DOE Bioenergy Technologies Office (BETO)
2015 Project Peer Review

Managing the Microbial Ecology of a Cyanobacteria-Based Photosynthetic Factory Direct!

Bruce E. Rittmann, Director
Swette Center for Environmental Biotechnology
Biodesign Institute at Arizona State University

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Algae Technology Area Review

Principal Investigator
Organization

This presentation does not contain any proprietary, confidential, or otherwise restricted information

Goal Statement

- Understand the factors controlling the productivity of a photobioreactor (PBR) that uses laurate-excreting *Synechocystis* in a Photosynthetic Factory Direct!
  - What controls laurate production by *Synechocystis*?
  - What controls laurate biodegradation by heterotrophic bacteria?
  - What strategies are needed to maximize net production?
- This work will lead to means to increase productivity and lower costs for microalgae-based biofuels.

Quad Chart Overview

Timeline

- December 1, 2012
- November 30, 2014
- 100% complete

Barriers

- Barriers addressed
  - Low product yield
  - Poor crop protection
  - High product cost

Budget

	Total Costs FY 10 – FY 12	FY 13 Costs	FY 14 Costs	Total Planned Funding (FY 15-Project End Date
DOE Funded	00	218,037	250,597	27,965
Project Cost Share (Comp.)*	00	55,699	59,886	10,540

Partners

- Partners
  - None

1 - Project Overview

- History
  - Originally a proposal submitted to NSF EFRI
  - Selected by DoE BETO for funding outside the NSF program
- Technical Direction
  - Enable the “Photosynthetic Factory Direct!”
  - Genetically modified *Synechocysistis* sp. PCC6803 excretes laurate, a jet fuel precursor
  - We want to maximize the net laurate productivity by:
    - Understanding photosynthetic productivity by *Synechocystis*
    - Understanding heterotrophic bacteria that biodegrade laurate
    - Testing systems to maximize laurate capture and minimize it biodegradation

2 – Approach (Technical)

1. Fundamental studies on what wild-type and modified *Synechocystis* produce by photosynthesis
   - Organic-C partitioning – biomass, SMP, laurate
   - Kinetic control – light, nutrients, pH

2. Identification and characterization of prevalent strains of heterotrophic bacteria in S*ynechocystis*-based PBRs
   - Phylogenetic diversity
   - Metabolic capability
   - Kinetics

3. Practical PBR strategy to minimize heterotrophs and improve laurate harvest

2 – Approach (Management)

- Study carried out totally within the PBR team of the Swette Center for Environmental Biotechnology

- PI – Bruce Rittmann
- Co-PI – Rosa Krajmalnik-Brown
- PhD Students – Alexander Zevin and Binh Nguyen
- Research Technologist – Megha Patel

- Two-year project, ended officially on Nov. 30, 2014.

3 – Technical Accomplishments / Results

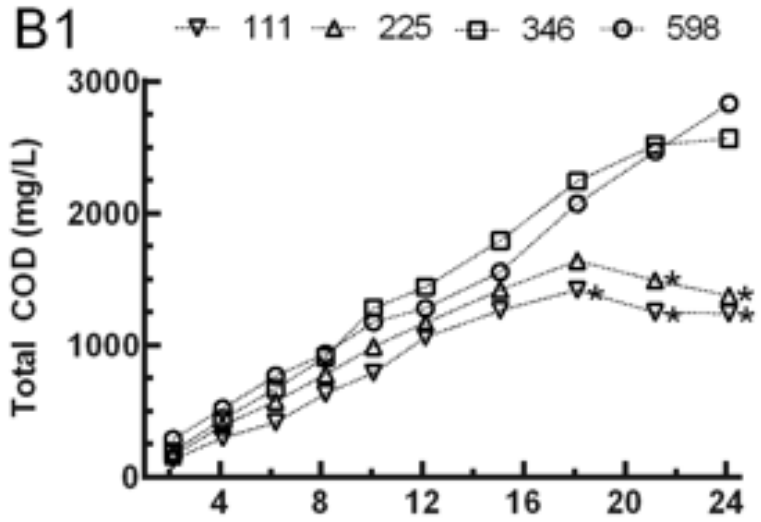
Due to time limitations, I present highlights from the project

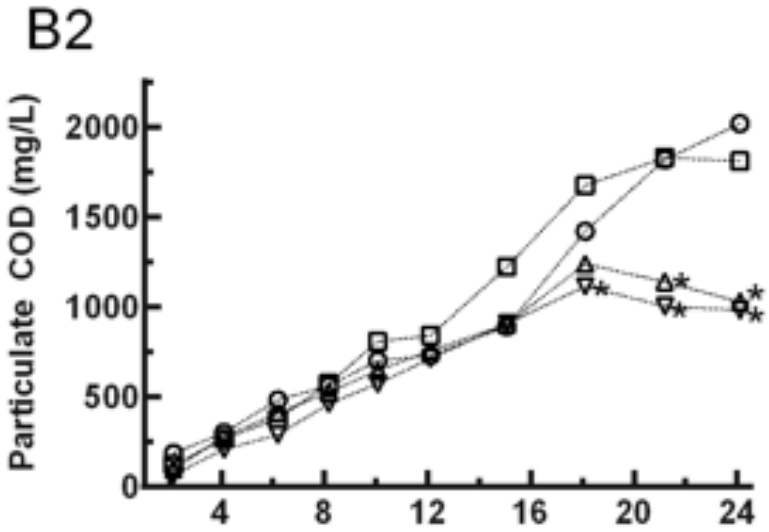
This is ~ 20% of what we did.

