

The logo for ABPDU features the letters 'AB' and 'PDU' in a bold, sans-serif font. A stylized, multi-colored flame or leaf-like graphic is positioned between the 'B' and 'P', with colors transitioning from orange to green.

**ADVANCED BIOFUELS  
AND BIOPRODUCTS**  
PROCESS DEVELOPMENT UNIT

March 6, 2019

ADO Program

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# Goals / Objectives of the ABPDU team

**The ABPDU will strongly enable and support the commercialization of industry-, academic- and DOE-driven biofuels and bio-products by providing a key technical resource and an agile, flexible team for process development and demonstration.**

**The project aims to have at least one industry sponsor per year commercially launch a biofuel / bio-product and secure private funding based on data generated at the ABPDU.**

# Quad Chart Overview – ABPDU Operations

## WBS # 2.6.1.101

### Timeline

- Project start date: FY2019
- Project end date: FY2021
- Percent complete: ~15%

Project was most recently merit-reviewed in FY18 for FY19 renewal / start. Anticipate next merit review for FY22 renewal.

### Budget

	Total Costs Pre-FY17	FY 17 Costs	FY 18 Costs	Total Planned Funding FY 19-21
DOE Funds*	\$37 M	\$2.5 M	\$2.5 M	\$7.5 M (\$2.5 M per year)

\*Includes \$17M in ARRA funding (2009)

### Barriers addressed

- ADO-A. Process Integration
- ADO-D. Technology Uncertainty of Integration and Scaling
- Ct-D. Advanced Bioprocess Development
- Ct-H. Gas Fermentation Development
- Ct-K. Developing Methods for Bioproduct Production
- Ct-L. Decreasing Development Time for Industrially Relevant Microorganisms

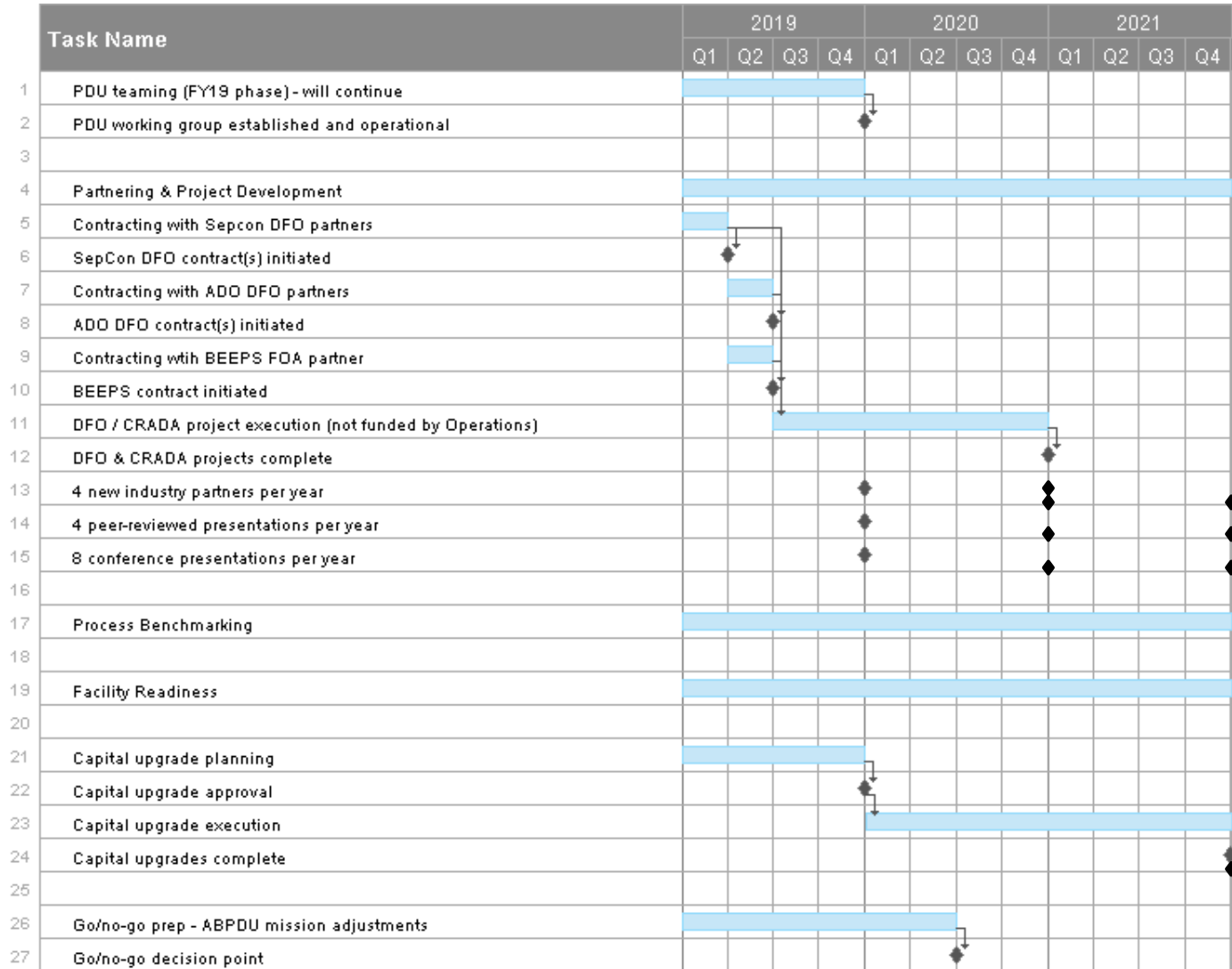
### Partners

- 41 Industry partners over FY14-19 to-date, including 33 US small businesses
- Formal AOP Lab partnerships as part of Agile BioFoundry, Separations Consortium, co-OPTIMA, Feedstock Conversion Interface, and FCTO BioH2 consortium projects

# Project budget overview

FY19 Tasks	Est. cost	Comments
Facility Readiness	\$1,250K	
Partnering and project development	\$750K	
Process Benchmarking and prototyping	\$400K	
PDU Teaming with other National Labs	\$100K	
<b>Total</b>	<b>\$2,500K</b>	<b>Q1 spending variance less than 4%</b>

# Project milestones and timeline



# 1 – Project Overview

# ABPDU enabling biofuel & bioproduct commercialization, verification and scaling

- 15,000 square foot Development & Demonstration Lab established by American Recovery and Reinvestment Act funds in 2009
- Closely engaged with DOE's EERE Bioenergy Technologies Office (BETO)



- A bio-process research incubator / accelerator – streamlined contracting, full cost recovery project fee structure, experienced team, and Bay Area location

