Lactic Acid Producing Methanotrophic Bacteria (LPMB) For Fermentation of Bio-Methane As A Biological Upgrading Technology (WBS 2.3.1.203)

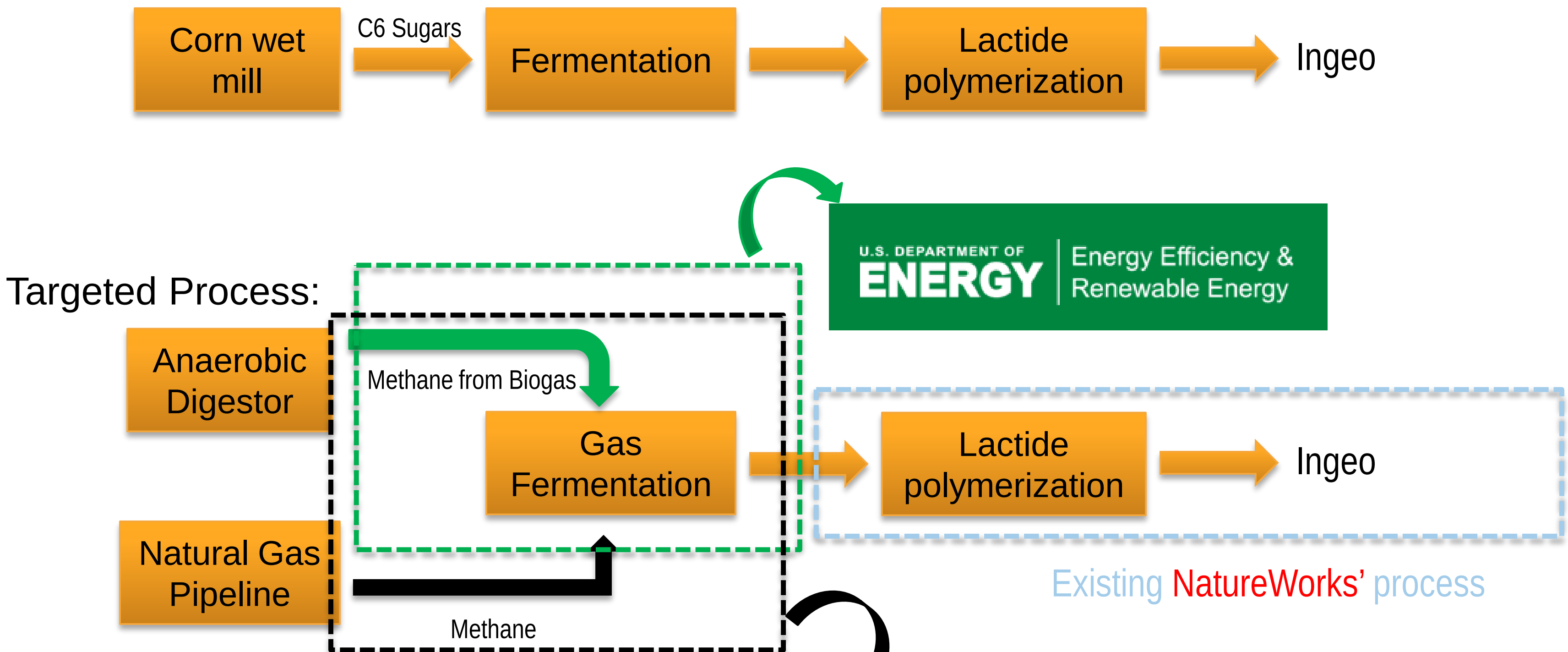
Date: **March 7, 2017**
Technology Area: **Waste to Energy**
Principal Investigator: **Ken Williams**
Organization: **NatureWorks, LLC**

U.S. Department of Energy (DOE)
Bioenergy Technologies Office (BETO)
2017 Project Peer Review

Big Picture…

- ***NTR is global leader in the development and commercialization of renewable/biodegradable polylactide polymers from plant sugars.***
- ***Collaborating with Calysta to develop biocatalyst/gas-phase fermentation process for conversion of methane to lactic acid***
- ***Developing a process from biogas enables production of completely renewable polylactides from biogenic methane,*** *and technological infrastructure for liquid transportation fuels*

Existing process:



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Goal Statement

- *Background: methane in biogas offers a renewable alternative to natural gas as a feedstock and intermediate in bioprocesses. This development effort is relevant to EERE's MYPP for developing commercially-viable, integrated waste-to-energy processes for the production of bioproducts enabling cost-competitive advanced biofuels production.*
- *Goal: Development of a commercially viable, disruptive fermentation process using methane in biogas and engineered methanotrophic bacteria for the production of lactic acid (HLA)*
- *Outcome: Demonstrate fermentation metrics at 2L scale that give lactic acid cost of goods produced (COGP) <$0.30/lb HLa.*
  - *techno-economic model at commercial scale (~400 MMlb/yr HLA) defines the sensitivity of lactic acid cost of goods produced (COGP) to a number of input variables and fermentation metrics*
  - *Advance and test biogas value chain capture, supply and deliver*

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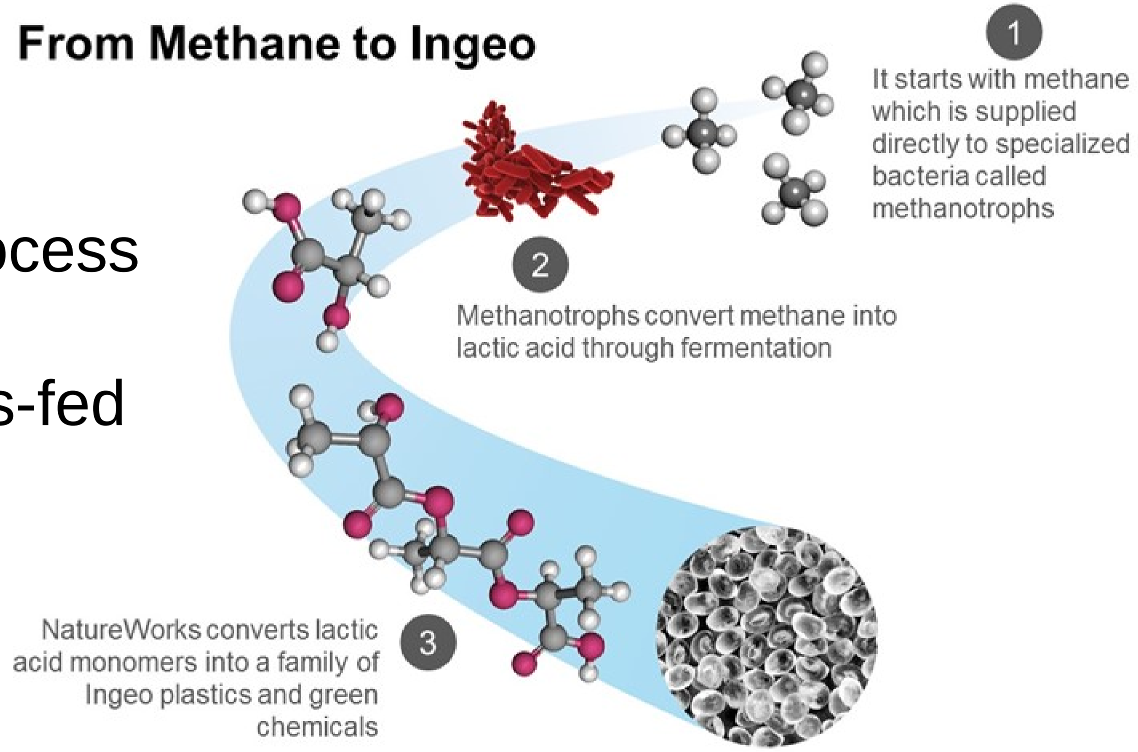
Goal Statement



Develop strains and process to enable disruptive, commercially-viable gas-fed fermentation process:

Commercial scale COGP < $0.30 lb/HLA

Example metrics in gas-fed fermentation process:
Titer: 1000 mM
Productivity: 2 g/L/hr
Yield: > 50% of theoretical (1.875 g HLA / g CH_4)

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We are committed to feedstock diversification:

Investment in innovation and R&D collaboration to grow our Ingeo feedstock portfolio

Performance materials made by transforming whatever are the right, abundant, local resources

GENERATION I

Today

Dextrose & Sucrose from cassava or corn starch, sugar cane or beet

"Bridging Crops"

Scaled & Operating C_6 sugar fermentation technology

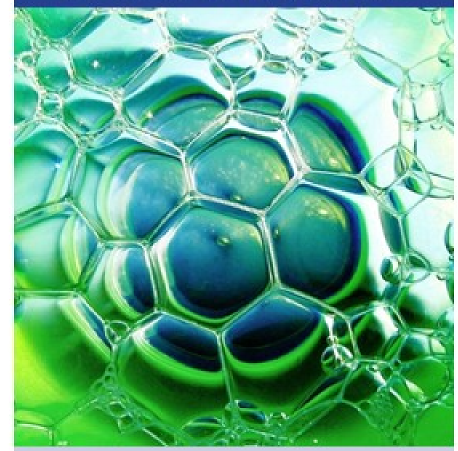
GENERATION II

Next 3-5 years

Lignocellulosics: Sugars from bagasse, wood chips, switch grass or straw.

"Purchase" strategy
C_6 sugars from 3rd party

GENERATION NEXT



And next?

CO_2 to lactic acid technology?

CH_4 to lactic acid technology?

"Develop" strategy
Methane fermentation

Quad Chart Overview

Timeline

- DOE Project start date: 5/1/2015
- DOE stagegate: 4/2017
- DOE Project end date: *est. 4/1/2019*
- Percent complete: ~50%

Budget

	Total Costs FY 12 –FY 14	FY 15 Costs ($)	FY 16 Costs ($)	Total Funding FY 15-16 ($)	Total Planned Funding (FY 17, $)
DOE Funded	-	448,310	801,690	1,250,000	TBD
Project Cost Share (Comp.) *		1,524,399	5,479,554	8,253,953	TBD

Barriers

- Bt-J: Catalyst Development
- Bt-K: Biochemical Conversion Process Integration
- Im-F: Cost of Production
- MYPP target addressed: < $3/GGE biofuel through enabling bioproduct production
- No supply of compressed raw biogas for fundamental R&D at 2L scale (1000s of scf required).

Partners

- Calysta (70% over BP1)
  Strain engineering and development, molecular biology, fermentation evaluation
- Standby Systems
  Biogas compression and procurement
- Blue Lake Waste Water Treatment Plant
  raw biogas source
- MN DEED (funding)

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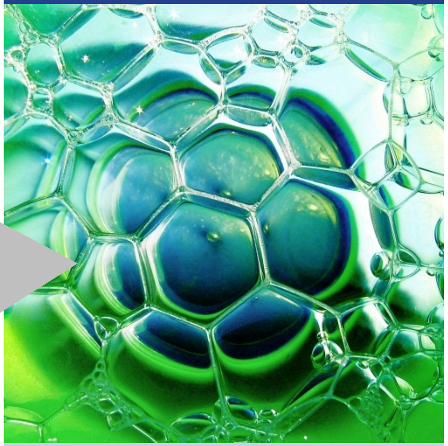
1 - Project Overview

Performance materials made by transforming whatever are the right, abundant, local resources

Investment in innovation and R&D collaboration to grow our Ingeo feedstock portfolio.