Take-home lessons are in blue.

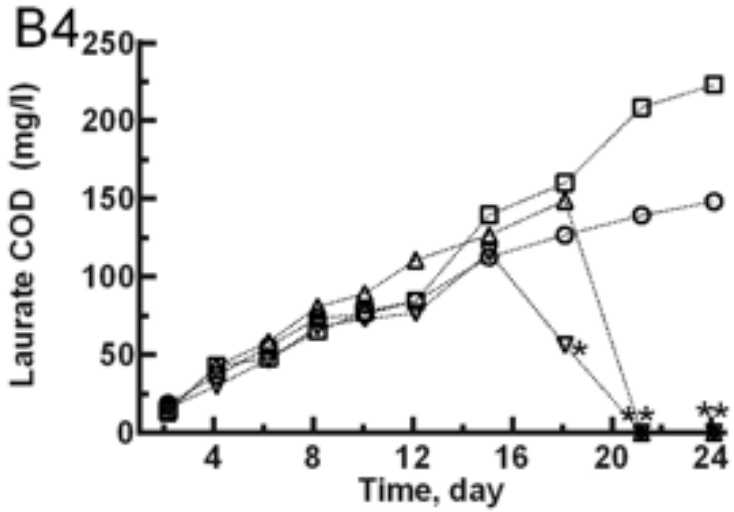
1. *Synechocystis*

Laurate Production by Modified *Synechocystis*



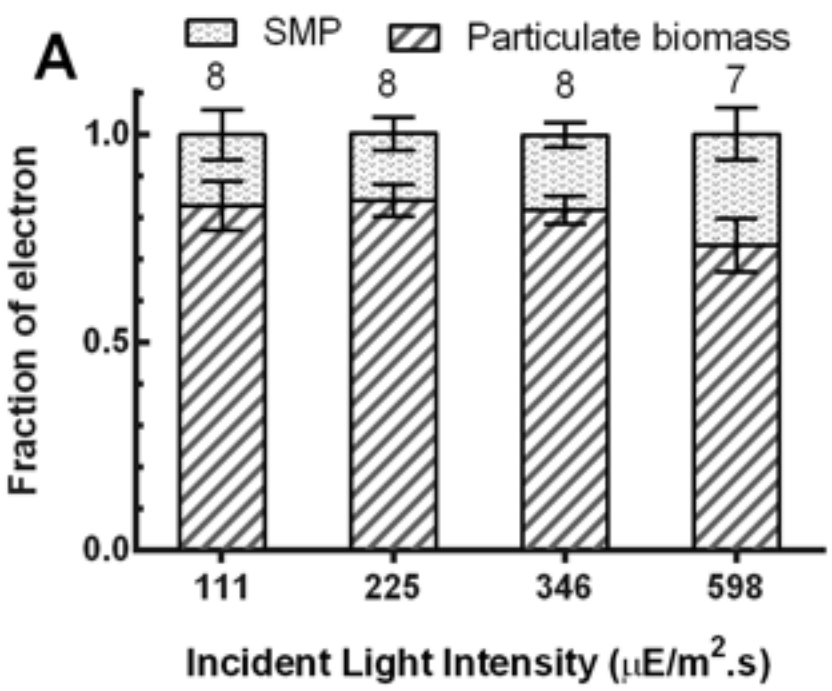


- Laurate production is proportional to biomass (PCOD) growth.
- Laurate is produced at less than 10% of PCOD production.
- Two batch runs showed biodegradation of laurate.

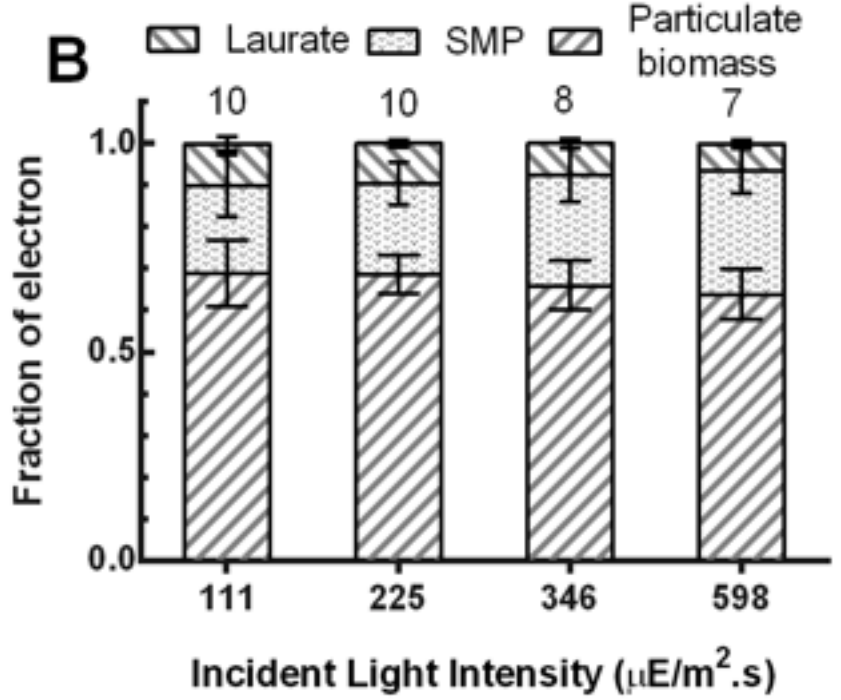


What does *Synechocystis* produce?

