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 the 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:

1. Corn wet mill ➔ C6 Sugars ➔ Fermentation ➔ Lactide polymerization ➔ Ingeo

Targeted Process:

1. Anaerobic Digestor ➔ Methane from Biogas ➔ Gas Fermentation ➔ Lactide polymerization ➔ Ingeo

**Existing NatureWorks’ process**
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
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 CH₄)
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

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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)
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

And next?

- CO₂ to lactic acid technology?
- CH₄ to lactic acid technology
2 – Approach (Technical)

Path to commercial COGP

- 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

Techno-economic analysis

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

Lab Today

- >$1/lb COGP

Process Development

- Mass transfer, reactor design, scale up

biocatalyst + process

Commercial Plant Future

- ~$0.30/lb COGP

Strain Development

- Productivity, selectivity, inhibition

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

GAP in conversion achievable in Lab vs Pilot Scale

Pilot Plant helps continue down the curve through improved methane conversion

$2.49
Explore + Feasibility Lab Scale

$1.14
Proof of Concept Lab Scale

$0.90
Pilot Scale Project Implementation $5-10 MM

$0.80

$0.50

$0.35

$0.30

= Completed in lab

= Stage gate milestone

% R&D Progress

COPG @ scale & $/MMBtu methane

time

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2 – Approach (Technical)

**Existing technology good enough?**

- **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.
Potential bottlenecks
Improved interfacial flux/mass transfer will be addressed by reactor scale up in a later stage of the project.

Address potential mass transfer/reducing equivalent limitations: experiments indicate not a limitation in 2L format.

Identify and overexpress key bottleneck enzymes in central metabolism.

Downregulate pdc and other pyruvate utilizing enzymes; downregulate TCA enzymes.

Downregulate other routes from formaldehyde (e.g., glycogen).

Adapt organism to tolerate higher external concentration of lactate while maintaining ability to produce lactate.

Introduce an active transporter to reduce intracellular lactate concentrations.

Process to sequester produced lactate.

Select/adapt variants with improved general tolerance to lactate.

Address limitations in ldh by identifying variants with better productivity and reduced product inhibition.

In vitro characterization of the ldh enzymes currently being used.

Address limitations in ldh by identifying variants with better productivity and reduced product inhibition.

Current Activities
Completed Activities
Planned Activities

R&D to date
Executive Oversight
CEO (NTR) & CEO (CE)

Sponsor

Technical Oversight
Calysta (2 members)
NTR (2 members)

Intellectual Property Oversight
Calysta (2)
NTR (2)

Program Management
Calysta (1)
NTR (1)

Project Teams (Phase 1)

Organism Development
Team Lead

Reactor Development
Team Lead

Product Recovery
Team Lead

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

• Biweekly Team Meetings
• Monthly Project Meetings with all Teams
• Team direction set by WBS reviewed/revised monthly

Lead: Joint
Joint
NTR
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
Lactic Acid Production from Biogas

• In addition to methane, biogas contains CO$_2$, H$_2$S and other components that may inhibit growth and/or lactate production.

• **Our strains are able to grow and produce lactate from biogas.**

Typical Biogas composition:
- 60 % methane
- 39 % carbon dioxide
- ~100 ppm H$_2$S
- 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**
Lactic Acid Production from Methane and Biogas

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

Fermentation (2L)

CO₂ inhibition on rate and titer

simulated biogas (60% CH₄/40% CO₂)

methane
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.
4 – Relevance

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)
Methane Sources

A 21st century shift from fossil-fuel to biogenic methane emissions indicated by $^{13}$CH$_4$

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


*Corresponding author. E-mail: hinrich.schaefer@iwaka.co.nz

‡Present address: Lassey Research & Education, Wellington, New Zealand.

§Present address: LowerHZ, Pinnwurt, 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 hydroxylyl 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

NatureWorks

naturally advanced materials

© 2014 NatureWorks
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
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
The Key Challenge…

LA production is limited by strain tolerance to LA and/or toxicity resulting from its production.
Focus on Higher Titers

Current Activities
- Adapt organism to tolerate higher external concentration of lactate while maintaining ability to produce lactate

Completed Activities
- Introduce an active transporter / upregulate native transporter
- Improve lactate efflux / reduce influx

Planned Activities
- 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
Acknowledgements (The Team)

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Daniel Wu
Earl Solis

Enrique Baliu
Bo Kim
Le Tran
Yelena Stegentseva
Berke Akgun
Son Nguyen
Sonny Zhang
Judy Su
Melissa Nhan
Eric Luning
Brandon Doss
Lisa Newman