- June 2013:
  - Long Term R&D Partnership Established Between NatureWorks & Calysta
- November 2013:
  - World's First Lab Scale Lactic Acid Production Demonstrated with engineered methanotrophic bacteria
- October 2014:
  - $2.5MM DOE Funding leveraging ~$10MM investment from NatureWorks on core R&D
- Jan 2016
  - $250k investment from MN DEED leveraged $675k investment in new MN based methane fermentation lab
  - Hiring gas-fed fermentation group, 6 high-earning professionals

GENERATION NEXT



And next?

CO_2 to lactic acid technology?

CH_4 to lactic acid technology

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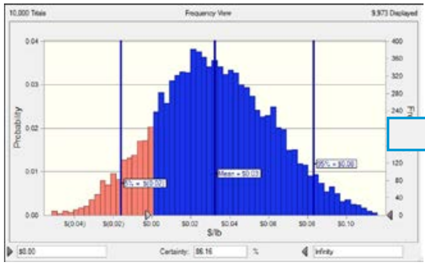
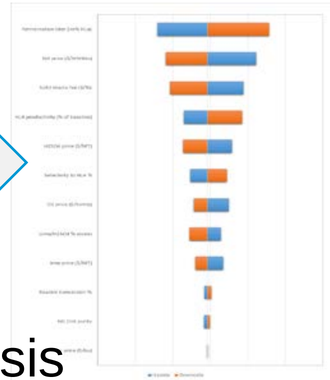
2 – Approach (Technical)

Path to commercial COGP





Techno-economic analysis

- Facility modeled at >100 MMlb/yr HLA
- Major fermentation metric inputs include yield, productivity and titer
- Baseline model at $6/MMBtu methane price
- Input only fermentation metrics based on validated lab/experimental data

- Yield (biggest COGP driver during initial development, variable and fixed OPEX, CAPEX)
- Titer (variable OPEX and CAPEX)
- Productivity (CAPEX and fixed OPEX)

Process Development
mass transfer, reactor design, scale up

Lab
Today

>$1/lb COGP

biocatalyst + process

Commercial Plant
Future

~$0.30/lb COGP

Strain Development
productivity, selectivity, inhibition

Requires iterations for coupled system

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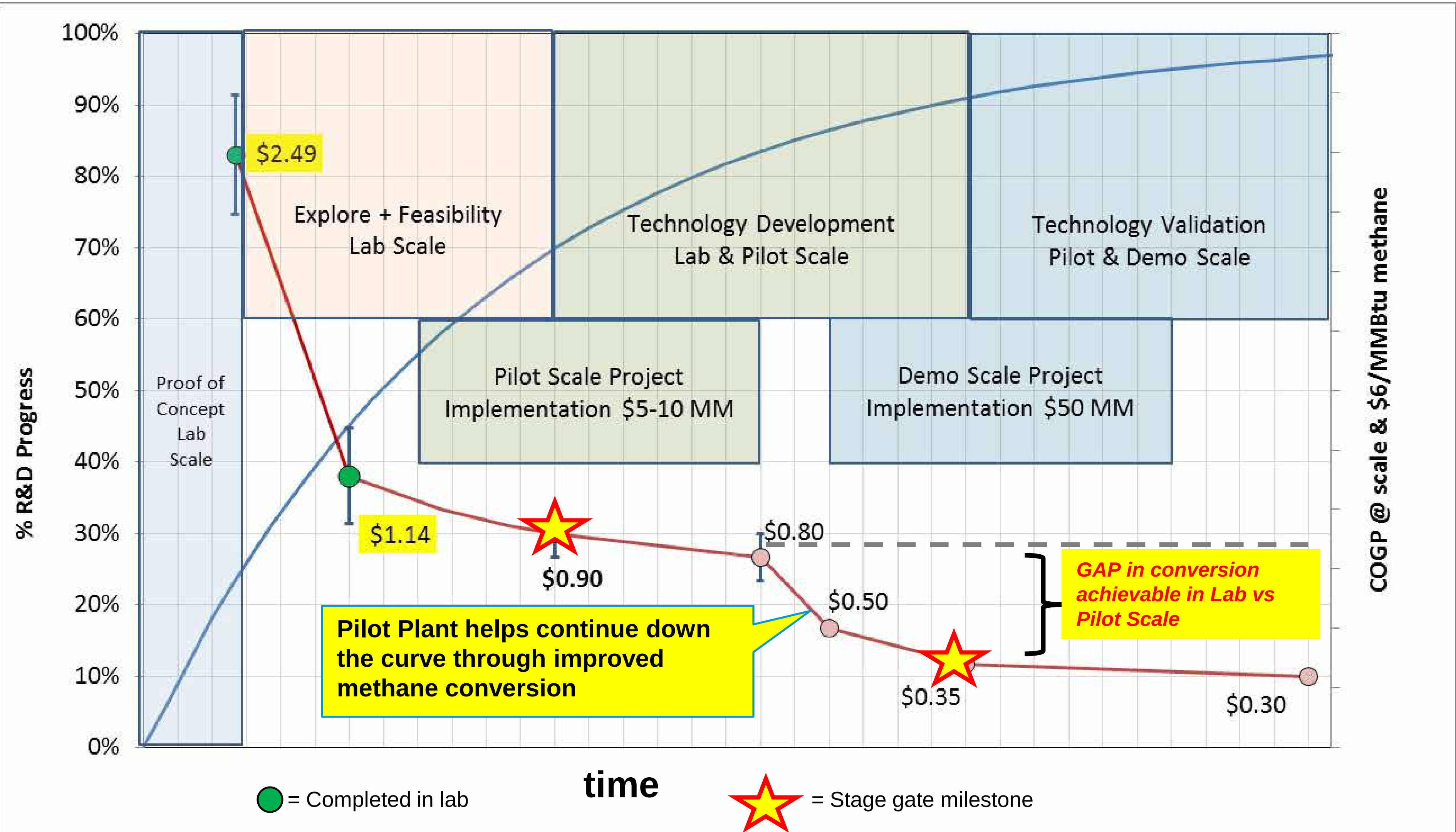
Project Plan…approach to less than $0.30/lb (see slides 34-35 for more information)



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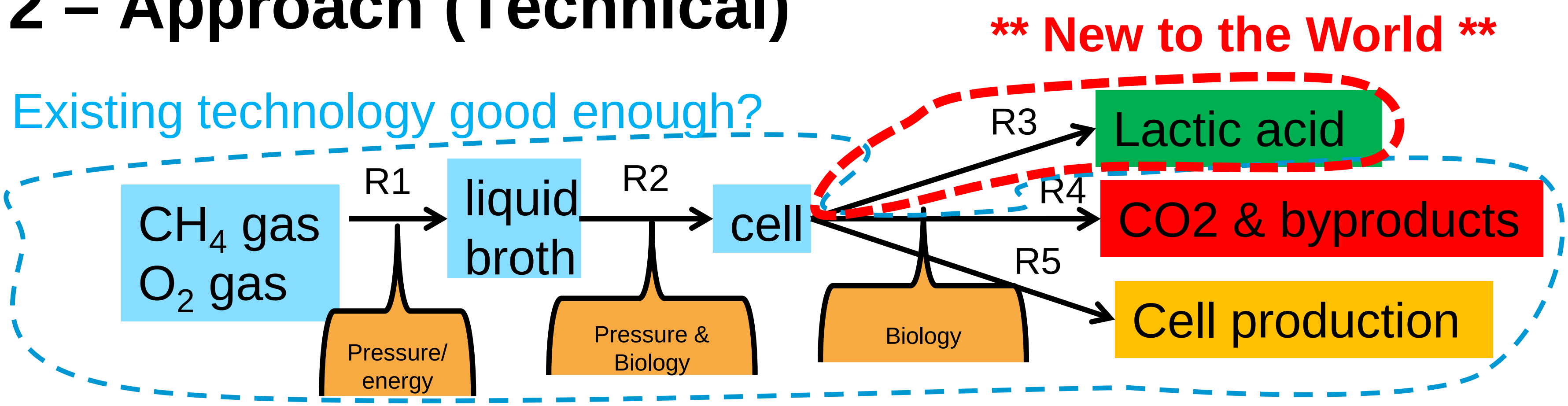
2 – Approach (Technical)



- **Calysta:** creation of plasmid-borne and chromosomally integrated strains, biochemistry of LDH, metabolic engineering
- **NatureWorks:** fermentation process development and strain adaptation
- Fermentations must overcome a series of *resistances* to drive feedstock conversion toward desired product.
- Methane fermentation is a gas-based fermentation, and a key pathway resistance (R1) is methane transfer into the aqueous fermentation media.
- Our 2L lab scale fermenters enable the genetic engineering team to evaluate resistance for pathways to cell growth, lactic acid and other by-products (R3, R4, and R5); but the COGP metric at scale greatly depends on yield, titer, and productivity considering all resistances simultaneously.