Wild-Type



Laurate-Excreting



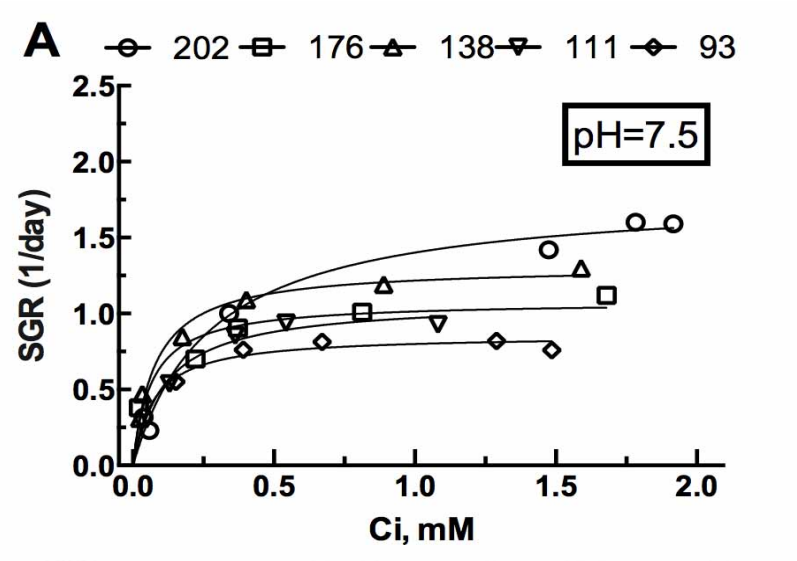
Both strains release at least 20% soluble microbial products (SMP), which is not a useful product.

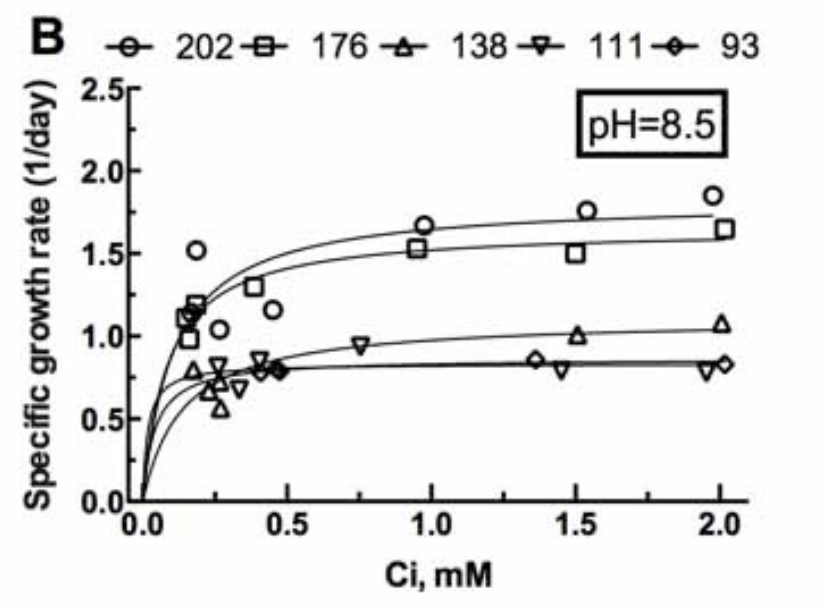
Laurate-excreting strain releases more SMP and ≤ 10% laurate.

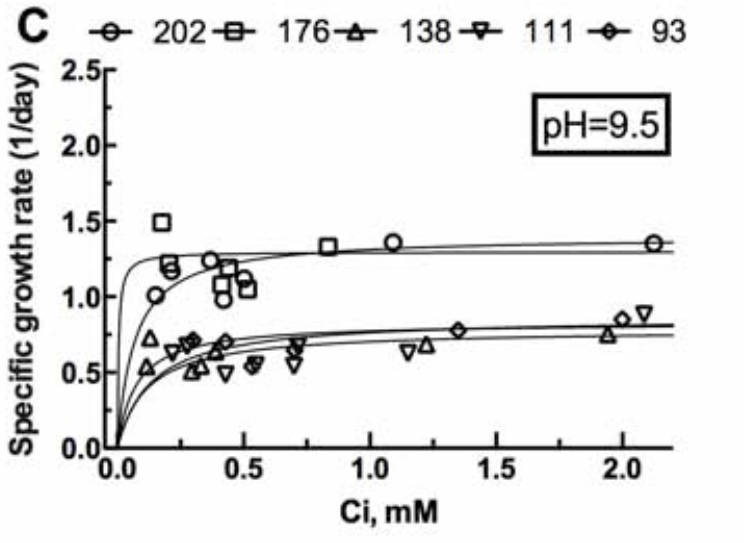
High incident light intensity shifts partitioning to SMP and away fr0m laurate.