# Broad product, technology and collaborator base

## Biofuels & biomass

## Materials & chemicals

## Food & health

## Environment & Ag

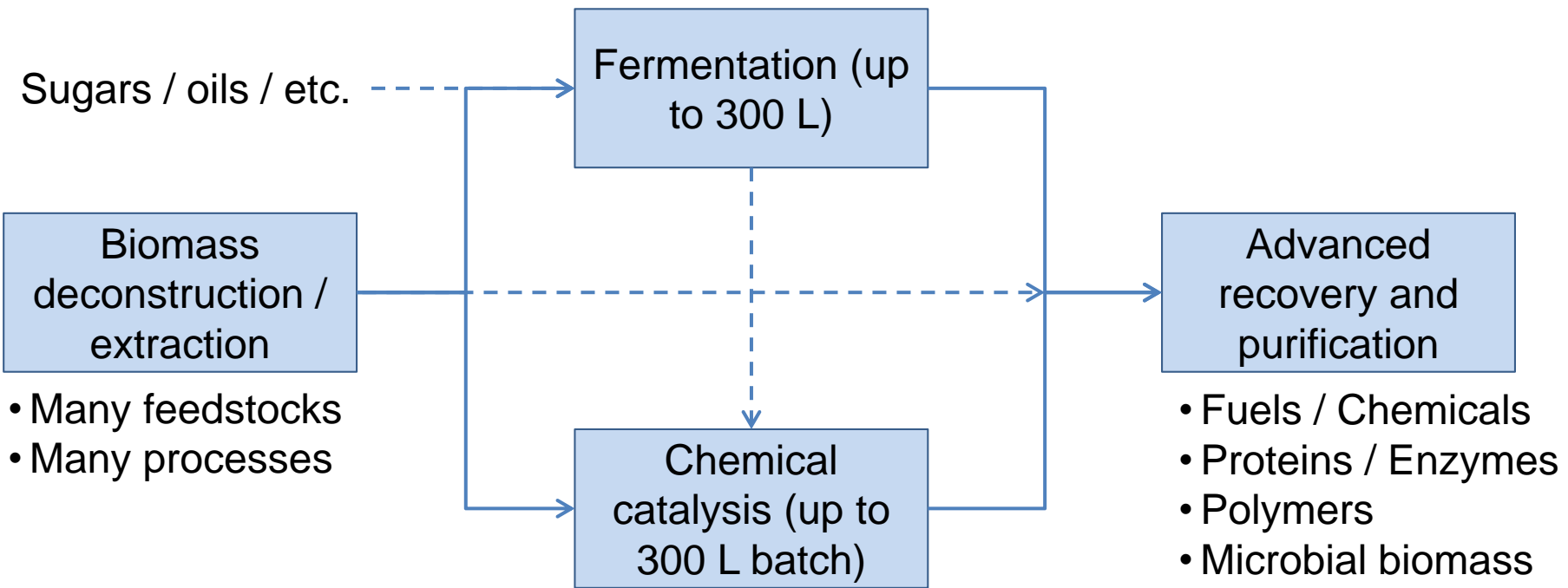
*New since last peer review*



## 2 – Technical approach

# Bench-scale & pre-pilot prototyping, benchmarking and cross-training prior to partner process execution

- Process verification, integration and techno-economic analysis across varied bio-process configurations, feedstocks and products



- Can focus on individual unit operations or several processes in succession

# The “why” - addressing gaps with our partners moving from TRL 2-3 into the TRL 4-6 range

- **Transition from lab- to bench-scale bioreactor process development**
  - Cell bank and seed train
  - Media formulation
  - Technology transfer – inbound and outbound
- **Analytical method implementation**
  - Sample and data sharing, troubleshooting, method development
- **Pilot-scale biomass handling, fermentation, & downstream processing**
  - Primary recovery and separations (S/L, L/L, S/L/L, volatiles)
  - Purification
  - Chemical finishing and polishing
  - Packaging and stability
- **Definition of target product profile, QA/QC specs, and TEA**

# Facility at a glance – from bench-to-pilot scale



## 2 – Management approach

# Project development in close concert with IPO / SPO / OCFO



**ABPDU Mission:**  
Partner with researchers from industry, the National Labs, and academia to optimize and scale technologies for bio-based chemicals / materials / fuels commercialization.