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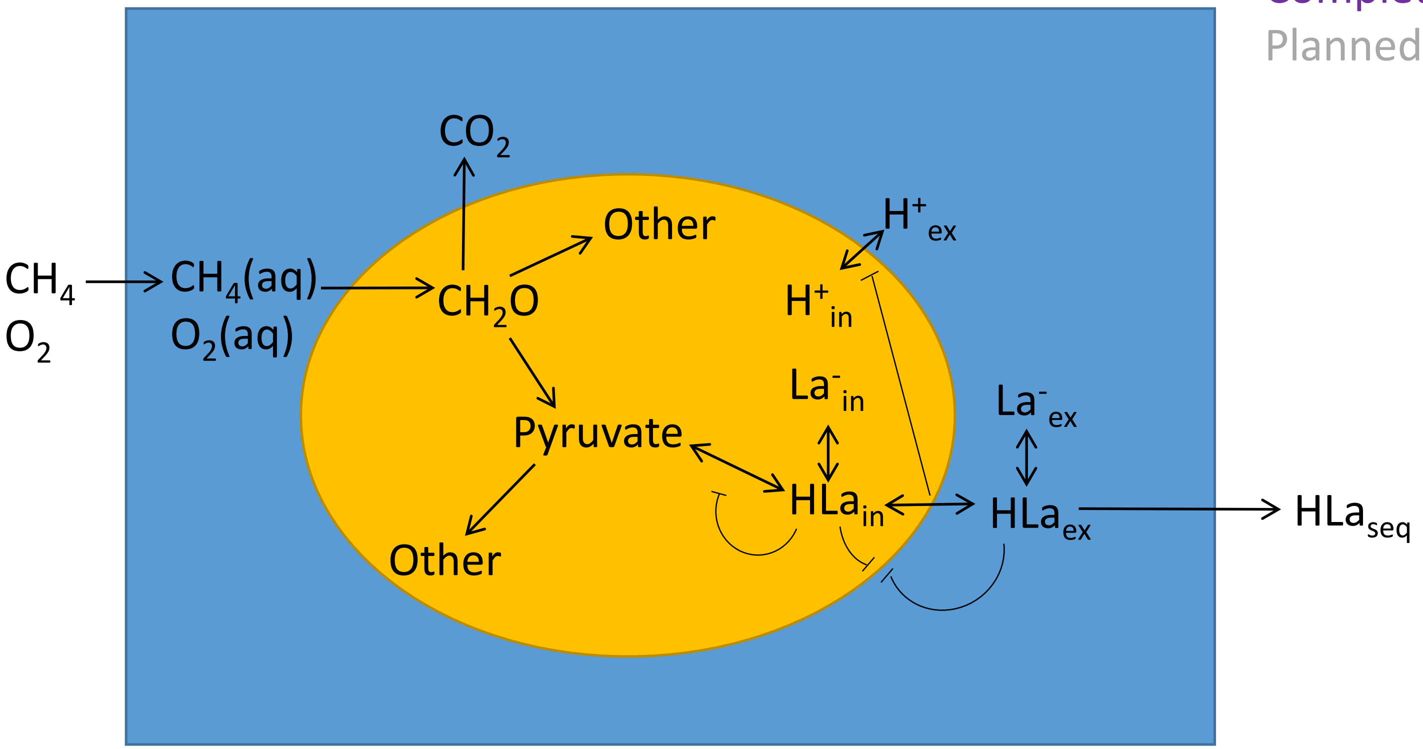
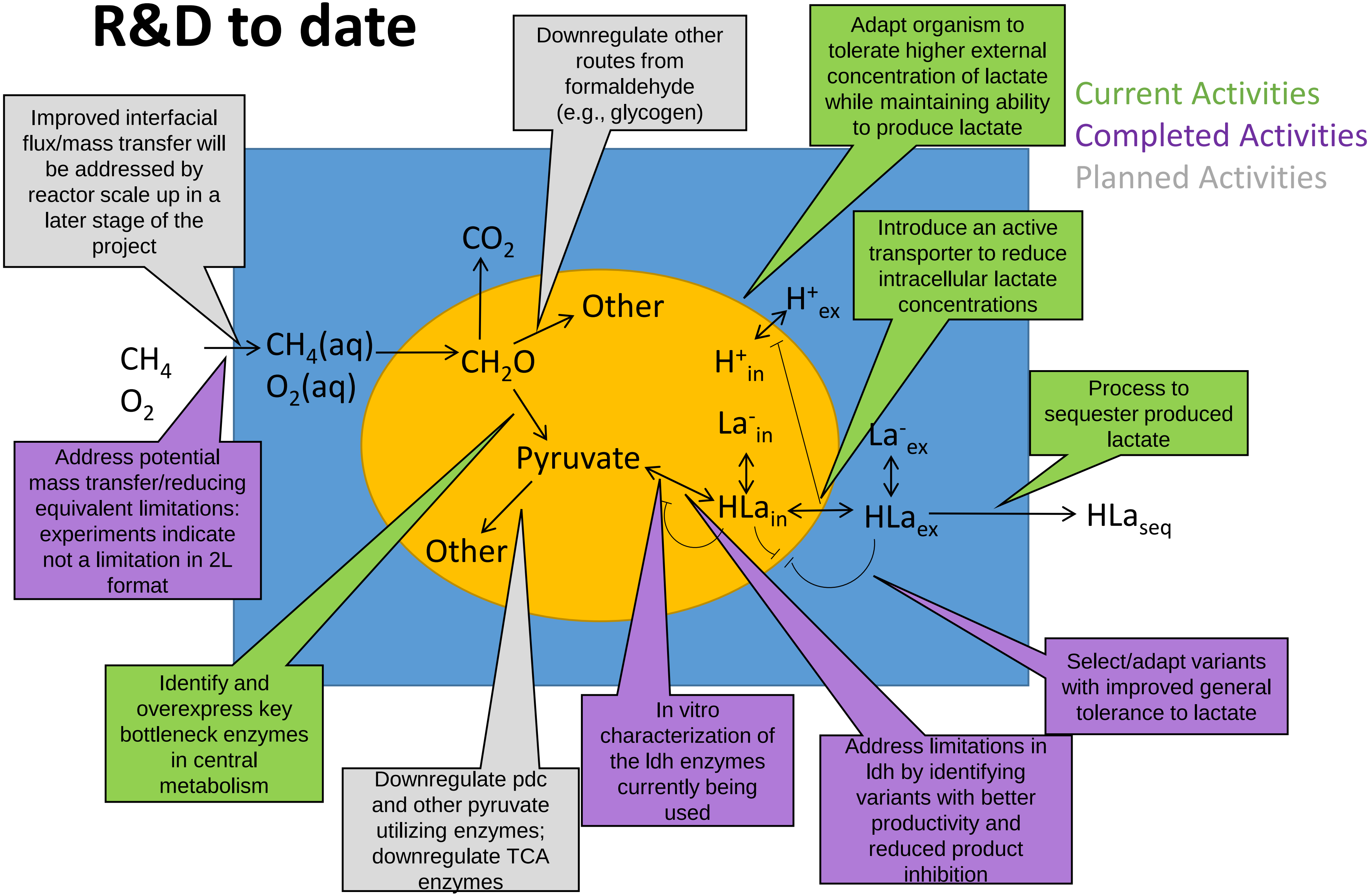
2 – Approach (Technical)

Potential bottlenecks

Current Activities
Completed Activities
Planned Activities



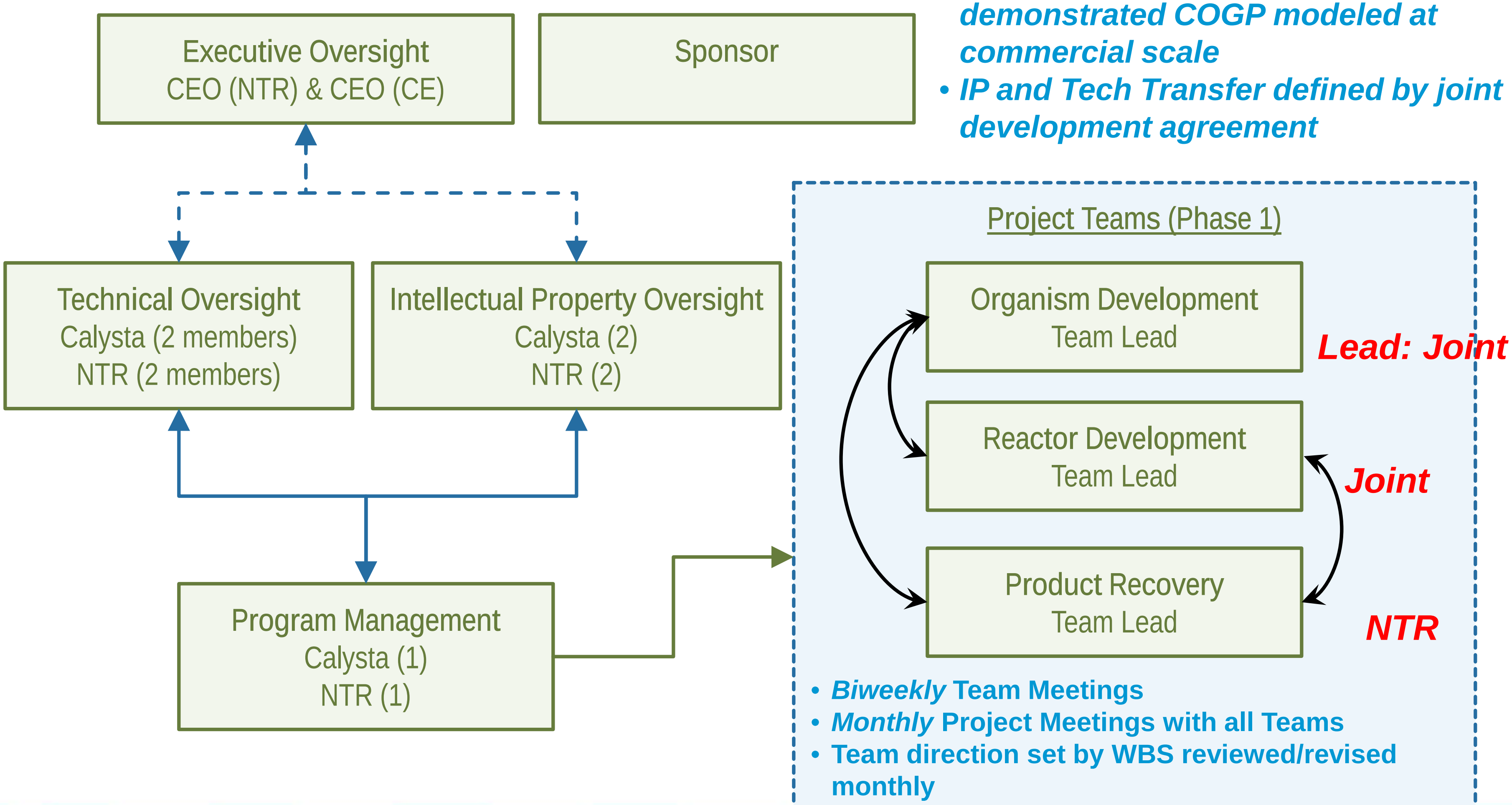
R&D to date



Improved interfacial flux/mass transfer will be addressed by reactor scale up in a later stage of the project
Downregulate other routes from formaldehyde (e.g., glycogen)
Adapt organism to tolerate higher external concentration of lactate while maintaining ability to produce lactate
Current Activities
Completed Activities
Planned Activities
Introduce an active transporter to reduce intracellular lactate concentrations
CO_2
Other
H^+_{ex}
CH_4
O_2
$CH_4(aq)$
$O_2(aq)$
CH_2O
H^+_{in}
La^-_{in}
La^-_{ex}
Process to sequester produced lactate
Address potential mass transfer/reducing equivalent limitations: experiments indicate not a limitation in 2L format
Pyruvate
HLa_{in}
HLa_{ex}
HLa_{seq}
Other
Identify and overexpress key bottleneck enzymes in central metabolism
Downregulate pdc and other pyruvate utilizing enzymes; downregulate TCA enzymes
In vitro characterization of the ldh enzymes currently being used
Address limitations in ldh by identifying variants with better productivity and reduced product inhibition
Select/adapt variants with improved general tolerance to lactate

2 – Approach (Project Management and Team Structure)

- ***Internal stagegates based on demonstrated COGP modeled at commercial scale***
- ***IP and Tech Transfer defined by joint development agreement***



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3 – Results:
What we've accomplished in under 3 years