Therefore, moderate LI and get *Synechocystis* to produce less SMP!

pH and Inorganic C



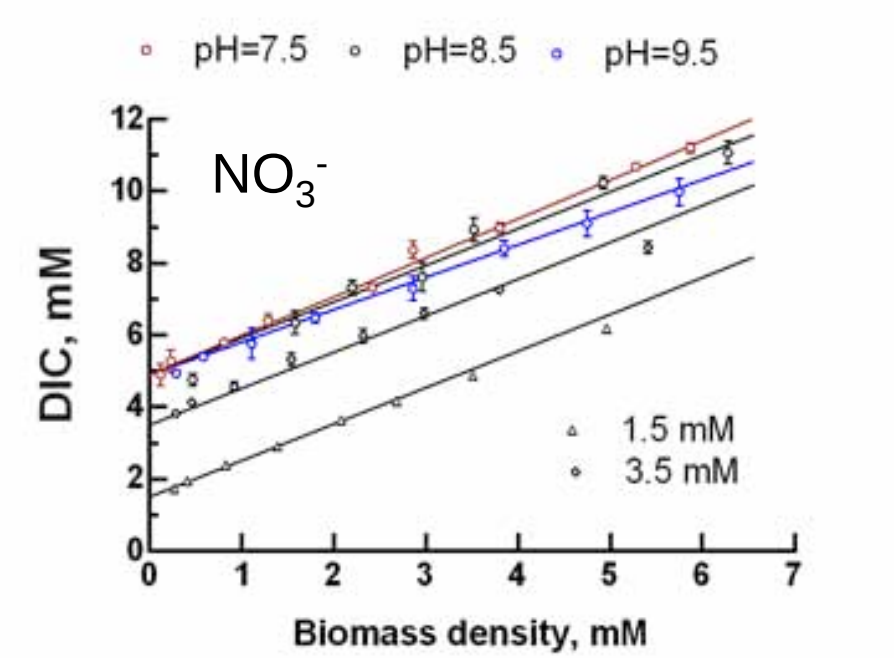


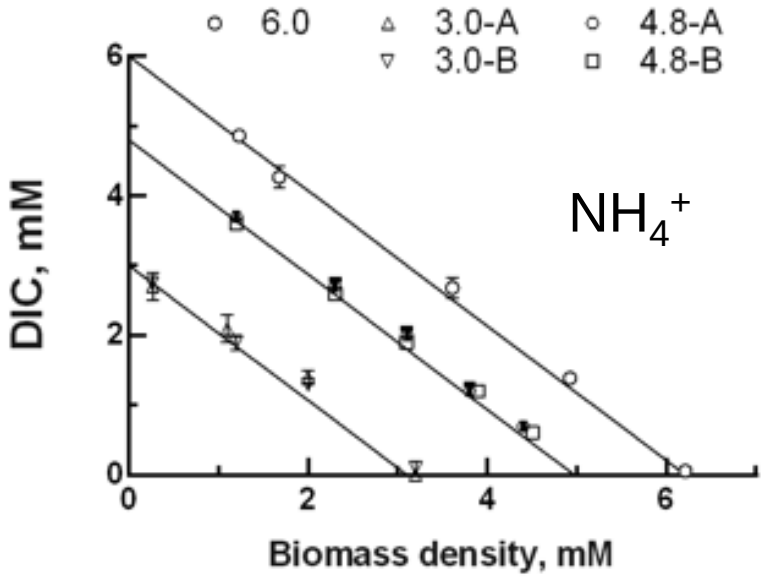


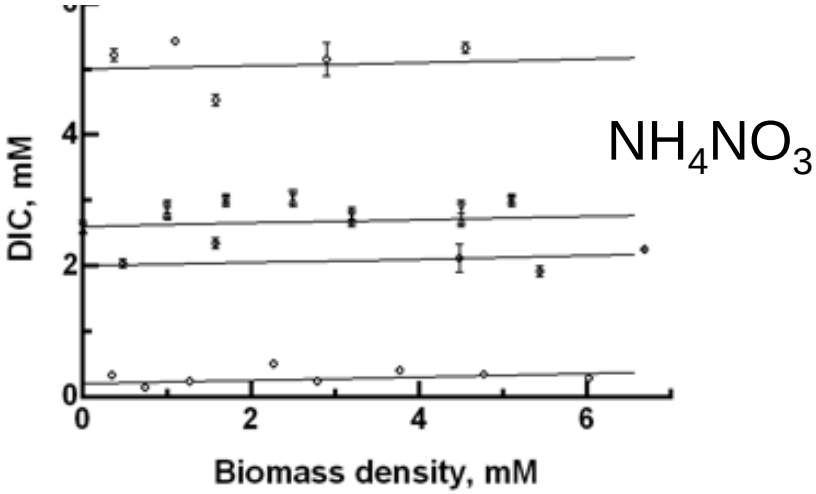
For the laurate-excreting strain, the optimal pH is 8.5, and C_i ≤~0.2 mM (= 2.4 mgC/L) seriously slows photosynthesis.

No need to have very high C_i, but pH control is of true value. C_i ~ 1 mM = 12 mgC/L and pH of 8 – 9 are good.