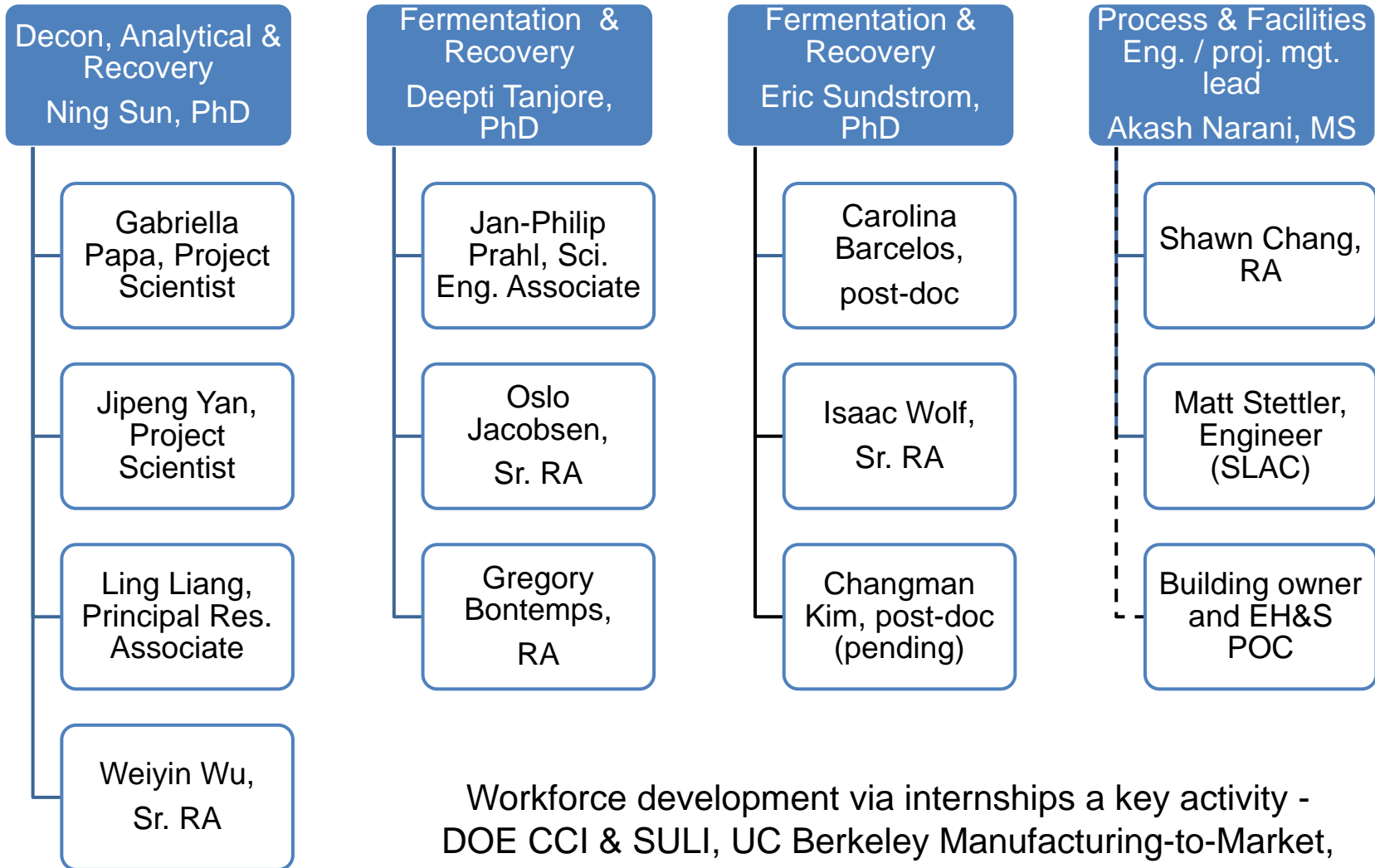


Strategic Partnerships Office



- Program Head regularly attends BETO quarterly meetings, PDU working group, BETO consortium team meetings and listening days
- Close monitoring of budget and milestones ABPDU SPP and CRADA projects with industry partners
- Weekly core team meeting and all-hands staff meetings
- Program Head is member of Biological Systems & Engineering Division leadership team and the Biosciences Executive Committee at LBNL.
- Utilization / occupancy tracked monthly and reported quarterly to BETO in narrative progress reports

# Multi-disciplinary and cross-trained team



Workforce development via internships a key activity - DOE CCI & SULI, UC Berkeley Manufacturing-to-Market, Biotech Partners, etc.



## 3 – Technical Accomplishments/ Progress/Results

# Critical upgrades for fermentation and downstream processing



Ambr250 HT automated bioprocess dev't system



Custom vacuum system for Andritz pretreatment & extraction vessel



TestUnit M20 (RO, NF, UF, MF)

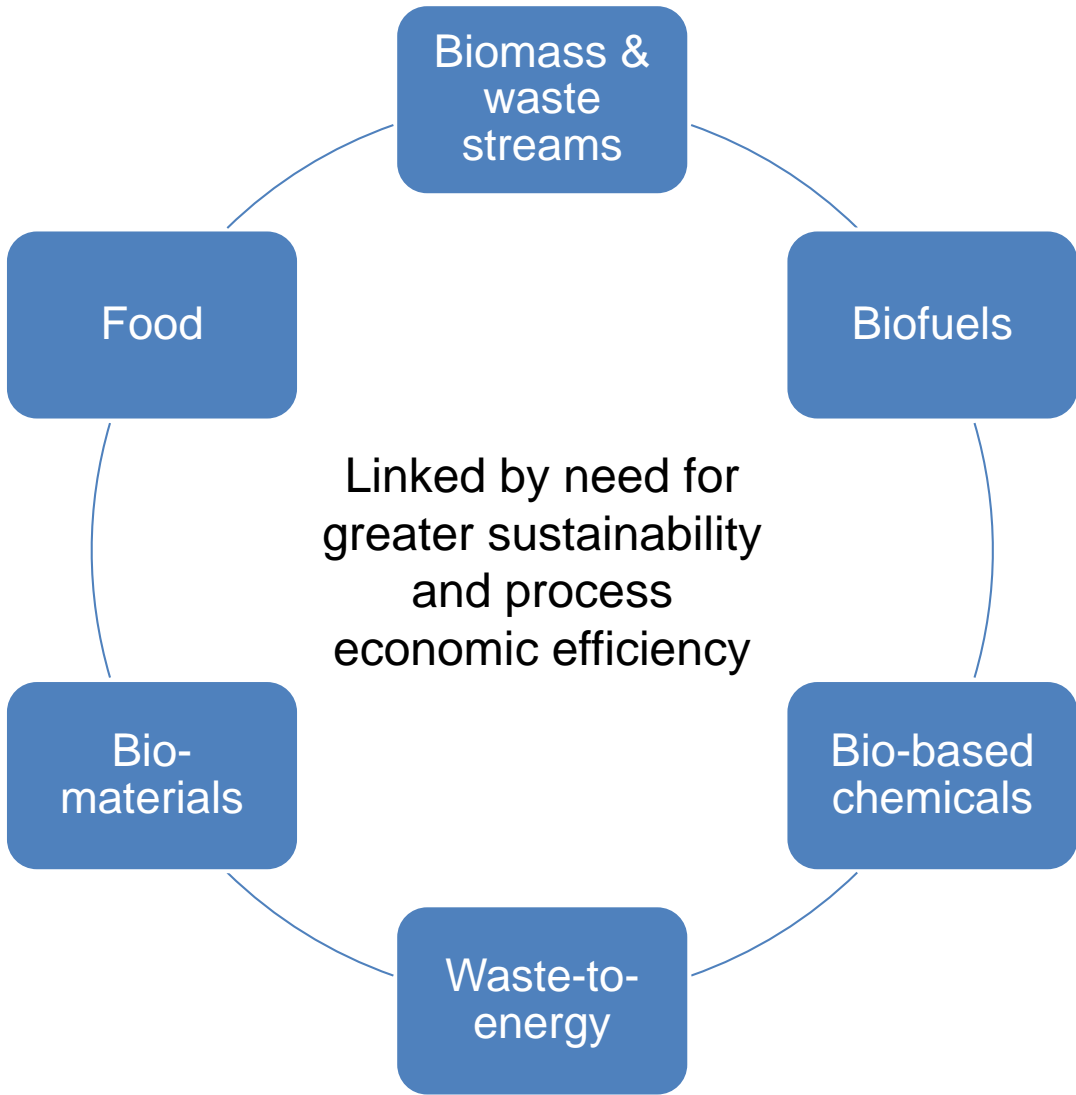


# Key outcomes and success factors

- 3 commercial product launches announced by our partners
- Over 20 different microbial hosts prototyped at different bioreactor scales from 2 L to 300 L – **rigorous biosafety review for each**
  - Canonical industrial hosts
  - Novel organisms for bioprocess utilization
  - Single and consortium cultures
- Over 40 different molecules and products tested – **rigorous process hazard analysis before every project**

## 4 - Relevance

# Driving innovation across several sectors of bioeconomy



# Working across wide array of BETO & Federal programs

Collaborations with other Labs and PDUs for process development & benchmarking



Mission-relevant industry projects from other agencies via subaward funding



Strong ties and project activities working with different DOE offices



# Key outcomes for private sector collaborators

- Numerous partners have set up their own labs or pilot plants and secured private financing while / after working with ABPDU
- Product launches with commercial / pre-commercial scale-up & scale-down