- Iterative testing of starting LDHs in plasmid format → generation of lead strains via chromosomal integration of best candidates
- Inducible commercially relevant promoter system validated
- Moved from (1) idea to (2) proof of concept to (3) technology transfer at 2L scale (pre-commercial)
- Successful Patent Application on engineered methanotroph strain
- 5 order of magnitude improvement to titer in under 3 years at 2L scale
- Built gas-fed fermentation lab (~$1M investment) and world-class fermentation/biology team at NTR
- Strong partnership with DOE-BETO and MN-DEED for non-dilutive funding
  - $1.25M from DOE-BETO / $1.25 M potential for budget period 2
  - $250k from MN DEED in loans/forgivable loan

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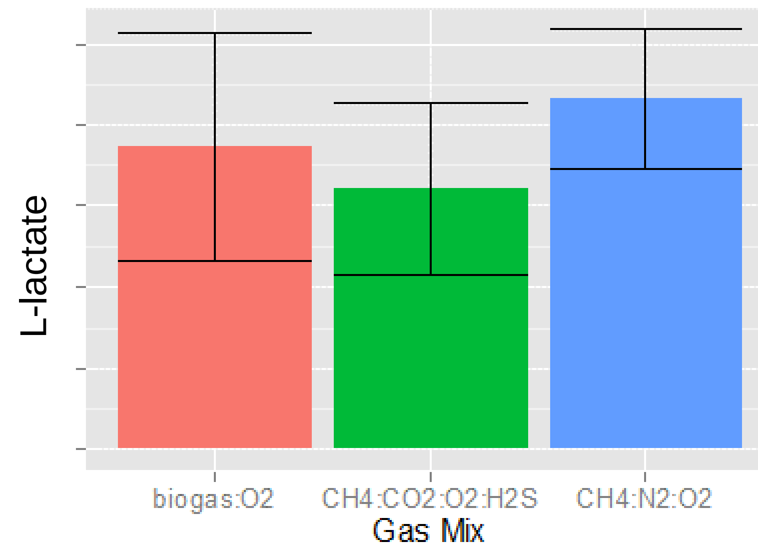
Lactic Acid Production from Biogas

- In addition to methane, biogas contains CO_2, H_2S and other components that may inhibit growth and/or lactate production.
- <u>Our strains are able to grow and produce lactate from biogas</u>.

Typical Biogas composition:
- 60 % methane
- 39 % carbon dioxide
- ~100 ppm H_2S
- Trace Si



Methane diluted with either nitrogen or carbon dioxide produces similar amounts of L-lactate to biogas → *no measurable toxicity up to 100 ppm H2S*

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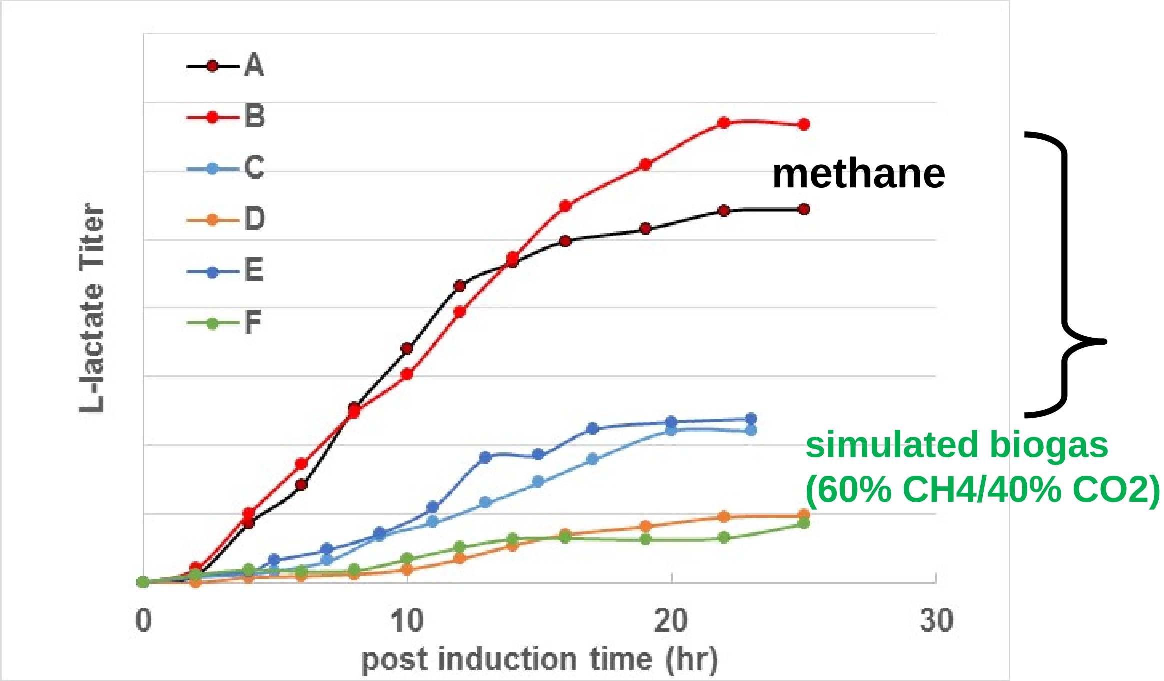
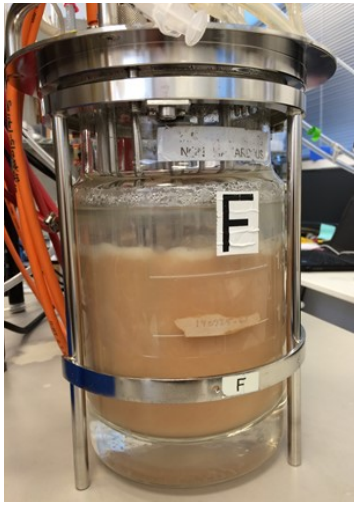
Lactic Acid Production from Methane and Biogas

…World's first demonstration of L-lactic acid production from an engineered strain (Nov 2013)



CO_2 inhibition on rate and titer

Fermentation (2L)



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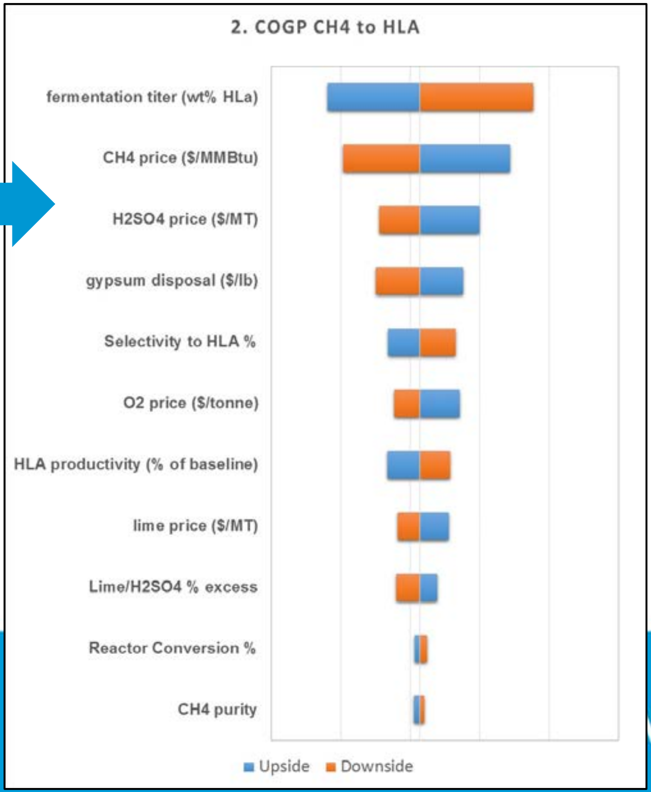
Progress toward performance metrics

	Productivity g/L/h	Yield % theoretical	Titer mM	COGP $/lb	Comment
Best cumulative performance	[redacted]	[redacted]	[redacted]	$1.14	Demonstrated
Phase I target	0.40	12	140	$0.90	Pilot authorization
Phase 2 target	0.50	14	167	$0.80	Pilot start-up
Commercial target	2.00	50	1000	$0.35	Pilot validation

We made significant progress towards achieving Phase I performance metrics for fermentation yield and productivity (technical feasibility), but achieving the target lactic acid titer remains the key challenge to address commercial viability



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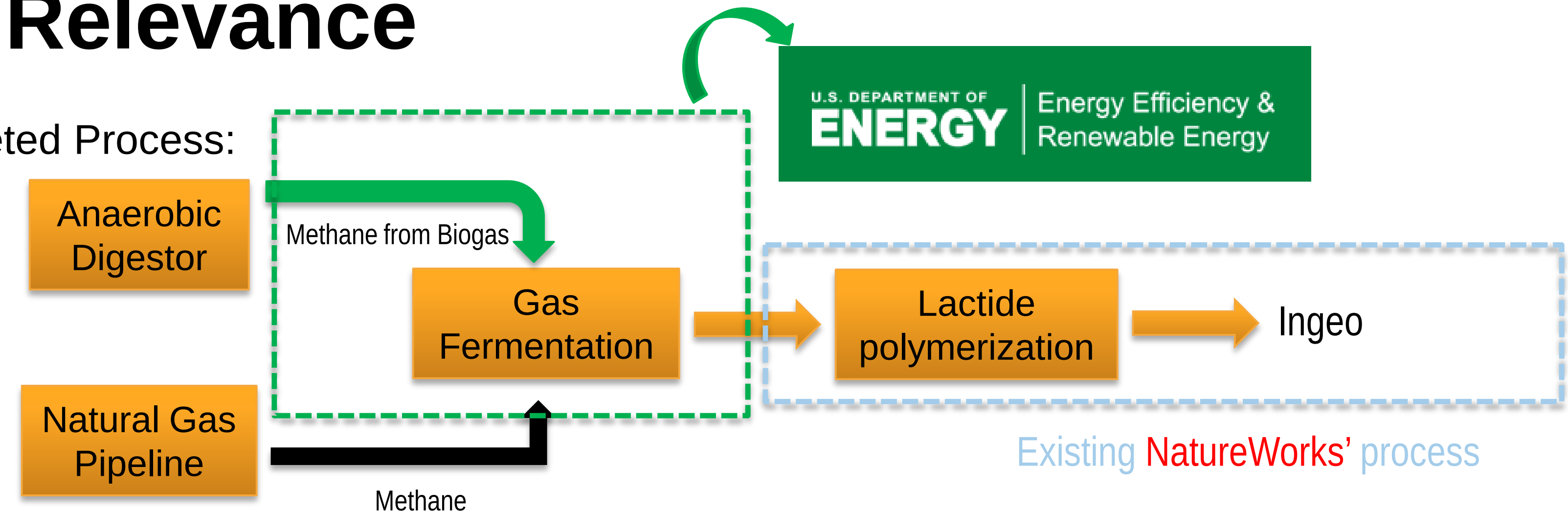
4 – Relevance

Targeted Process:



Supporting cost-effective biofuel production through integrated bioproduct development

- *Supports BETO's mission to < 3$/GGE biofuel by creating commercially relevant co-product from waste stream at integrated biorefinery*
- *Project metrics and targets driven by commercial scale TEA and NatureWorks significant technical and commercialization experience*
- *Leverages NatureWorks existing biopolymer production technology and developed commercial markets*
- *This project successfully developed first of kind biogas supply chain from WWTP and identifies valuable opportunity for underutilized biogas **(see slides 36-38)** .*
- *DOE identified lactic acid as platform chemical and the right price point enables billion pound downstream chemical markets made from oil today (e.g., acrylic acid)*

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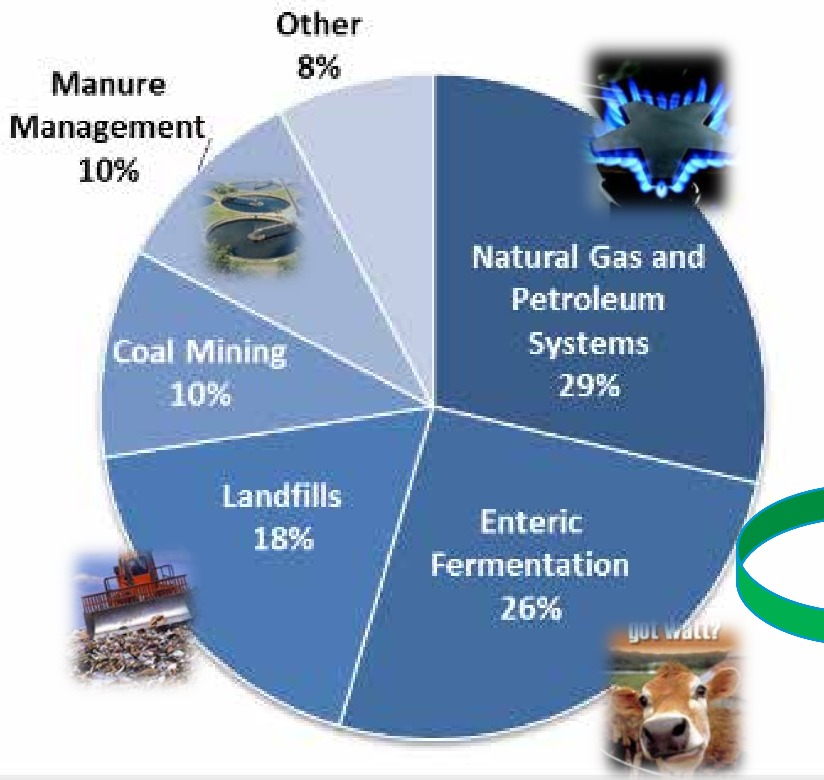
Methane Sources

U.S. Methane Emissions, By Source



Note: All emission estimates from the *Inventory of U.S. Greenhouse Gas Emissions and Sinks: 1990–2013.*

Science REPORTS

Cite as: H. Schaefer *et al.*, *Science* 10.1126/science.aad2705 (2016).

A 21st century shift from fossil-fuel to biogenic methane emissions indicated by $^{13}CH_4$

Hinrich Schaefer,[1*] Sara E. Mikaloff Fletcher,[1] Cordelia Veidt,[2] Keith R. Lassey,[1†] Gordon W. Brailsford,[1] Tony M. Bromley,[1] Edward J. Dlugokencky,[3] Sylvia E. Michel,[4] John B. Miller,[3] Ingeborg Levin,[2] Dave C. Lowe,[1‡] Ross J. Martin,[1] Bruce H. Vaughn,[4] James W. C. White[4]

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‡Present address: LoweNZ, Plimmerton, New Zealand.

Between 1999 and 2006, a plateau interrupted the otherwise continuous increase of atmospheric methane concentration [CH_4] since pre-industrial times. Causes could be sink variability or a temporary reduction in industrial or climate sensitive sources. We reconstruct the global history of [CH_4] and its stable carbon isotopes from ice cores, archived air and a global network of monitoring stations. A box-model analysis suggests that diminishing thermogenic emissions, probably from the fossil-fuel industry, and/or variations in the hydroxyl CH_4-sink caused the [CH_4]-plateau. Thermogenic emissions didn't resume to cause the renewed [CH_4]-rise after 2006, which contradicts emission inventories. Post-2006 source increases are predominantly biogenic, outside the Arctic, and arguably more consistent with agriculture than wetlands. If so, mitigating CH_4-emissions must be balanced with the need for food production.

See slides 36-38 (Appendix)

Locally harvested biogas for R&D Program

Partnering in MN to Make it Happen

METROPOLITAN COUNCIL | Standby Systems inc. | NatureWorks ingeo: naturally advanced materials | mn EMPLOYMENT AND ECONOMIC DEVELOPMENT | U.S. DEPARTMENT OF ENERGY Energy Efficiency & Renewable Energy

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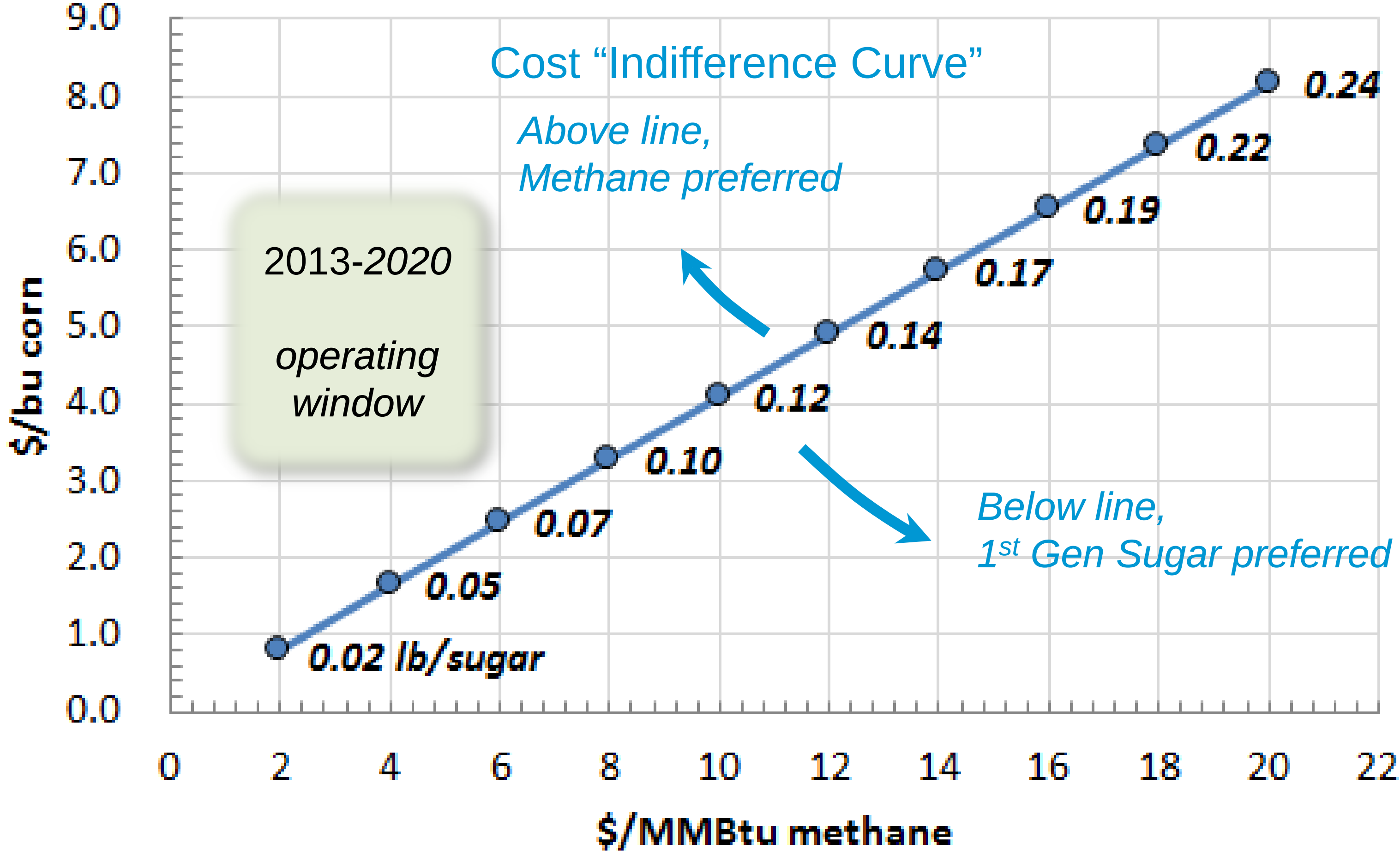
Opportunity for reduced feedstock cost …



Calculation assumptions:
Heating value of methane = 20,000 BTU/lb, methane to lactic yield = 80% of 1.875 g/g theoretical / 90% sugar to lactic acid yield

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5 – Future Work (Technical Strategy)

- Organism Development Team focused on increasing production by increasing tolerance to LA
- The mechanism of tolerance to organic acids is poorly understood, especially for methanotrophic bacteria and while targeted metabolic engineering has been used successfully to increase optical purity or yield by reducing the formation of byproducts, random mutagenesis and evolutionary adaptation have had better success at increasing tolerance.
- Informed by BP1 lessons learned, BP2 workplan will be based on:
  - Random mutagenesis/adaptation of the strain to increasing concentrations of LA or decreasing pH
  - Identifying genes that can be amplified to increase tolerance/production
  - Evaluating and selecting the best LDH for the process
  - Product recovery team focused on HLA sequestration approach (outside of BETO funded program but complimentary and synergistic)
- BP2 Milestone Target: 500 mM (45 g/L) titer enables significant improvements in associated metrics

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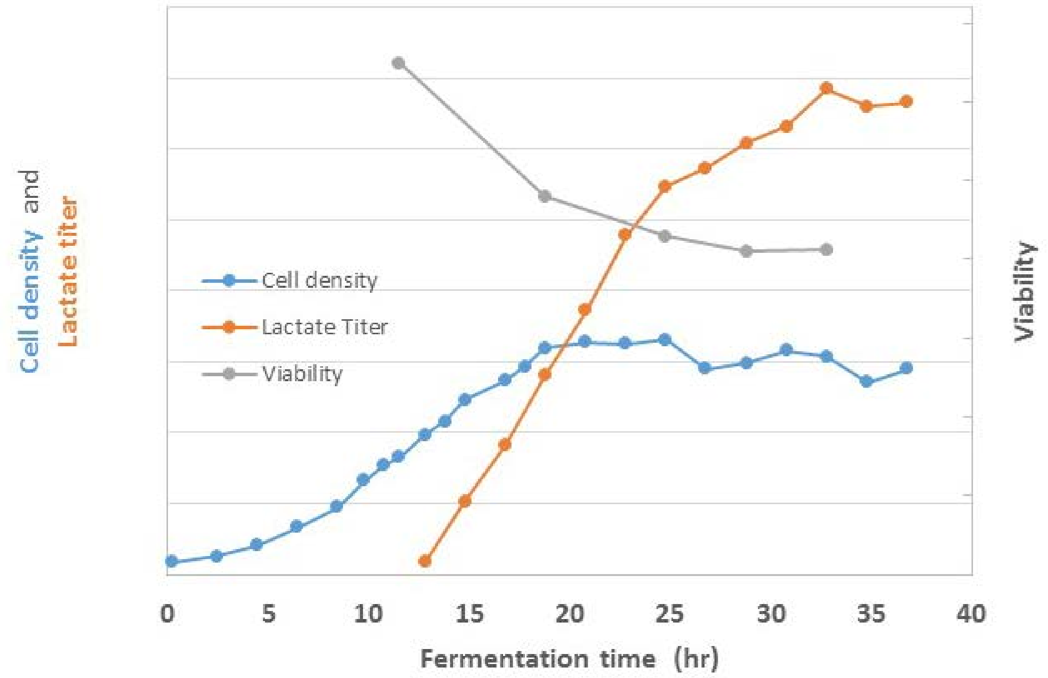
The Key Challenge…