Controlling pH and C_i





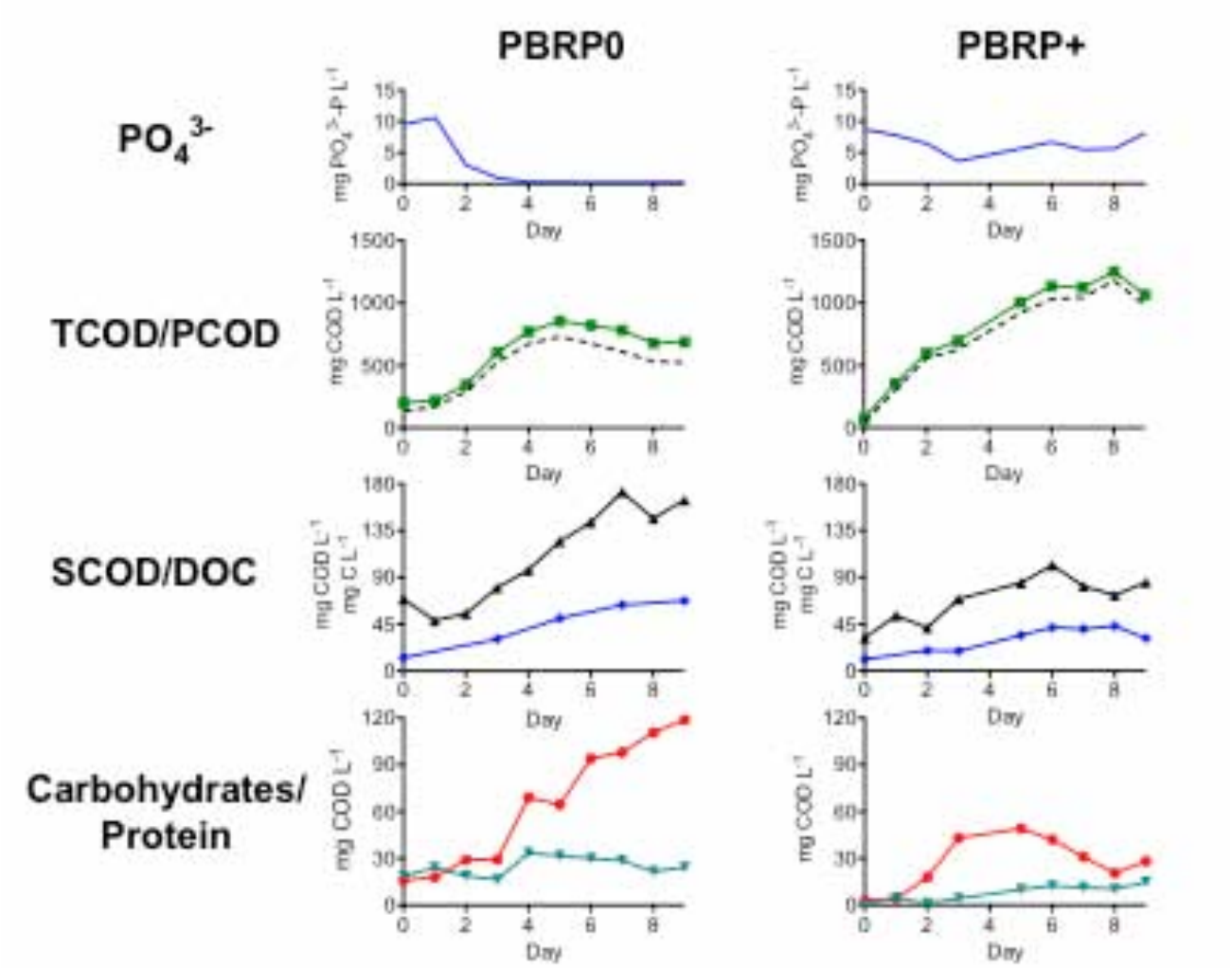


We developed and verified a model that shows that C_i is controlled by the N source in a pH-stat.

Using NO_3^- causes Ci to rise with photosynthesis.

Only NH_4NO_3 holds a constant C_i.