**Geltor**

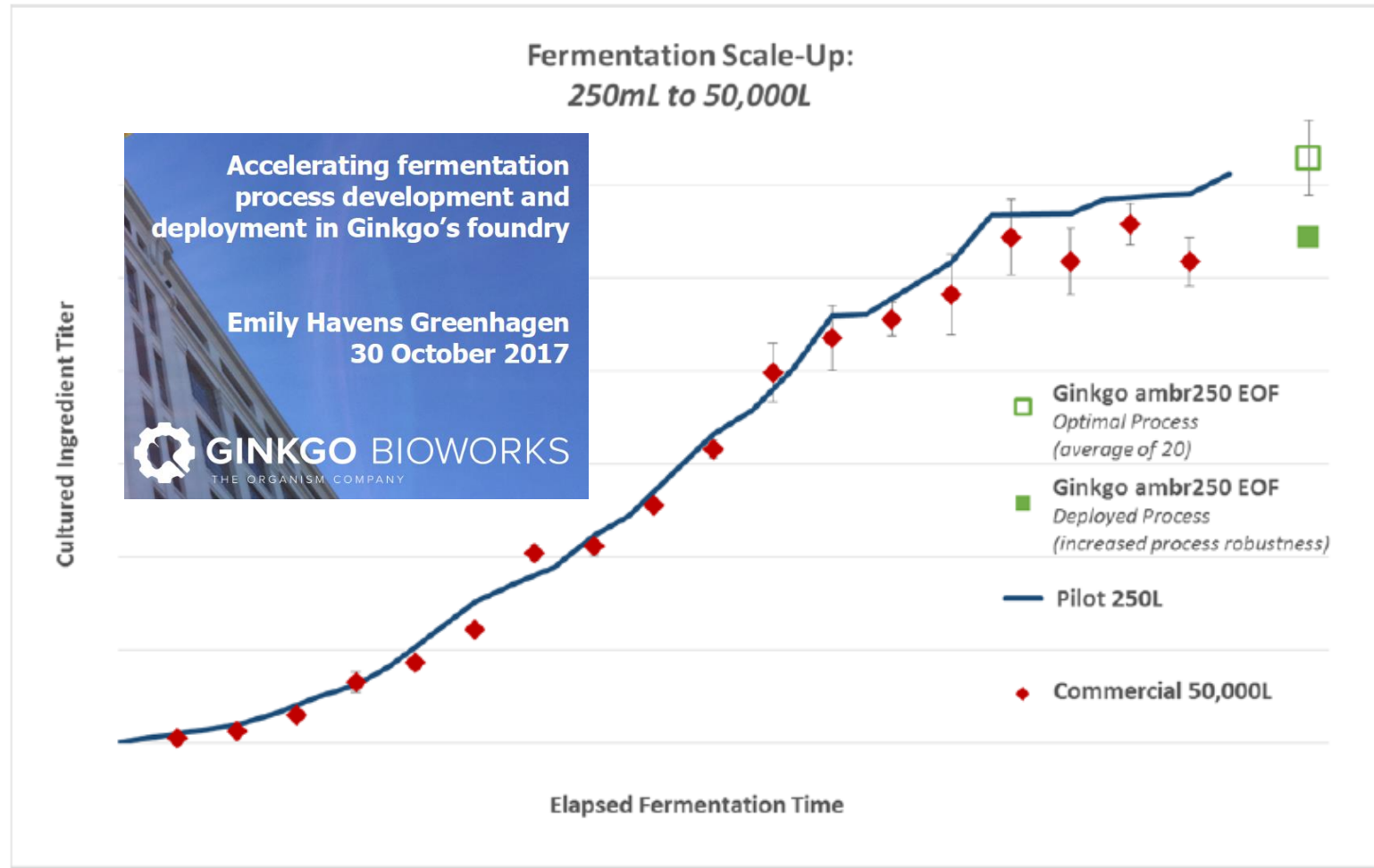


RIPPLE FOODS



**GINKGO  
BIOWORKS™**  
THE ORGANISM COMPANY

## Successful scale-up exceeding commercial target





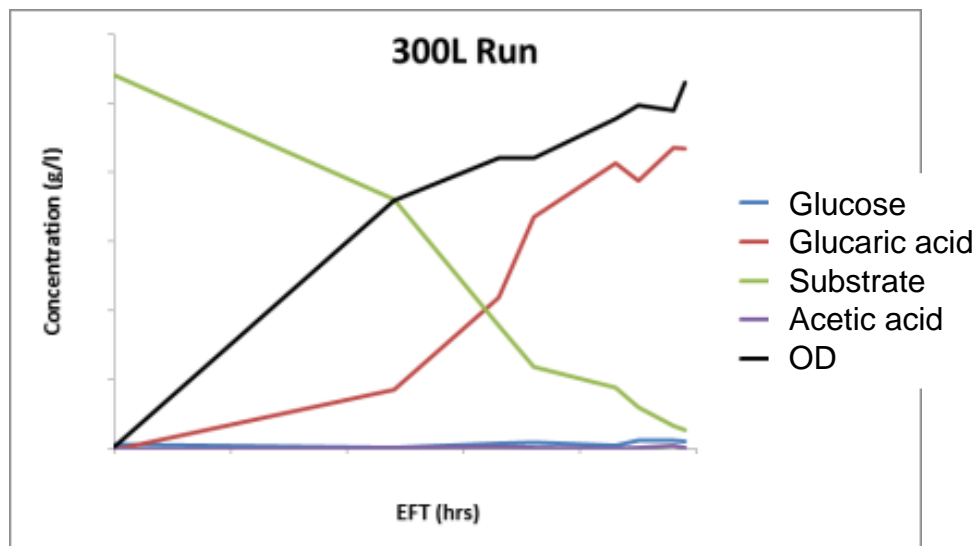
# Scaling up “Top Value Added Chemicals From Biomass” – BETO Seedling & SBV

## Fermentation goals met:

- Successfully achieved target glucaric acid titer in bench-scale fermentation during tech transfer
- Demonstrated even better fermentation performance at 300 L scale with nearly 2-fold increase in titer
- > 95% mass balance closure at pilot scale



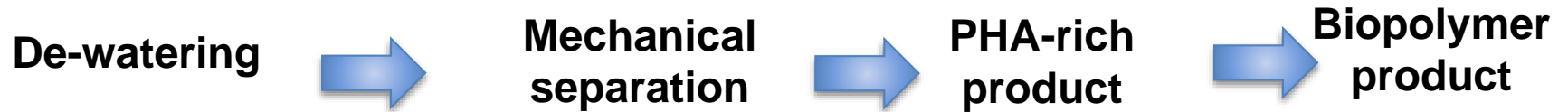
Pilot scale run at 300 L



Crystallized product (> 1 kg)

# Methane to bioplastics – SPP & BETO SBV & SepCon CRADA projects

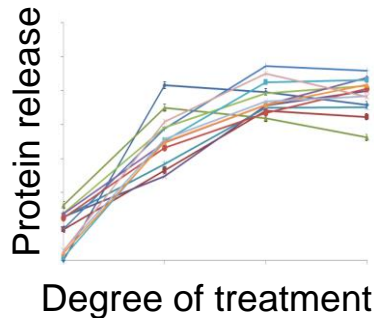
- Developed downstream recovery process to dewater fermentation broth and recover polyhydroxyalkanoate (PHA)-rich product
- Identified optimized conditions for de-watering and mechanical separation
- Achieved biopolymer-rich product with >95% purity