LA production is limited by strain tolerance to LA and/or toxicity resulting from its production



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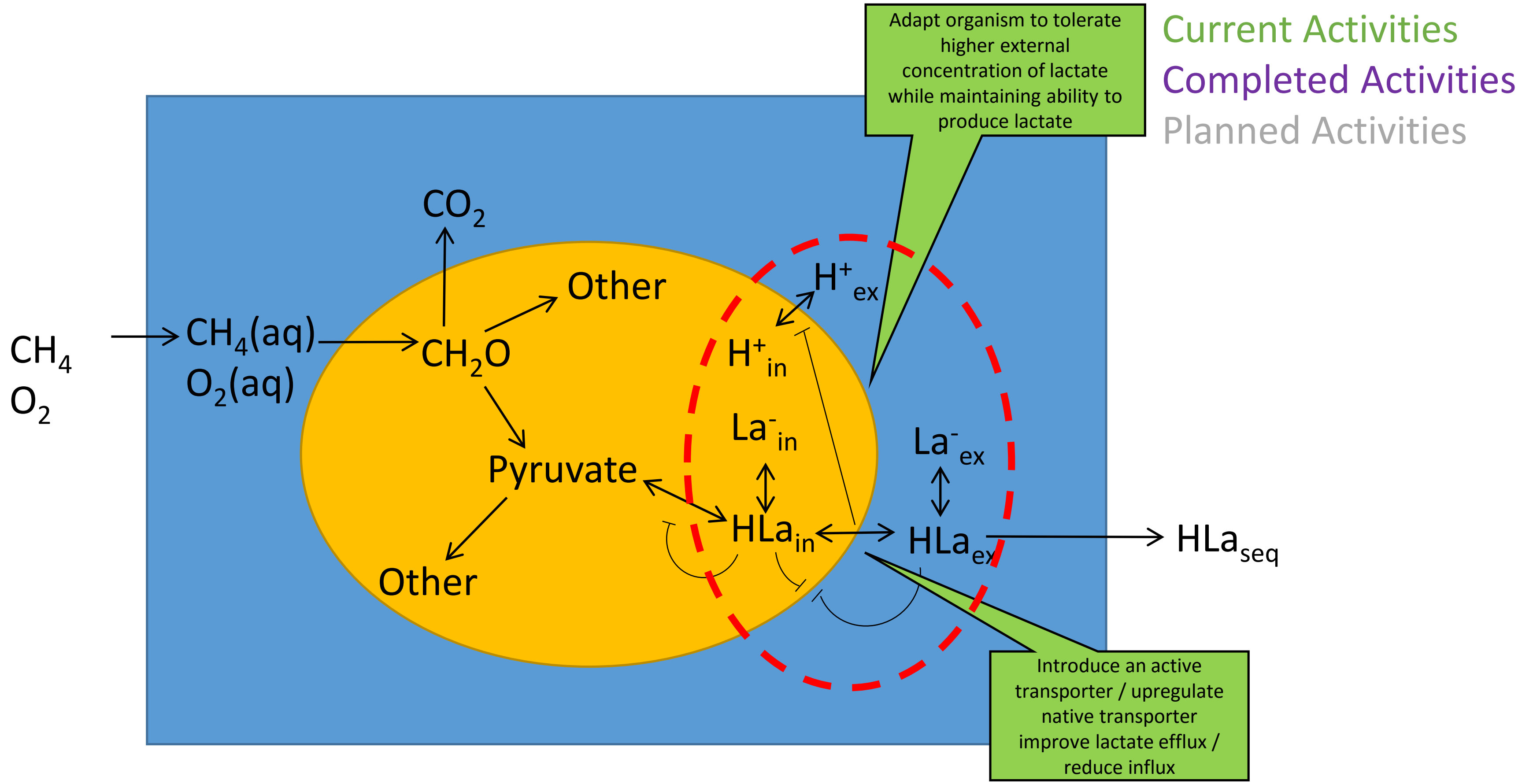
Focus on Higher Titers



Methane to Lactic Acid -- Recapping

- NatureWorks and Calysta developing methane to HLA process (joint R&D effort)
- Expected cost: $multi-MM development cost, larger program includes MN State partnership
- Expected timeline: multi-year effort to pilot plant
- Goals: significantly lower Ingeo™ cost and breakthrough on viable bioproducts enabling cost competitive biofuels (DOE MYPP)
- NatureWorks continues to lead real project with significant $ currently invested, including advancing and testing real biogas value chain (***slides 36-38***)
- Continued leadership towards sustainable/renewable US BioEconomy, consistent with BETO MYP, DOE-USDA *Billion Ton Study*, and multi cross agency BioEconomy Blueprint, Advanced Manufacturing Initiative, and Presidential EPA Green Chemistry

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Acknowledgements (The Team)

NatureWorks — ingeo: naturally advanced materials

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Paloma Rueda
Daniel Wu
Earl Solis

Enrique Baliu
Bo Kim
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Berke Akgun
Son Nguyen
Sonny Zhang
Judy Su
Melissa Nhan
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Minnesota Department of Agriculture
Kevin Hennessy

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Additional Slides

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Responses to Previous Reviewers' Comments

- If your project is an on-going project that was reviewed previously, address 1-3 significant questions/criticisms from the previous reviewers' comments (refer to the 2015 Peer Review Report, see notes section below)
- Also provide highlights from any Go/No-Go Reviews

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Publications, Patents, Presentations, Awards, and Commercialization

- Lori Giver (VP, R&D, Calysta), Bioenergy 2015, Washington, DC (24 July 2015)
  – https://energy.gov/sites/prod/files/2015/07/f24/giver_bioenergy_2015.pdf
- Ken Williams (Program Director, NatureWorks), Minnesota Renewable Energy Roundtable, Morris, MN (3 Nov 2015)….
  – http://www.auri.org/assets/2015/11/Ken-Williams.pdf
- Ken Williams, Methane Bioengineering Summit, San Diego, CA (1 Sept 2015)
  – http://www.methanesummit.org/
- U.S. Patent Application No. 14/898,948 / WO2014205146 A1
  – Title: COMPOSITIONS AND METHODS FOR BIOLOGICAL PRODUCTION OF LACTATE FROM C1 COMPOUNDS USING LACTATE DEHYDROGENASE TRANSFORMANTS
  – www.google.com/patents/WO2014205146A1?cl=en
- ***Please see Appendix slides for status of technology transfer and commercialization efforts***

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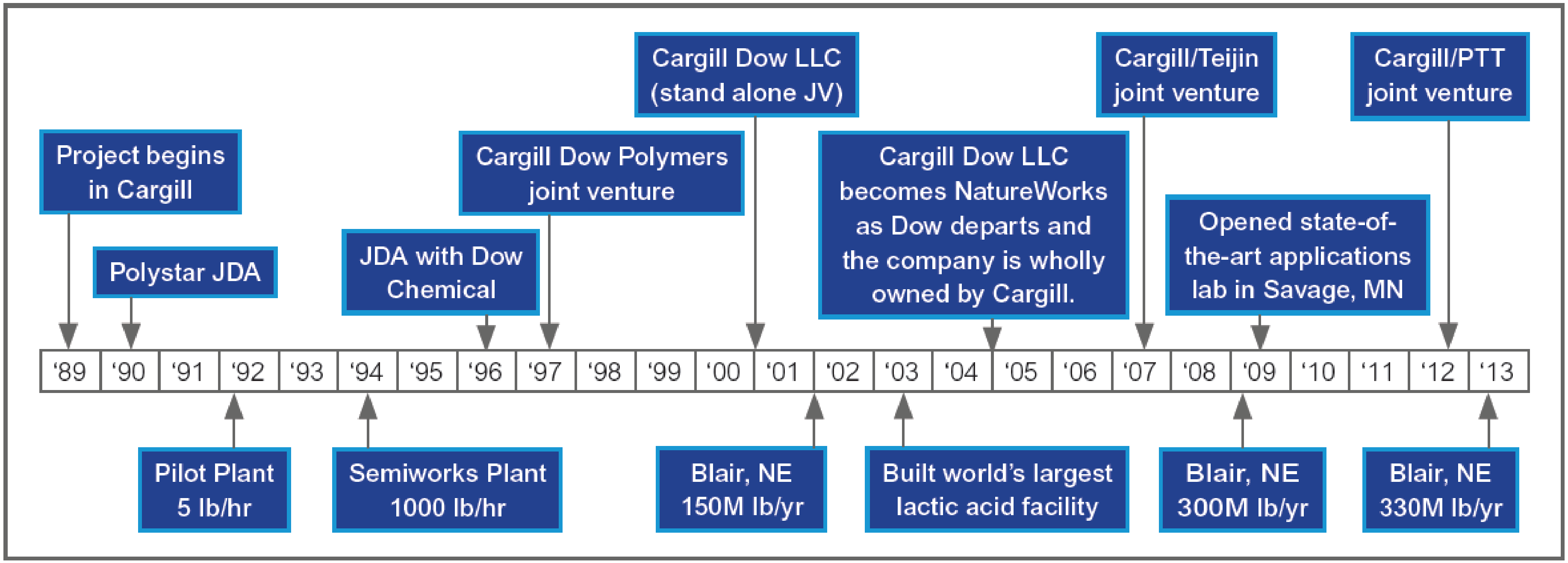
Timeline for
Commercial Scale Manufacturing

It always takes longer than "they" say….