Phosphate Limitation

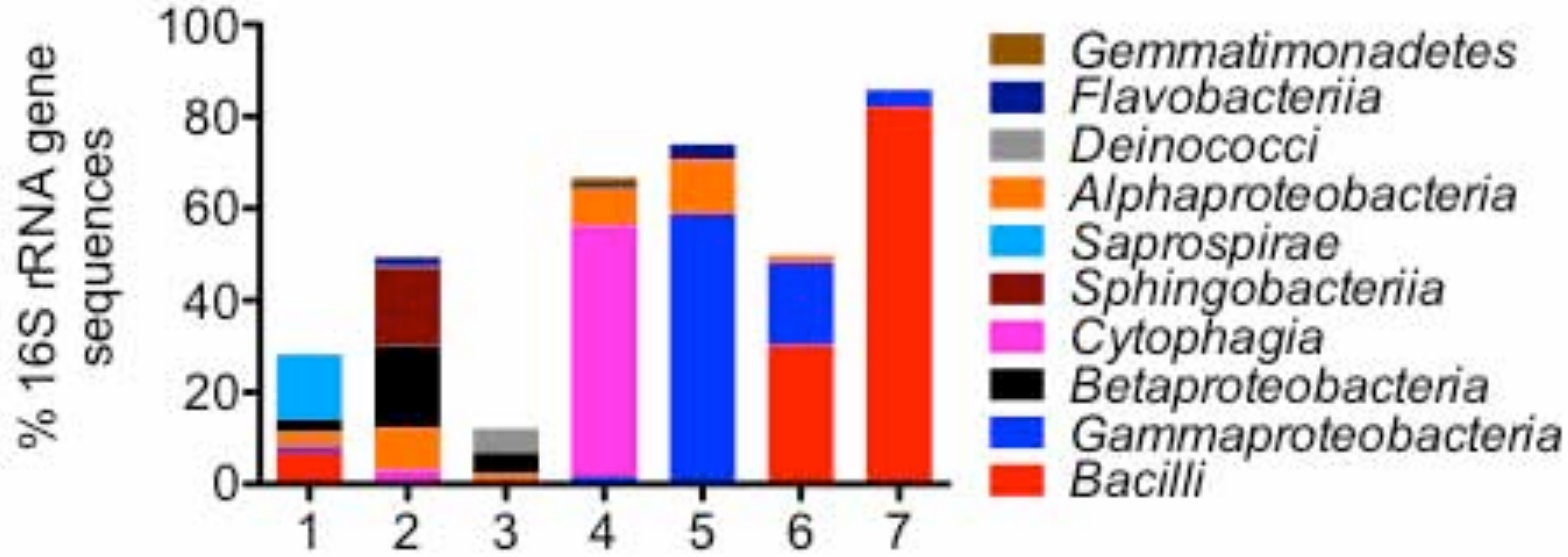


Depleting phosphate (PBR0) shows dramatic loss of biomass growth (PCOD) and increase of SMP (SCOD) and carbohydrates.

Avoid prolonged P depletion!

2. Heterotrophs

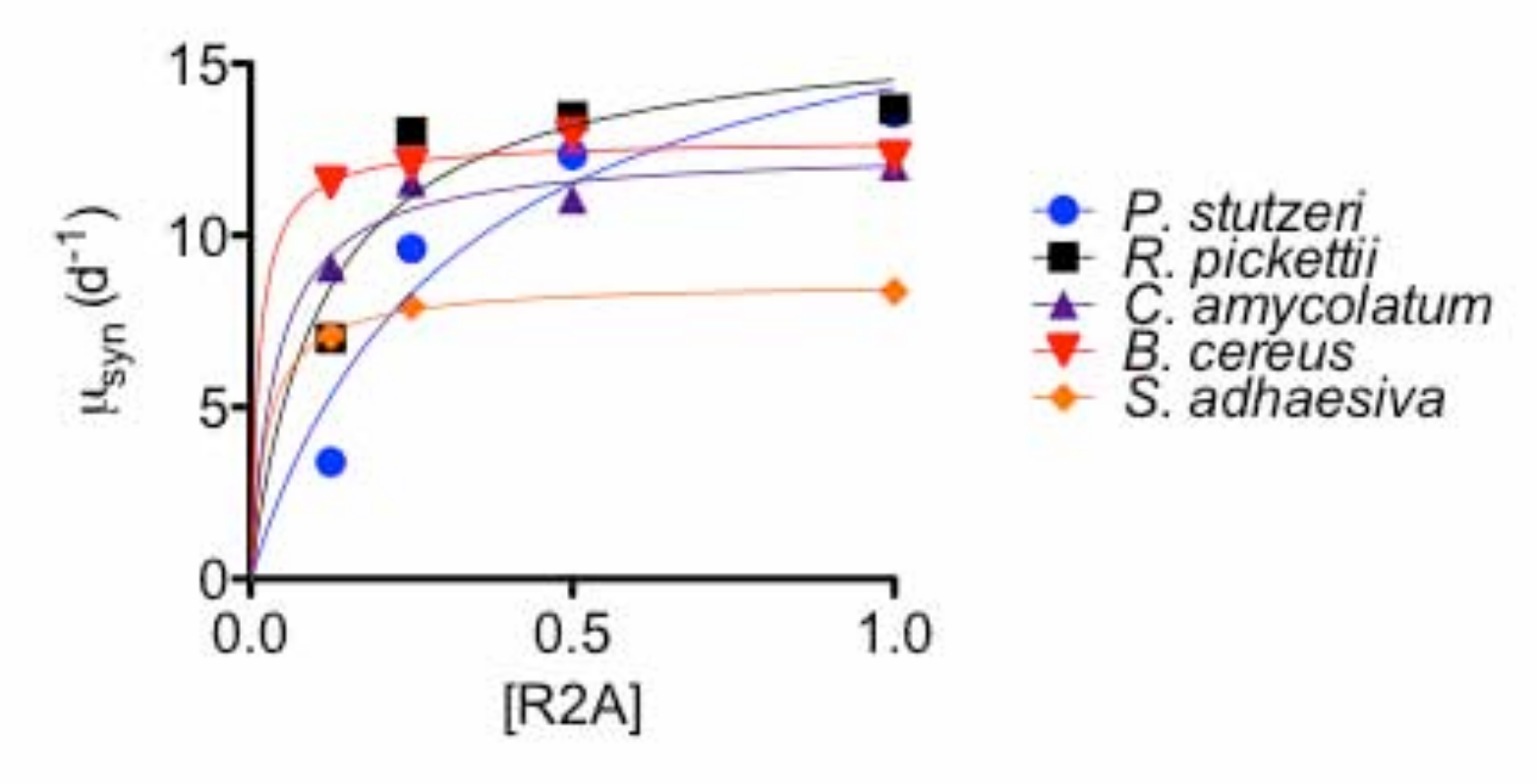
Heterotrophs in PBRs



Phylogenetic distribution at the class level of heterotrophic bacteria in seven *Synechocystis*-based PBRs by Illumina high-throughput sequencing.

Great diversity means that the ability to live on SMP from *Synechocystis* is widespread.

Heterotrophic Catabolism



Five PBR isolates grew rapidly on the complex medium R2A. This is a reason why they survive well in PBRs.