Increased rate of de-watering by 5x → from 8 to 1.5 hours for a 500L batch



Protein released demonstrated cell lysis



>95% purity



Met internal quality control standards for pure product



## 5 – Future Work

# Growth along new partnerships and technologies

- Review ADO RFI, industry trends, and program priorities with BETO to identify technical needs and opportunities to prioritize with capital upgrades or improvements (e.g. gas fermentation, continuous chemical catalysis, electrochemical bioprocessing, etc.)
- Develop more incubator / accelerator and academic / university collaborations and project proposals



- Augment US small business projects with larger company alliances
- Develop and implement Masters in Bioprocess Engineering Program with UC Berkeley

# New projects just starting or pending award / contract

Privately sponsored

Federally funded

**CALYSTA**



INVIZYNE TECHNOLOGIES

protera

**KOCH**  
 BIOLOGICAL SOLUTIONS

Kiverdi



LBNL LDRD for  
 electrochemical biomass  
 production

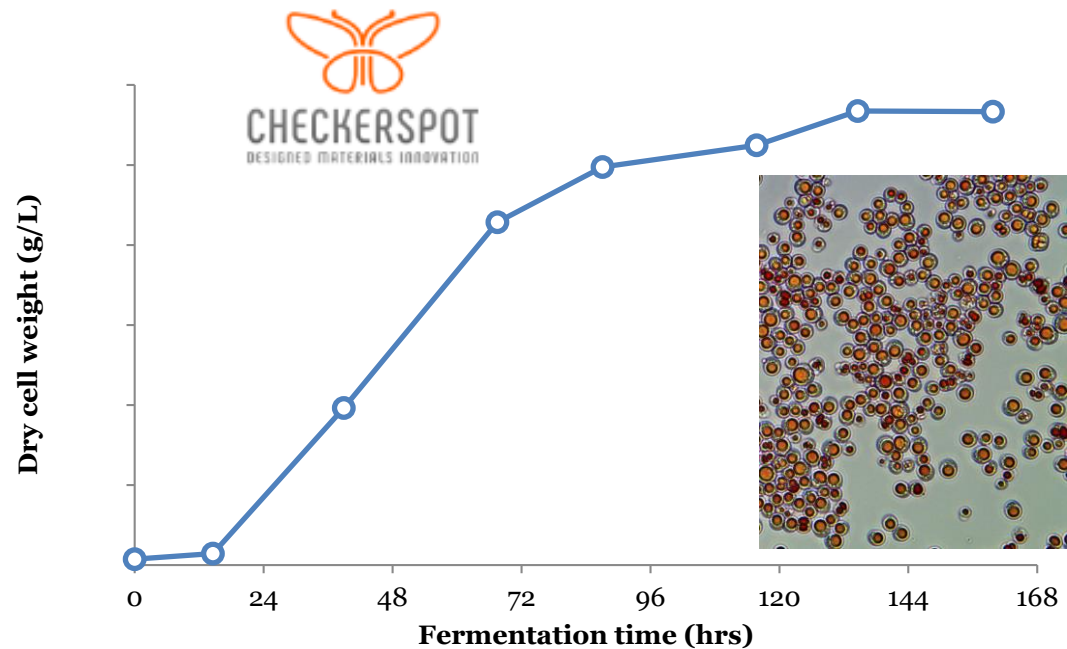
**MIT**

Massachusetts Institute of Technology®

# Checkerspot seedling → ongoing SPP collaboration

## Fermentation goals met:

- Very high dry cell weight achieved in fed batch fermentation
- Extremely high lipid content recorded in resulting biomass
- Documented fermentation performance of base strain and two alternative hosts



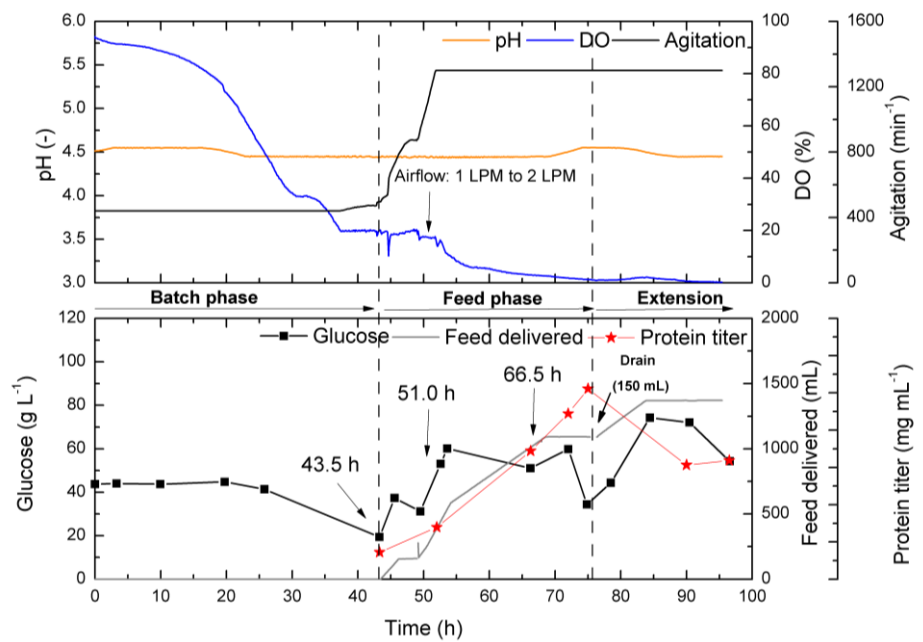
Process development at 2L scale

# Perfect Day seedling → Ongoing SPP collaboration

## Perfect Day

### Seedling goals met:

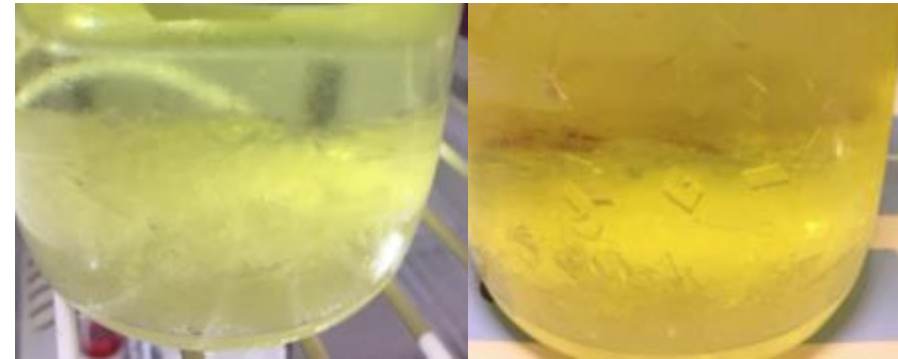
- Low-cost sustainable feedstocks resulted in comparable or even higher product titers compared to conventional feedstocks
- Reproducibility of results was demonstrated
- Recovery process for protein concentration was developed at bench scale