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Energy Efficiency & Renewable Energy

Jim Hoch
Horia Dinulescu

Blue Lake WWTP
Carol Mordorski
Scott Joseph

Lisa Hughes
John Shoffner

DOE BETO
Christine English
Jessica Phillips
Brandon Hoffman

Corinne Young

Kevin Hennessy
Additional Slides
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
Publications, Patents, Presentations, Awards, and Commercialization

- Ken Williams (Program Director, NatureWorks), Minnesota Renewable Energy Roundtable, Morris, MN (3 Nov 2015)…. 
- Ken Williams, Methane Bioengineering Summit, San Diego, CA (1 Sept 2015)
  - http://www.methanesummit.org/
  - Title: COMPOSITIONS AND METHODS FOR BIOLOGICAL PRODUCTION OF LACTATE FROM C1 COMPOUNDS USING LACTATE DEHYDROGENASE TRANSFORMANTS
- Please see Appendix slides for status of technology transfer and commercialization efforts
Timeline for Commercial Scale Manufacturing

It always takes longer than “they” say….

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
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<td>'89</td>
<td>Project begins in Cargill</td>
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<td>'90</td>
<td>Polystar JDA</td>
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<td>'91</td>
<td>JDA with Dow Chemical</td>
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<td>'92</td>
<td>Pilot Plant 5 lb/hr</td>
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<td>'93</td>
<td>Cargill Dow Polymers joint venture</td>
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<td>'94</td>
<td>Semiworks Plant 1000 lb/hr</td>
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<td>'95</td>
<td>Cargill Dow LLC (stand alone JV)</td>
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<tr>
<td>'96</td>
<td>Blair, NE 150M lb/yr</td>
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<tr>
<td>'97</td>
<td>Built world’s largest lactic acid facility</td>
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<tr>
<td>'98</td>
<td>Blair/Teijin joint venture</td>
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<td>'99</td>
<td>Cargill Dow LLC becomes NatureWorks as Dow departs and the company is wholly owned by Cargill.</td>
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<tr>
<td>'00</td>
<td>Blair, NE 300M lb/yr</td>
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<td>'01</td>
<td>Cargill/PTT joint venture</td>
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<tr>
<td>'02</td>
<td>Blair, NE 330M lb/yr</td>
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<tr>
<td>'03</td>
<td>Opened state-of-the-art applications lab in Savage, MN</td>
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NatureWorks’ journey to commercialization
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

1.7 Billion lb
ingenious
natural selection

Market and Innovation Progression

3D printing

Durables

Semi-durables

Single Use

naturally advanced materials
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
Where Are We Globally?
2 – Approach (Program Management)

Program Leadership Team

1. Organism Development Team

2. IRIS Development Team

3. Product Recovery Team

4. NTR Fermentation Lab Install

5. Funding Capture

6. DOE BETO Grant

Other Grants TBD

7. Pilot FEED Goal: Basic Engineering Package for Pilot

Capital Authorization for Pilot Plant

Key:

- Project
- R&D/Eng.
- Construction
- Funding/Govt. Aff.
2 – Approach (Program Stagegate Structure)

DOE helps funding through TRL 5

Project Definition

“Proof of Concept”

Phase 0
Lab

“Explore” + “Feasibility”

Phase 1
Lab

Phase 2: Feasibility
Pilot

DOE 2 yr Biogas Program
Explore & Feasibility

DOE Funds

R&D

Pilot FEED

May 2015

Up to $2.5 MM

TBD

28 mo

X mo

Running Pilot

Cap. Auth. for Pilot

>8 yr

Phase 3:
Pilot / Demo

Phase 4:
Pilot / Demo

Phase 5:
Demo

Phase 6:
Construction

Phase 7:
Plant Startup

Commercial Plant

“Develop” + “Plan”

“Execution” “Startup”

~$ MM

$ MM/yr

$ MM/yr

$ MM

$ MM

$ MM

$ MM

$ MM

$ MM

~$ MM

Full Plant

Demo

Plant FEED

Pilot (+ Lab Capex)

Demo FEED

12 mo

8 mo

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What does “bio-methane to Ingeo” look like ...
Locally harvested biogas for R&D Program

Partnering in MN to Make it Happen

Blue Lake Waste Water Treatment Plant
Shakopee MN
Biogas Compression and Collection (Jan 2015 and June 2016)

Raw biogas from WWTP AD

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

~1000 scf to support 2L fermentation evaluation
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