1
Day 2
Day 3

0  2000

minera-9  minira-11  minira-7  minira-6  minira-11  minira-3
minira-5  minira-9  minira-5  minira-6  minira-5
minira-6  minira-11  Ws2  minira-7  minira-11  minira-9
minira-7  Ws2  minira-3  minira-6  minira-9  Ws2
Ws2  minira-15  minira-16  minira-10  minira-15  minira-14
minira-10  minira-15  minira-12  Ws2  minira-16  minira-15
minira-12  Ws2  minira-15  minira-14  minira-10  Ws2
minira-10  minira-18  minira-10  minira-15  minira-12  Ws2
REP

Light (μ mol m⁻² s⁻¹)

Day 1
Day 2
Day 3

Novel “bipolar” responses
Just use fluctuating light as a REP, we obtain 10-fold higher phenotype “hit rate”. Adding other parameters (e.g. temperature) gives us even more.
Environmental Photobioreactor (ePBR)/Sensor Matrix

- Simulates incrementally conditions experienced in under field conditions
- High throughput mode (easy to use, inexpensive, etc.).
- Can measure key photosynthetic and growth parameters
Controlled mixing to simulate raceway or similar situations.

This is important:
Mixing tends to increase productivity (light? Nutrients?, CO$_2$, O$_2$?)
But introduces fluctuations in conditions, particularly light
Energetically costly. Should be balanced/optimized.
Complex effects of mixing/settling on photosynthesis

DECREASE at top

INCREASED at bottom
>3,500 genes (proteins)

Reproducible phenotypes

- 2%
- 98%

No obvious phenotypes

Novel (emergent)

- ~30%

More severe

- ~40%

Similar

- ~30%

Emergent

- >20%

<80%

No visible phenotype

Just use fluctuating light as a REP, we obtain 10-fold higher phenotype “hit rate”. Adding other parameters (e.g. temperature) gives us even more.
THE PHYCOSPHERE: Microbial community within an algal habitat

- Shawn R. Starkenburg, Armand E. Dichosa, Momo Vuyisch, and Pulak Nath. Los Alamos National Lab

- Development of a novel microfluidics enabled (micro) co-culturing platform to dissect complex microbiomes

- Simultaneous, high throughput screening and identification of species-to-species interactions

Production Ecology
Synthetic Ecology
Systems Ecology

Impacts
Isolate growth-promoting bacteria in an algal phycosphere
New insight into the mechanisms of endosymbiosis
Improve the productivity and increase the efficiency of an algal biofuel production system
Technology platform is widely applicable to interrogate other microbiomes and interactions: wastewater treatment, host-pathogen interactions
Bacterial community profile of algal cultures

Genera:
- Roseisalinus
- Phaeobacter
- Loktanella
- Ruegeria
- Roseivivax
- unciliated Rhodobacteraceae
- Porphyrobacter
- Alcanivorax
- Oceanicola
- Capnocytophaga
- Jannaschia
- Flavobacterium
- Rhodovulum
- Roseobacter
- Roseovarius
- Ketogulonicigenium
- Pannonibacter
- Rhizobiales
- Cytophaga
- Elizabethkingia
- Rhodopirellula
- Leeuwenhoekella
- Unclassified Proteobacteria

Abundance (%)

Tetraselmis Laboratory Culture
Nannochloropsis Production Culture
# Bacteria DNA in Algal Cultures

<table>
<thead>
<tr>
<th>Bacteria*</th>
<th>Plant/Algae Associated Function</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas fluorescens</em></td>
<td>antibiotics /anti-fungal properties [1]</td>
</tr>
<tr>
<td><em>Agrobacterium sp. H13-3</em></td>
<td>plant rhizosphere isolate [2]</td>
</tr>
<tr>
<td><em>Stenotrophomonas maltophilia</em></td>
<td>plant seed endophyte [3]</td>
</tr>
<tr>
<td><em>Pseudomonas putida</em></td>
<td>soil saprophyte/ antibiotic producer [4]</td>
</tr>
<tr>
<td><em>Enterobacter cloacae</em></td>
<td>Plant growth promoting/root colonizer [6]</td>
</tr>
<tr>
<td><em>Acinetobacter sp. ADP1</em></td>
<td>soil bacterium, chemoheterotroph</td>
</tr>
<tr>
<td><em>Pseudomonas mendocina</em></td>
<td>Soil bacterium/ alginate producer [7]</td>
</tr>
<tr>
<td><em>Pseudomonas protegens</em></td>
<td>plant protection bacteria [8]; <em>P. fluorescens</em> relative</td>
</tr>
<tr>
<td><em>Enterobacter asburiae</em></td>
<td>plant growth promoting rhizobacteria [9]</td>
</tr>
<tr>
<td><em>Pelagibacterium halotolerans</em></td>
<td>marine isolate [13]</td>
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<tr>
<td><em>Agrobacterium fabrum</em></td>
<td>plant pathogen relative [14]</td>
</tr>
<tr>
<td><em>Dinoroseobacter shibae</em></td>
<td>algal symbiont [15]</td>
</tr>
<tr>
<td><em>Enterobacter sp. 638</em></td>
<td>plant growth promoting endophyte [16]</td>
</tr>
</tbody>
</table>
1. Co-capture single algal and bacterial cells in GMDs
2. Encapsulate in defined media to generate MDs
3. Cultivate and FACS analyze algal growth
4. High-throughput FACS selection (chlorophyll, starch, lipids, DNA)
FACS Isolation of Bacterial Symbiont