# ZymoChem seedling → SBV and pending ABF BEEPS FOA

## Goals met:

- Performed two 4 x 2L campaigns of anaerobic fed-batch fermentation and obtained equivalent microbial performance
- Established growth and metabolism profiles using glucose, hydrolysate and other industrial feedstocks
- Verified cell metabolism and biosynthetic pathway performance unaffected by product in fermentation broth
- Crystallization and evaporation tested for product recovery



*Small scale crystallization test*

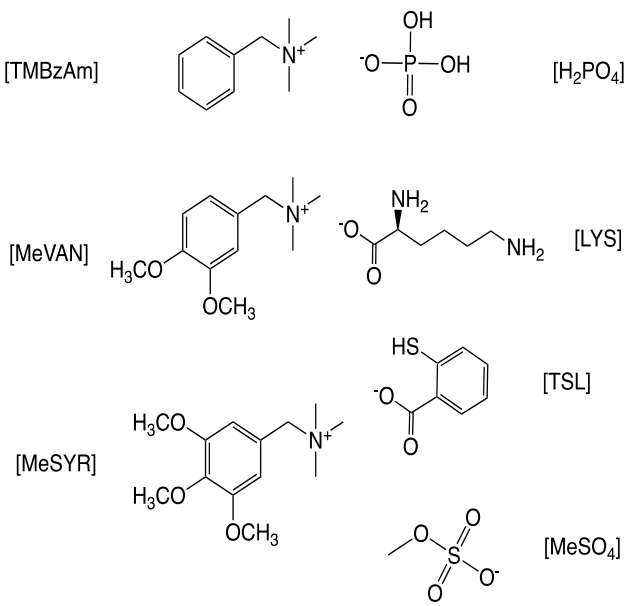
**ZYMOCHEM**



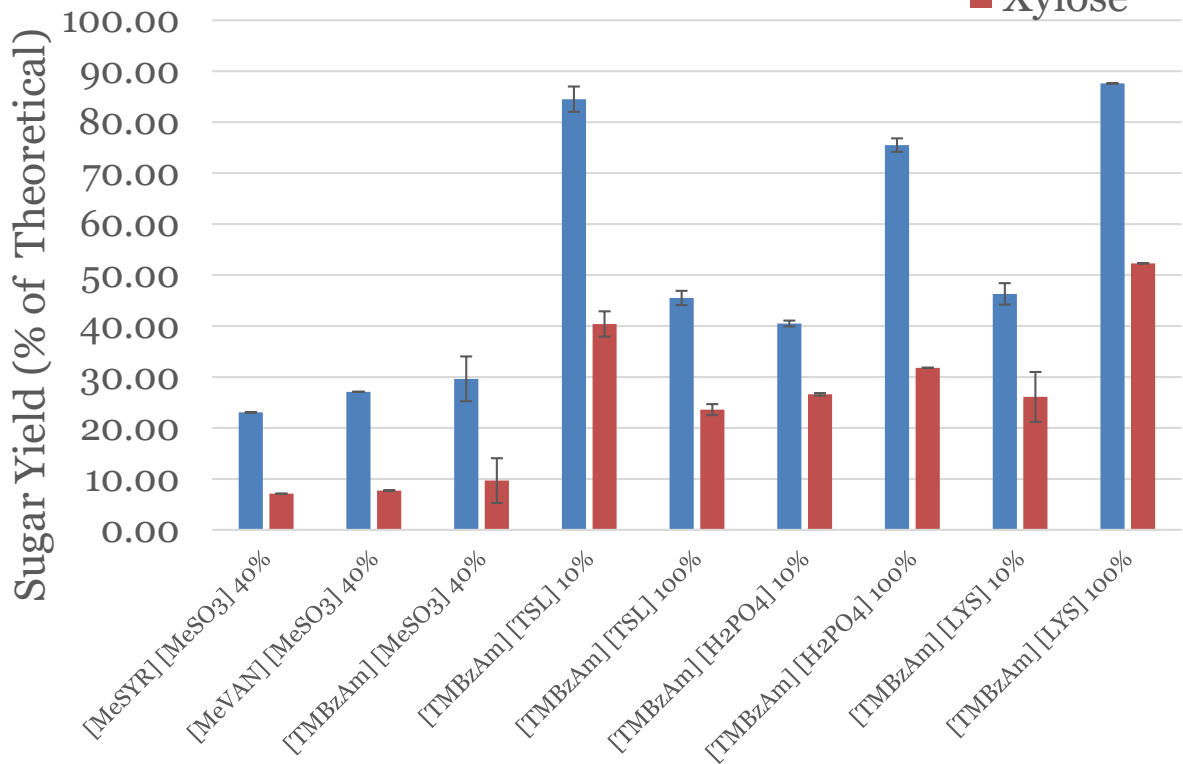
# Illium Seedling → pending TCF CRADA



## Examples of the synthesized ILs



## Sugar Yields from Switchgrass



- Protic ammonium ILs were prepared from lignin derived precursors (e.g. vanillin, syringaldehyde)
- Glucose and xylose yields from biomass pretreatments with protic ILs are comparable to gold standard ILs such as [C<sub>2</sub>C<sub>1</sub>mim]OAc

# Broader collaboration moving forward

PDU working group – cross-training, site visits and joint industry outreach



Leveraging other Berkeley Lab resources for DOE- and industry-sponsored projects



# Summary

# Enabling bio-economy and BETO partners to succeed

**Overview** – Partnered with > 40 companies and several other academic and National Lab researchers across wide spectrum of sectors and technologies.

**Management approach** – Project development in close coordination with LBNL OCFO, SPO, IPO stakeholders as well as BETO management and other PDUs.

**Technical approach** – Flexible unit op utilization. Continuing focus on equipment upgrades, procurement, safety, and preventative maintenance to avoid downtime.

**Progress** – Installed and commissioning high-throughput bioreactor system as well as new filtration test unit / skid. > 20 microbial host types cultivated. > 40 products.

**Relevance** – Addressing key BETO and industry challenges & barriers in Process Integration and Technology Uncertainty of Integration and Scaling. Three bioproducts commercialized based on processes developed and scaled at ABPDU.

**Future work** – Successfully execute ongoing and upcoming CRADA and SPP projects. Grow collaboration with other PDUs & BETO projects. RFI & capital upgrades to address upcoming DOE and industry capability needs.

## Additional slides

# Responses to prior reviewer comments (from FY17)

Comment 1: “This is a nice effort to establish another pilot facility (closer to where it is needed) to enable small businesses and national laboratories with quicker and cost-effective turnaround on their small-scale fermentations... Crosstalk with the other pilot plants, bench-scale validation, and analytics seem to be gaps where the PI and BETO are encouraged to facilitate better in the future with good handover of protocols, techniques, etc. Overall, this project has made good progress and is on the right track to fulfill BETO’s goals.”