NatureWorks' journey to commercialization

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Who we are

- World's first and largest bioplastics producer
- World-scale plastics facility
- 2002 Winner - Presidential Green Chemistry Challenge
- DOE partner (1998-2008) to develop world-scale biorefinery (over $18M in DOE support)
- Proprietary portfolio of Ingeo bio-polymers & intermediates
- Peer reviewed LCA, strong eco-profile
- Global customer base and product adoption
- Ingeo applications with breadth across markets, geographies, and retail applications

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ingenious
natural selection

Market and Innovation Progression

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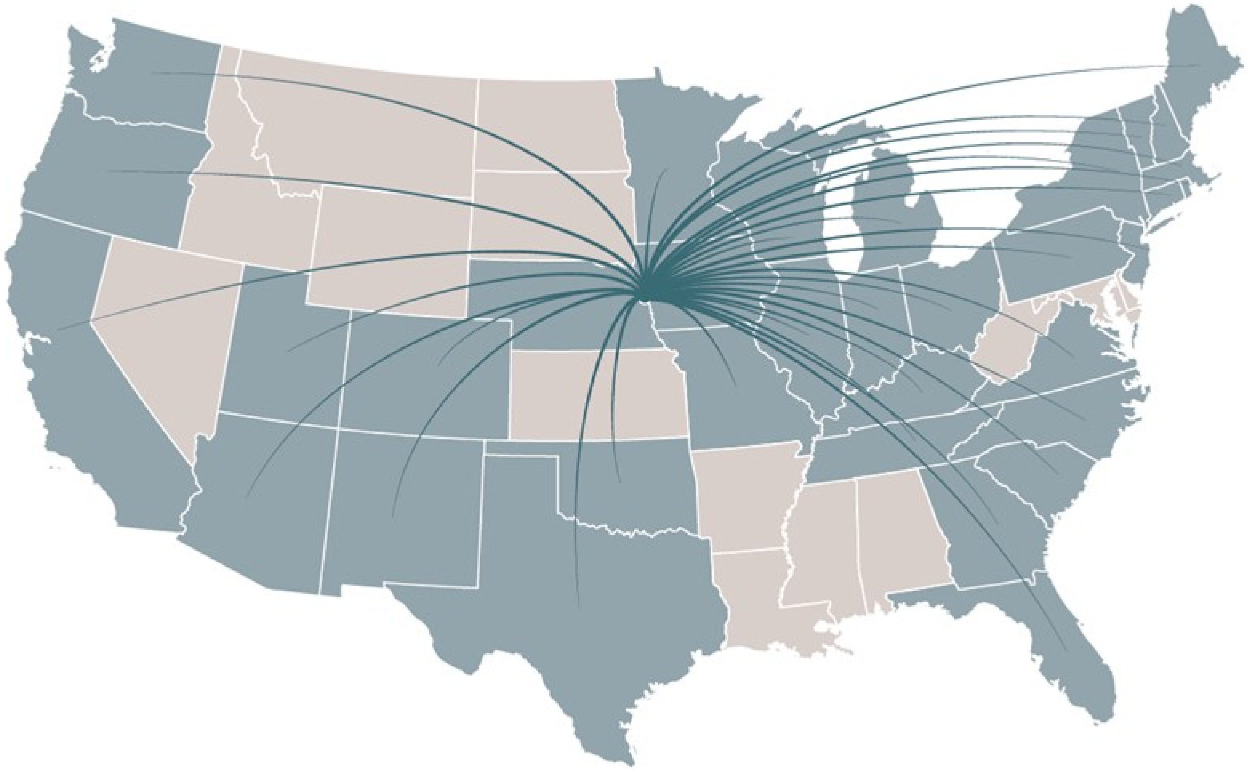
Where Are We Nationally?

NatureWorks is *fueling* green jobs and innovation in the national bioeconomy



Manufacturers in 36 states produce products using Ingeo and retailers like Walmart & Target feature Ingeo packaging or products in all 50 states

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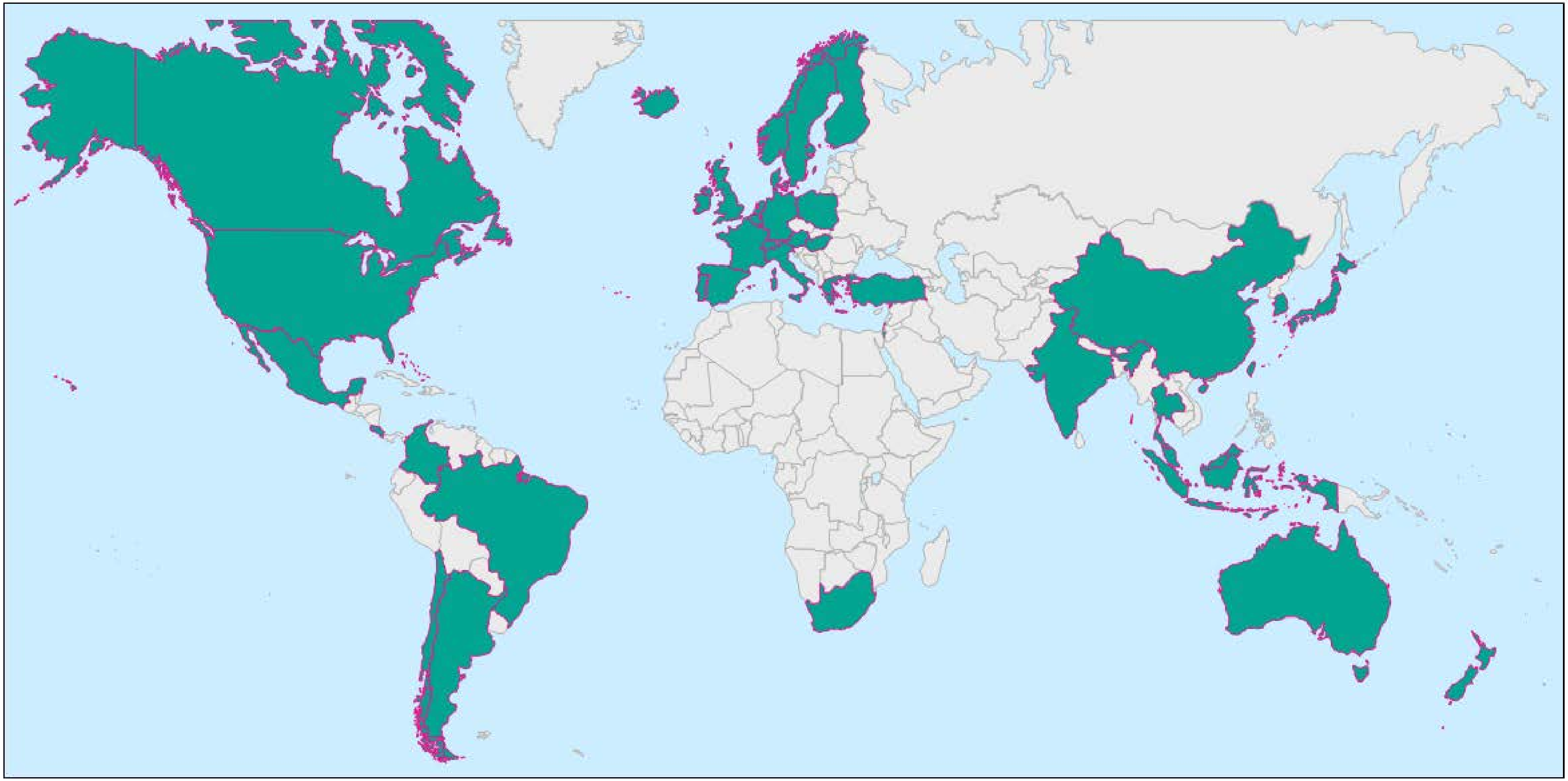
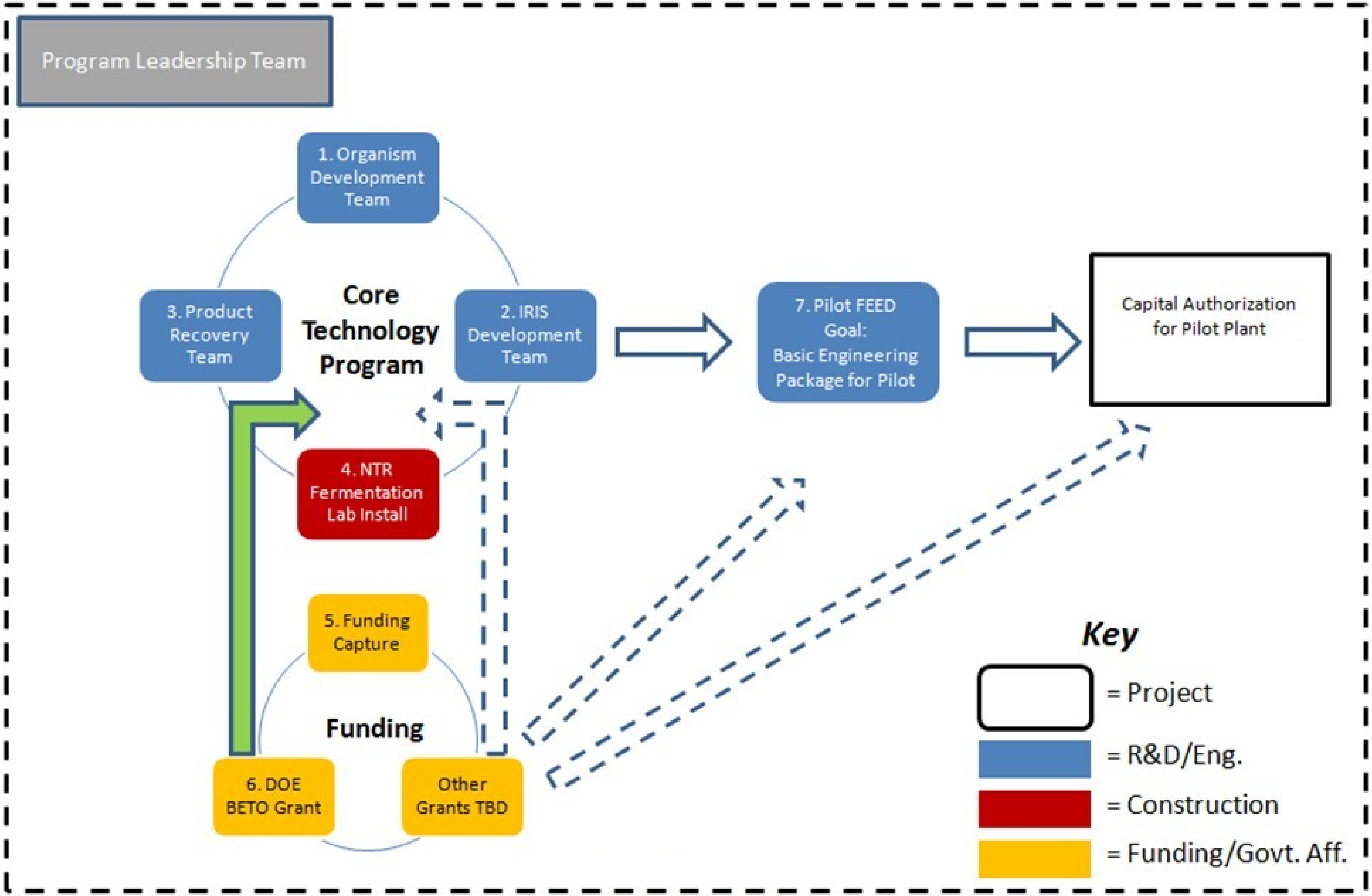
Where Are We Globally?