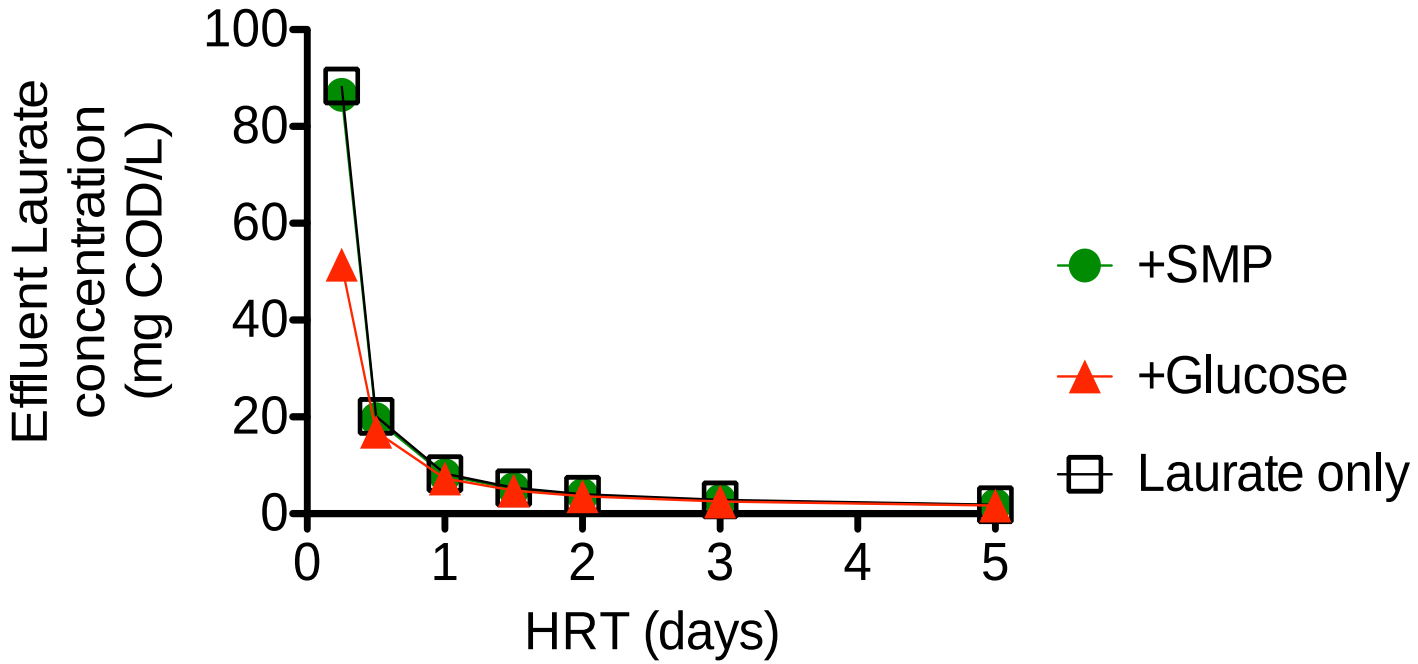
Heterotroph Catabolism Kinetics

Table C1. Kinetic constants for growth of B2 and *ENR1* on acetate, laurate, and glucose.

		Acetate/Laurate		**Glucose**		**SMP**
Constant	**Units**	***P. stutzeri***	***1-Ac***	***P. stutzeri***	***1-Ac***	***1-Ac***
Y	mg COD/mg COD	0.51/0.5	0.37/0.52	0.52	0.51	0.3
K	mg COD/L	2.4/8.8	18.8/34.5	44.06	0.17	187.3
μ_{max}	1/d	6.0/8.8	7.2/5.7	7.3	6.5	1.7
q_{max}	mg COD/mg COD*d	11.8/17.6	19.5/11.0	13.9	12.7	5.7
S_{min}	mg COD/L	0.04/0.1	0.7/0.62	0.62	0.003	11.7
$[\vartheta_x^{min}]_{lim}$	d	0.17/0.11	0.38/0.18	0.14	0.16	0.63
K_{Pi}	mg P/L	0.045	0.18			

Isolated strains consumed rapidly and grew well on acetate, laurate, glucose, and SMP harvested from PBRs. SMP gave slowest growth.

Hard to Wash Out Heterotrophs



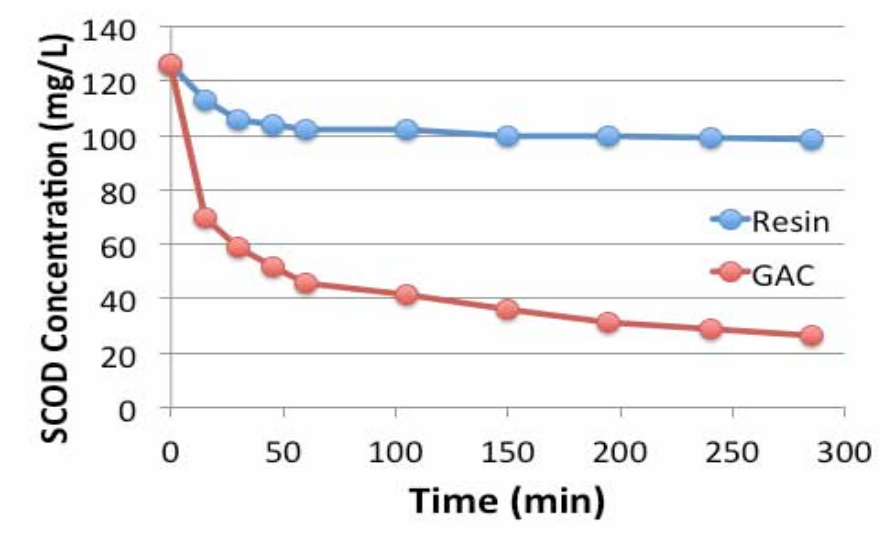
Modeled effluent laurate concentrations for a PBR with an initial laurate concentration of 100 mg COD/L and with either 10 mg COD/L SMP or 10 mg COD/L glucose.