Comment 2: “ABPDU facilities and staff are versatile, having worked on bioprocesses to make chemicals, materials, biomass, and protein products. Initiating a system of project coordination with other pilot facilities (e.g., NREL) in the national laboratory system is highly encouraged.”

Comment 3: “The ABPDU has had success generating business for their facility and also in reducing costs to BETO. However, their interaction with other larger-scale facilities within the program (NREL’s Process Development Unit and NREL bench scale) seems limited or nonexistent, to the point that it appears more competitive than collaborative. It would streamline operations if these valuable facilities were more coordinated.”

Response: A clear need was identified for better integration, coordination and collaboration between the ABPDU and other PDUs (e.g. IBRF at NREL, BFNUF at INL) and projects (Bench-scale integration and Analytical Method Dev’t at NREL). The ABPDU team has been working extensively over the past 2 years with staff from these teams on the ABF and FCIC BETO projects. We have also established a PDU working group to share best practices and foster teaming, joint projects, and cross-training between the Labs.

# Publications with partners since FY17 peer review (slide 1 of 3)

1. S. Patil, A. Narani, P. Coffman, T. Pray, D. Tanjore, and J. Rohrer. Thermo Scientific Application Note 1161. Improved Method for Determination of Biofuel Sugars by HPAE-PAD.
2. S. Patil, A. Narani, P. Coffman, T. Pray, D. Tanjore, and J. Rohrer. Thermo Scientific Application Note 72210. Fast determination of biofuels sugars by HPAE-PAD.
3. Narani A, Coffman P, Gardner J, Li C, Ray AE, Hartley DS, et al. Predictive modeling to de-risk bio-based manufacturing by adapting to variability in lignocellulosic biomass supply. *Biores Tech.* 2017;243:676-85. doi: <http://dx.doi.org/10.1016/j.biortech.2017.06.156>.
4. L. Liang, C. Li, F. Xu, Q. He, T. Luong, J. Yan, B. A. Simmon, T. R. Pray, S. Singh, V.S. Thompson, and N. Sun (2017). Conversion of Cellulose Rich Municipal Solid Waste Blends Using Ionic Liquids: Feedstock Convertibility and Process Scale-up. *RSC Adv.*, 2017,7, 36585-36593 DOI:10.1039/C7RA06701A
5. Yaegashi J, Kirby J, Ito M, Sun J, Dutta T, Mirsiaghi M, Sundstrom E, Rodriguez A, Baidoo E, Tanjore D, Pray T, Sale K, Singh S, Keasling J, Simmons B, Singer S, Magnuson J, Arkin A, Skerker J, and Gladden J (2017). *Rhodospiridium toruloides*: A new platform organism for conversion of lignocellulose into terpene biofuels and bioproducts. *Biotechnology for Biofuels*. 2017 10:241 <https://doi.org/10.1186/s13068-017-0927-5>
6. Timo Schuerg, Jan-Philip Prah, Raphael Gabriel, Simon Harth, Firehiwot Tachea, Chyi-Shin Chen, Matthew Miller, Fabrice Masson, Qian He, Sarah Brown, Mona Mirshiaghi, Ling Liang, Lauren M. Tom, Deepti Tanjore, Ning Sun, Todd R. Pray and Steven W. Singer (2017). Xylose induces cellulase production in *Thermoascus aurantiacus*. *Biotechnol Biofuels* 10:271. DOI 10.1186/s13068-017-0965-z

# Publications with partners since FY17 peer review (slide 2 of 3)

7. L. Liang, C. Li, Q. He, F. Tachea, D. Tanjore, T. Luong, M. Somma, N. D'Alessio, T. R. Pray, and N. Sun (2018). Upgrading of Post-consumer Absorbent Hygiene Products for Bioethanol Production. *ACS Sustainable Chem. Eng.* DOI: 10.1021/acssuschemeng.7b03931
8. L. Wendta, J. A. Murphy, W. A. Smith, T. Robb, Q. Nguyen, D. W. Reed, A. E. Ray, L. Liang, Q. He, N. Sun, A. Hoover, and Q. Nguyen (2018). Compatibility of high-moisture storage for biochemical conversion of corn stover: storage performance at laboratory and field scales. *Front. Bioeng. Biotechnol.* <https://doi.org/10.3389/fbioe.2018.00030>
9. P. Coffman, N. Macaffrey, J. Gardner, S. Bhagia, R. Kumar, C. Wyman, and D. Tanjore. (2018). In situ Rheological Method to evaluate Feedstock Physical Properties throughout Enzymatic Deconstruction. *Front. Energy Res.*, 02 July 2018. <https://doi.org/10.3389/fenrg.2018.00053>
10. Sundstrom, E., J. Yaegashi, J. Yan, F. Masson, G. Papa, A. Rodriguez, M. Mirsiaghi, L. Lian, Q. He, D. Tanjore, T. Pray, S. Singh, B. Simmons, N. Sun, and J. Gladden (2018). Demonstrating a separation-free process coupling ionic liquid pretreatment, saccharification, and fermentation with *Rhodospiridium toruloides* to produce advanced biofuels. *Green Chem.*, 2018,20, 2870-2879. DOI:10.1039/C8GC00518D
11. Yuzawa, R., M. Mirsiaghi, R. Jovic, T. Fujii, F. Masson, V. Benites, E. Baidoo, E. Sundstrom, D. Tanjore, T. Pray, R. Davis, J. Gladden, B. Simmons, L. Katz, and J. Keasling (2018). Microbial production of short-chain ketones using hybrid polyketide synthases *Nature Communications* volume 9, Article number: 4569 <https://www.nature.com/articles/s41467-018-07040-0>



# Publications with partners since FY17 peer review (slide 3 of 3)

12. J Yan, L Liang, TR Pray, N Sun (2018). Book chapter: Impacts, Challenges and Economics of Ionic Liquid Pretreatment of Biomass in *Biomass Preprocessing and pretreatment: mechanical, Chemical and thermal methods* CRC Press/ Taylor & Francis Group. Edited By Jaya Shankar Tumuluru.
13. M Wehrs, J-P Prah, J Moon, Y Li, D Tanjore, JD Keasling, T Pray and A Mukhopadhyay (2018). Production efficiency of the bacterial non-ribosomal peptide indigoidine relies on the respiratory metabolic state in *S. cerevisiae*. *Microbial Cell Factories* 201817:193 <https://doi.org/10.1186/s12934-018-1045-1>
14. A Narani, N.V.S.N. Murthy Konda, C-S Chen, F Tachea, P Coffman, J Gardner, C. Li, AE Ray, DS Hartley, B Simmons, TR Pray, D Tanjore (2019). Simultaneous application of predictive model and least cost formulation can substantially benefit biorefineries outside Corn Belt in United States: A case study in Florida. *Bioresource Technology* Volume 271, January 2019, Pages 218-227. <https://doi.org/10.1016/j.biortech.2018.09.103>
15. M. Wehrs, D. Tanjore, T. Eng, J. Lievense, TR Pray and A Mukhopadhyay (2019). Engineering Robust Production Microbes for Large-scale Cultivation. *Trends in Microbiology* – in press.