2 – Approach (Program Management)



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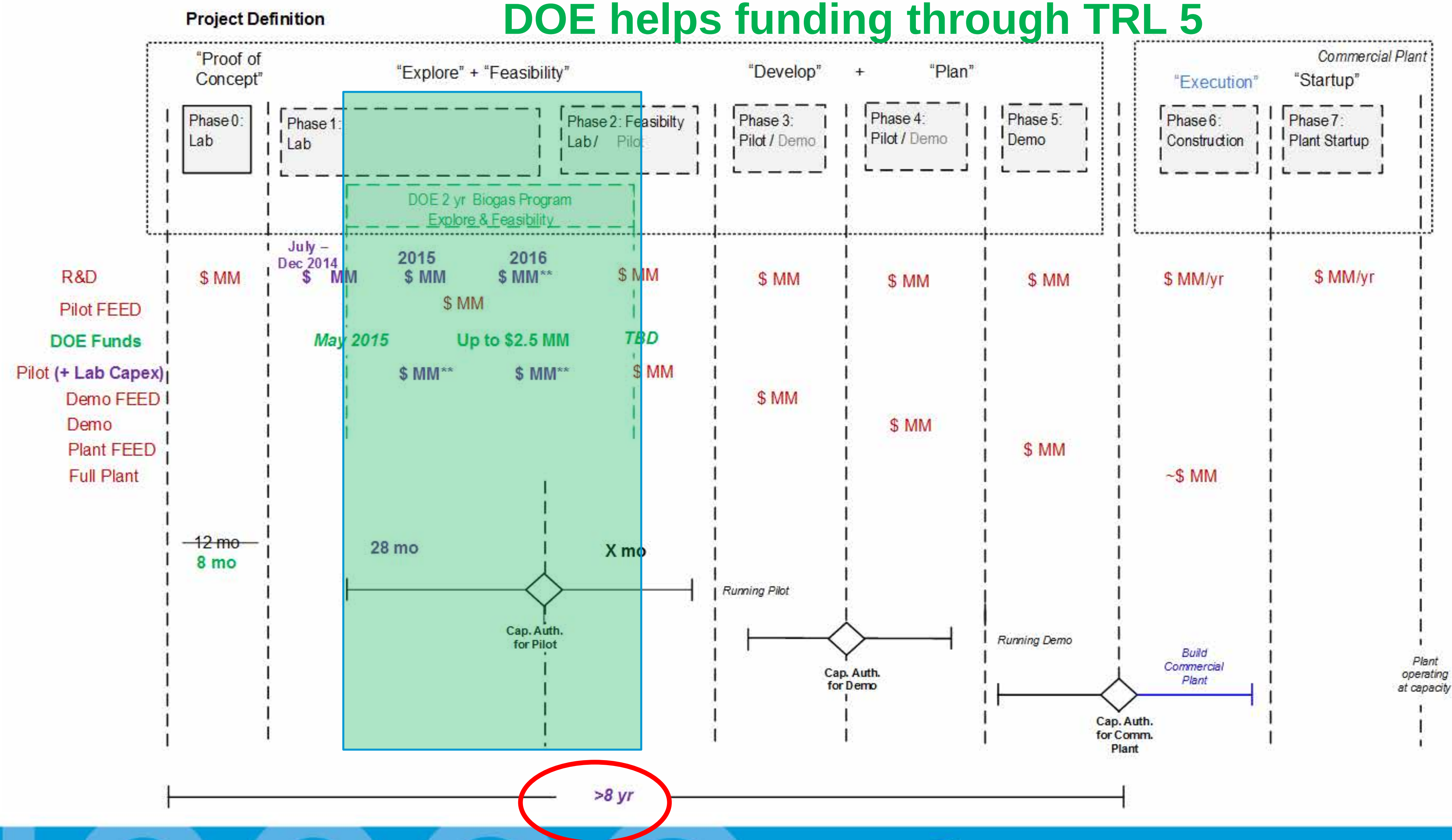
2 – Approach (Program Stagegate Structure)



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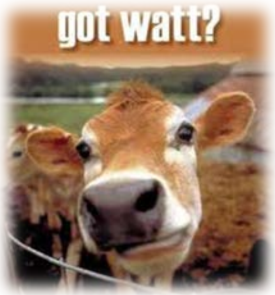
What does "bio-methane to Ingeo" look like …

Green House Gas


Bio-Methane is the R&D focus of this project

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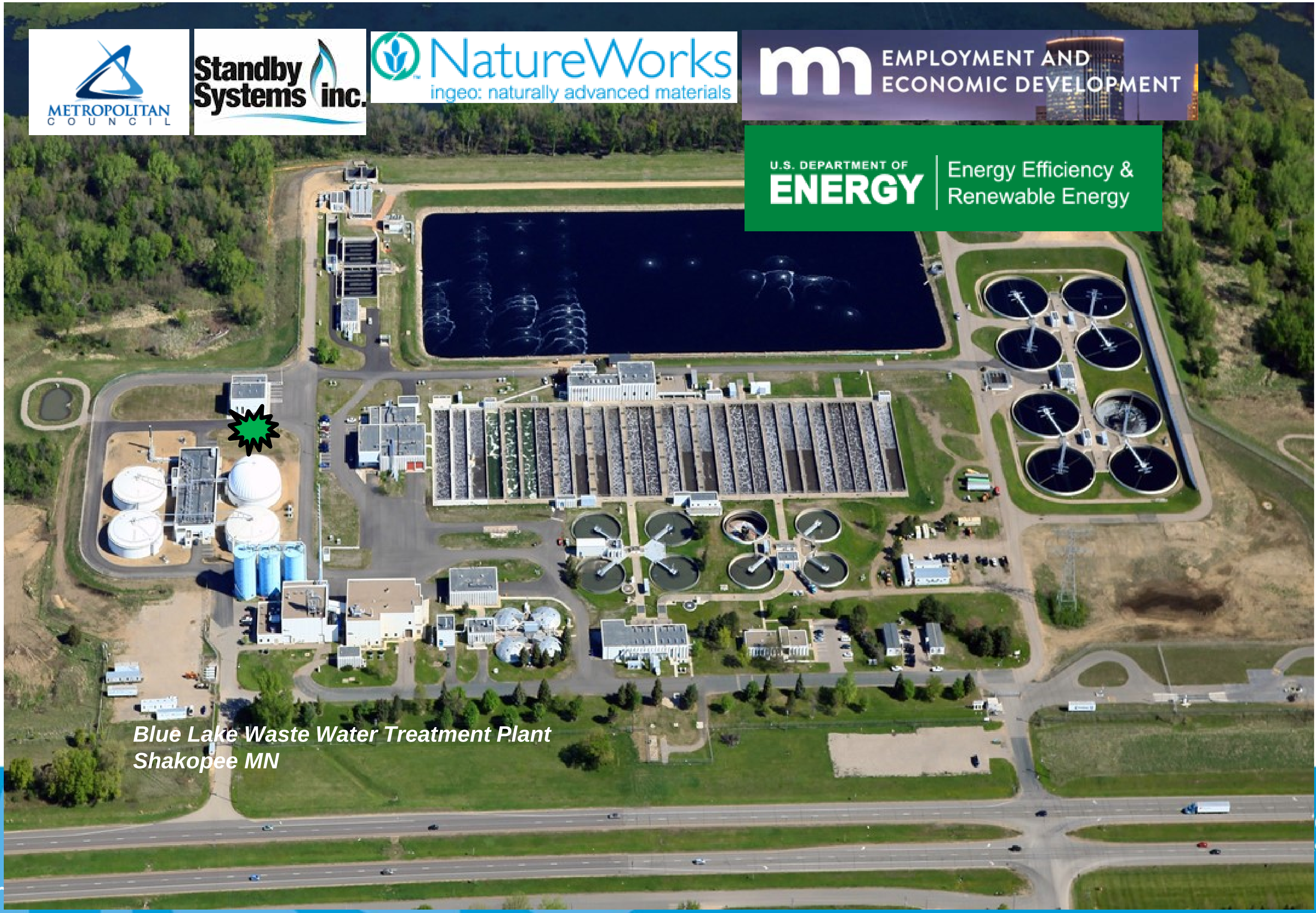
mn EMPLOYMENT AND ECONOMIC DEVELOPMENT

Fermentation → Lactic Acid → Lactide → Ingeo →

Locally harvested biogas for R&D Program

Partnering in MN to Make it Happen

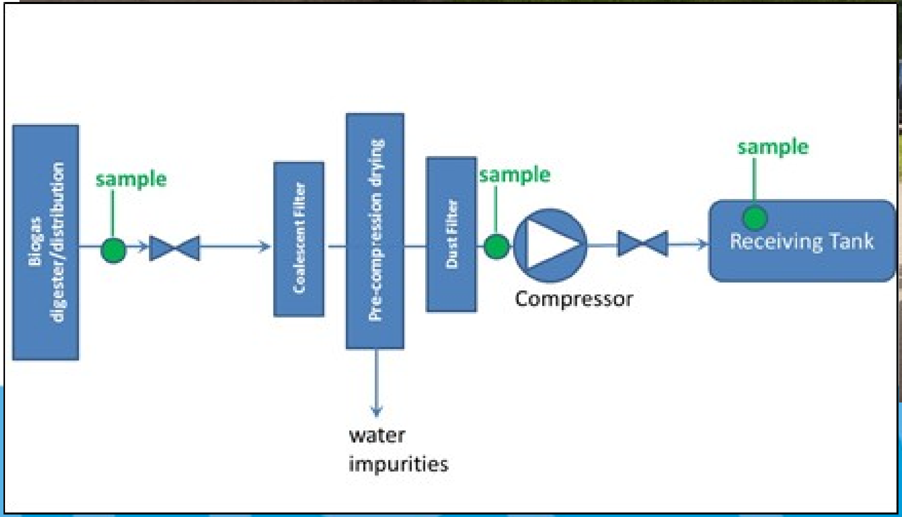


Biogas Compression and Collection (Jan 2015 and June 2016)

Raw biogas from WWTP AD

Cooled to 42ºF, dried with dessicant bed, and compressed to 2500 psig

~1000 scf to support 2L fermentation evaluation



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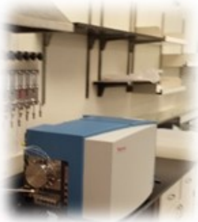
Technology Transfer To NatureWorks Fermentation Lab

- Safe installation of equipment and procedures (MOC, EAP, BHP)
- Off-gas Analysis and GC FID
- Research Cell Bank
- Cell and media characterization
- Dissolved gas analysis
- Serum Bottles:
  - Commissioned May 2016
- Eight 2L fermentation tanks:
  - Commissioned June 2016



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