1. Bacterial Identification
2. Genome Assemblies
3. Secondary Cultivation
4. Targeted Enrichment
5. Signature Detection Assays

Starkenburg, Dichosa, Vayisch, Nath
Pan evaporation rates in NM are 8-9 feet (2.75 m) per year

For large-scale systems the make up water requirements required fresh water (non-existent), brackish ground water or produced water, and/or adoption of hyper-saline growth conditions

Enclosed systems prevent evaporative water losses but experience passive solar heat gain

Red lines – $T_{opt}$ Galdieria sulphuraria
Green lines – $T_{opt}$ Chlorella sorokiniana
Requirements for Highly *Productive* and *Stable* Cultures
Identify Critical Biological Phenotypes

1. Heterotrophic phenotypes are highly desirable
   - Inoculum scale up is a major bottleneck for large-scale algae biotechnology
   - Heterotrophic growth allows inoculum production in fermenters yielding >100 g/L cell densities and ~1000-fold dilution into photoautotrophic systems (open or closed)
   - *Closed* cultures systems (PBRs) provide enhanced culture stability
   - Design systems that delay and/or minimize residence time in open raceway photoautotrophic conditions to minimize culture risk

2. Extremophile phenotypes are highly desirable
   - Extreme values of pH and/or temperature tolerance facilitate control over culture composition
Modular Bioreactors with Condensation Capturing

**Potential Benefits for Harvest/De-watering**

- Condensation rates of 2 Liters per square meter per day in New Mexico
- ~2% per day for 10 cm culture and 30% water recovery as condensate for 15 day batch culture
- 30% savings on harvest costs
Impact of Hydrothermal Biomass Processing on Cultivation Design Concepts

1. Compatible with a range of biochemical compositions – Liquefaction Bio-oil yields are 5-25% higher than lipid content, protein contributes to bio-oil formation
   Biller and Ross (2011) Potential yields and properties of oil from the hydrothermal liquefaction of microalgae with different biochemical content. Bioresource Technology 102: 215-225

2. Aqueous waste stream can be gasified and combusted for electricity and CO2 recycling

3. Nutrient re-capture from HTL water extractives (C & N) and solids (P) transforms scale expectations from algal-based waste-water treatment

4. Sequential HTT extracts starch prior to liquefaction
   Chakraborty M. et al. (2012) Concomitant extraction of bio-oil and value added polysaccharides from Chlorella sorokiniana using a unique sequential hydrothermal extraction technology. Fuel 95: 63-70
Acknowledgements

Funding:

- DoE - EERE
- National Alliance for Advanced Biofuels & Bioproducts
- RAFT – UA/NMSU/PNNL/Texas & AM
- REAP – NMSU, WSU, LANL + NMC & MSU, Algenol/Pan Pacific
- Air Force Research Laboratory (Wright Patterson)
- National Science Foundation (NM-EPSCoR RII4)
- Office of the Vice President for Research – NMSU

PBR Collaborators

- Algenol Biofuels
- Sapphire Biofuels
- Solix Biosystems
## Acknowledgements - 2

**The Team at NMSU**

### GOAL

The Phase 1 goal of the REAP project is to improve Algal Biomass Yield Improvements to produce 3,500 gallons of bio-crude oil per acre per year from algae feedstock within 30 months.

### NMSU PARTICIPANTS AND TASKS

<table>
<thead>
<tr>
<th>Cultivation and Harvesting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wayne Van Voorhies, Peter Lammers, Nick Csakan</td>
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<td><strong>Molecular Biology/VPR Office</strong></td>
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<th>Production System Ecology</th>
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<tr>
<td>Wiebke Boening</td>
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<td><strong>Fisheries and Wildlife</strong></td>
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<td>N. Nirmalakhandan</td>
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<tr>
<td><strong>Civil Engineering</strong></td>
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<td>Omar Holguin</td>
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<td><strong>Plant and Environmental Science</strong></td>
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</tbody>
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<th>Biomass Conversion to BioCrude Oil</th>
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<td>Shuguang Deng</td>
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<tr>
<td><strong>Chemical Engineering</strong></td>
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