- **Ning Sun:** AIChE Program Committee's Young Investigator Award for Innovations in Green Process Engineering (2017)
  - <https://www.aiche.org/community/bio/ning-sun>
- **Akash Narani:** AIChE 35 Under 35 award in Bioengineering Category (2017)
  - <https://www.aiche.org/resources/publications/cep/2017/august/aiche-r-35-under-35>
- **Todd Pray;** Director's Award for Exceptional Achievement in Technology Transfer (2018)
  - <http://recognition.lbl.gov/laureates/>

# Oral presentations since FY17 peer review (slide 1 of 3)

1. Todd Pray (invited) - Scaling Novel and Innovative Processes for Commercialization. BIO World Congress, Jul 24, 2017, Montreal, Canada
2. Todd Pray (invited) - Industrialization of Synthetic Biology and Metabolic Engineering. SIMB Annual Meeting, Aug 1, 2017, Denver, CO.
3. Todd Pray - ABPDU-JBEI collaboration overview. JBEI Annual Meeting, Sep 13, 2017, Monterey, CA
4. Ning Sun (invited) - Unlocking Biomass Energy: Process Development and Scale-up of Biomass Conversion to Advanced Fuels and Chemicals. Institute of Process Engineering, Chinese Academy of Sciences, Aug 14, 2017, Beijing, China
5. V. Estes, N. Mouncey, A. Juminaga, P. Pharkya, N. Hillson, B. Simmons, and **Deepti Tanjore** (Panel presentation / Berkeley Lab Workshop): Industrialization of engineering biology: from discovery to scale-up. Synbiobeta SF 2017. October 3-5, 2017. San Francisco, CA
6. N. Sun, Y. Lee, L. Liang, R. Kalb, D. Blauch, T. R. Pray, Aaron Socha (Oral presentation): Unlocking Biomass Recalcitrance Using Lignin based Ionic Liquids, 2017 AIChE Annual Meeting, October 30, 2017, Minneapolis, MN
7. Todd Pray (Panel presentation): Collaborative Bioprocess Development and Piloting for Industrial Chemicals, Fuels, Materials and Food Ingredient Production (Pilot Plant Best Practices Session). AIChE Annual Meeting. October 30, 2017. Minneapolis, MN.
8. Ning Sun (Invited Award Presentation): Unlocking Biomass Energy: Process Development and Scale-up of Biomass Conversion to Advanced Fuels and Chemicals, 2017 AIChE Annual Meeting, October 31, 2017. Minneapolis, MN

# Oral presentations since FY17 peer review (slide 2 of 3)

9. Ning Sun, L Liang, J. Yan, Q He, B A Simmons, C Li, V S. Thompson, S Singh, T R Pray (Oral presentation): Scale-Up and Process Intensification of Biomass Conversion Using Ionic Liquid Based Process, AIChE Annual Meeting. November 2, 2017. Minneapolis, MN
10. Eric Sundstrom, M. Mirsiaghi, F. Masson, F. Tachea, C. S. Chen, J. P. Prah, T. Pray, and D. Tanjore (Oral presentation): Accelerating the bioeconomy: minimizing capital investment through industry-government partnerships. Recent Advances in Fermentation Technology, October 31, 2017, Bonita Springs FL
11. Todd Pray (Panel presentation, invited): Advanced Biofuels Summit. ABLC. Feb 18, 2018, Washington, DC.
12. Eric Sundstrom and D. Tanjore (Oral presentation): "Test" and "Learn" in process research informs design strategy, Eco-bio Conference, March 3-6, 2018, Dublin, Ireland.
13. Ling Liang, J. Yan, Q. He, C. Gutierrez, T. Pray, C. Chu and N. Sun. (oral presentation). Efficient conversion of municipal solid waste to biofuels and bioproducts. 40th Symposium on Biotechnology for Fuels and Chemicals, Clearwater Beach, FL, April 29- May 2, 2018.
14. Jipeng Yan, L. Liang, T. R. Pray, E. Karp, J. E. Coons, G. T. Beckham, and Ning Sun (oral presentation). Characterization and Separation of Lignin Streams after Alkali and Ionic Liquid Pretreatment. 40th Symposium on Biotechnology for Fuels and Chemicals. April 29-May 2, 2018, Clearwater Beach, FL.
15. Ning Sun (oral presentation) Unlocking Biomass Energy: Process Development and Scale-up of Biomass Conversion to Advanced Fuels and Chemicals 2018 AIChE NorCal Symposium "Opportunities and Challenges in Process Scale Up" Emeryville, CA, May 11, 2018

# Oral presentations since FY17 peer review (slide 3 of 3)

16. Todd Pray (panel moderator and presentation). Bioprocessing Separations Technology to Move Renewable Chemicals and Biofuels to Market. BIO World Congress, Philadelphia, PA. July 17, 2018.
17. Todd Pray (Invited lecture): Rapid Bioprocess Prototyping, Scaling and Integration for SynBio. Synthetic Biology for Defense Conference. September 27, 2018. Arlington, VA.
18. Todd Pray. (Invited lecture): Rapid Bioprocess Prototyping, Scaling and Integration for SynBio. Synthetic Biology for Materials Conference. October 23, 2018. Arlington, VA.
19. Todd Pray. (Seminar): Rapid development and optimization of synthetic biology fermentation and downstream processes at ABPDU. Defense Manufacturing Conference. December 6, 2018. Nashville, TN.

# Patent applications

1. Reactor vessel useful for performing multiple pretreatments. Application number 62/080,968 filed 2014.
2. "Conversion of a soiled post-consumer cellulosic composition." Application number 62/193,701 filed 7/17/2015. International Patent Application Filed in matter "Conversion of post-consumer hygiene cellulosic stream for biorefinery," July 18, 2016. Patent Application Ser. No: PCT/US2016/042863
3. Novel Method to Produce a Polysaccharide Gel. U.S. patent application serial no. 62/480,117 filed on March 31, 2017
4. Efficient Conversion of Municipal Solid Waste to Biofuels and Bioproducts. Provisional application: 62/578,248. Filed Oct 27, 2017.

## Risk registry (from AOP)

Name	Status	Target Completion Date	Severity	Response	Description
Funding shortfall	Not active	9/30/19	High	Develop other funding sources; adjust service offered	Insufficient corporate sponsorship - Industry downturn or lack of need for services
Unplanned downtime	Active	9/30/19	Low	PM and contingency for repairs, backups and replacement	Periodic equipment and utility failures
Inadequate staffing	Monitor	9/30/19	Moderate	Training, team building, incentives	Increased team turnover

**Project scope change** – not applicable / no scope changes thus far