Only a very short HRT, which means a very fast specific growth rate, may be able to wash out heterotrophs and preserve laurate.

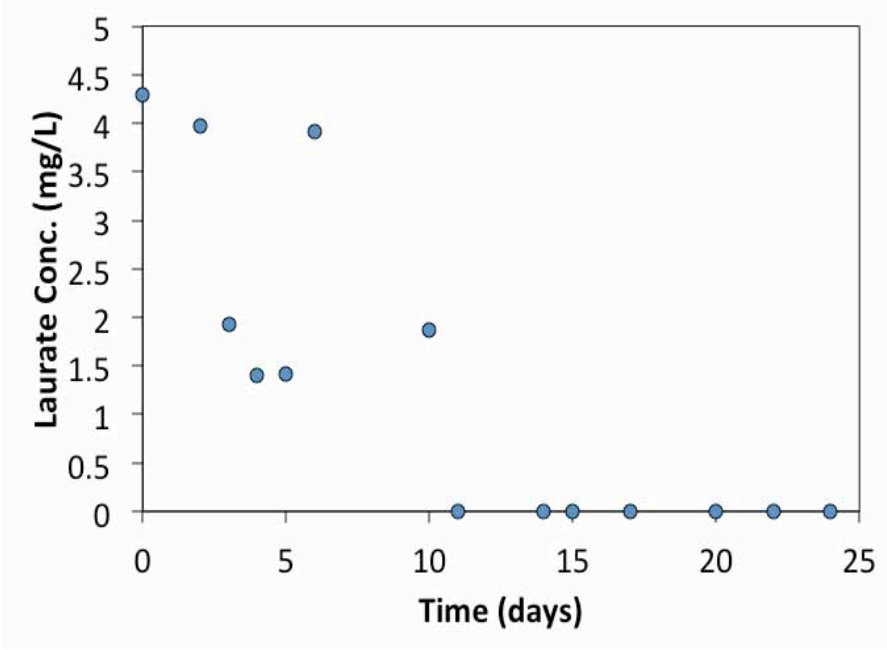
We need a PBR system able to give fast growth of *Synechocystis*. This goes well with the fact that laurate production is proportional to growth!

3. PBR System

SMP Removal and Laurate Harvest



Kinetics of SMP adsorption from membrane permeate of wild-type *Synechocystis* on MP-64 resin and F-300 GAC.



Laurate concentration in the effluent for the PBR with the resin and GAC columns throughout sequencing-batch operation.

A tandem of MP-64 ion-exchange resin (for laurate harvest) and F-300 GAC (for SMP adsorption) could forestall heterotrophic biodegradation.

Need to link this with a PBR system that allows a very fast specific growth rate and laurate-production rate.

4 and 5 – Summary and Relevance

- Laurate-excreting *Synechocystis* generates laurate in proportion to its growth rate. We want a fast specific growth rate.
- Synechocystis partitions more fixed C to SMP than to laurate, especially for high light intensity. We want to modify *Synechocystis* to release less SMP and to use a moderate light intensity.
- *Synechocystis* does not need a high C_i (<12 mgC/L), but it needs a pH around 8 – 9 and no P depletion. Control pH and use NH_4NO_3.
- *Synechocystis*-dominated PBRs have a wide diversity of heterotrophic bacteria.
- Many heterotrophic bacteria are able to consume complex organic materials, such as in SMP.
- Harvest of laurate onto ion-exchange resin and adsorption of SMP by GAC can forestall the onset of heterotrophic biodegradation of laurate.
- Long-term success will require a PBR able to sustain high specific growth rates (to wash out heterotrophs) and possibly modifications to *Synechocystis* so that it produces more laurate and less SMP.

Additional Slides

Publications, Patents, Presentations, Awards, and Commercialization

Publications to date (more expected)

- Nguyen, B., Rittmann, B.E. Electron partitioning into soluble organic products by wild-type and modified *Synechocystis* sp. PCC 6803. Submitted to *Algal Research.*
- Nguyen, B., Rittmann, B.E. Controlling dissolved inorganic carbon in photoautotrophic microalgae culture via the nitrogen source. Submitted to *Algal Research*
- Nguyen, B., Rittmann, B.E. Effects of inorganic carbon and pH on growth kinetics of *Synechocystis* sp. PCC 6803. Submitted to *Algal Research*
- Zevin, A.S., Masters,D.P., Rittmann, B.E., Krajmalnik-Brown, R Phylogenetic characterization of heterotrophic bacteria isolated and enriched from PBR cultures of *Synechocystis* sp. PCC6803. Submitted to *Appl. Environ. Microb*.
- Zevin, A.S., Rittmann, B.E., Krajmalnik-Brown, R. The inoculum source has a strong influence on microbial community structure in *Synechocystis*-based PBRs. Submitted to *Algal Res..*
- Zevin, A.S., Nam, T.G., Rittmann, B.E., Krajmalnik-Brown, R. Effects of phosphate limitation on soluble microbial products and microbial community structure in semi-continuous *Synechocystis*-based photobioreactors. Submitted to *Biotechnology and Bioengineering*