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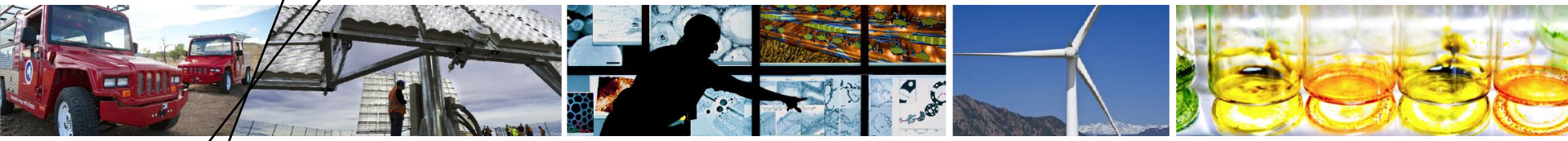
NATIONAL RENEWABLE ENERGY LABORATORY

Biological Upgrading of Waste Gases for Fuels and Chemicals



Philip Pienkos

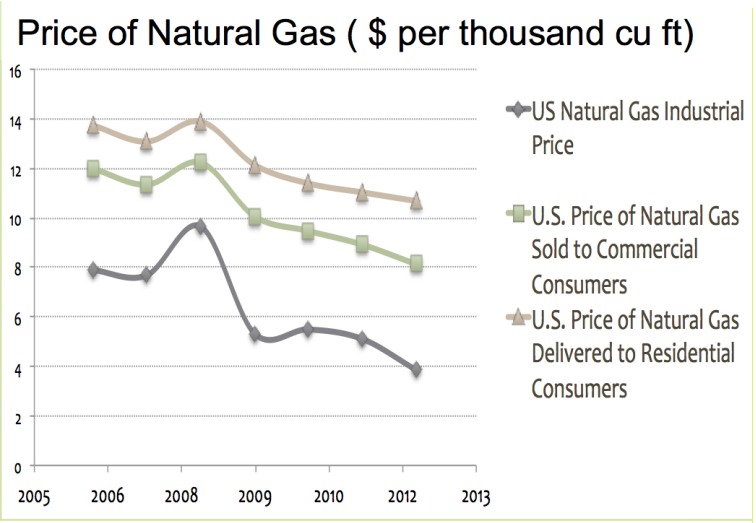
February 29, 2016

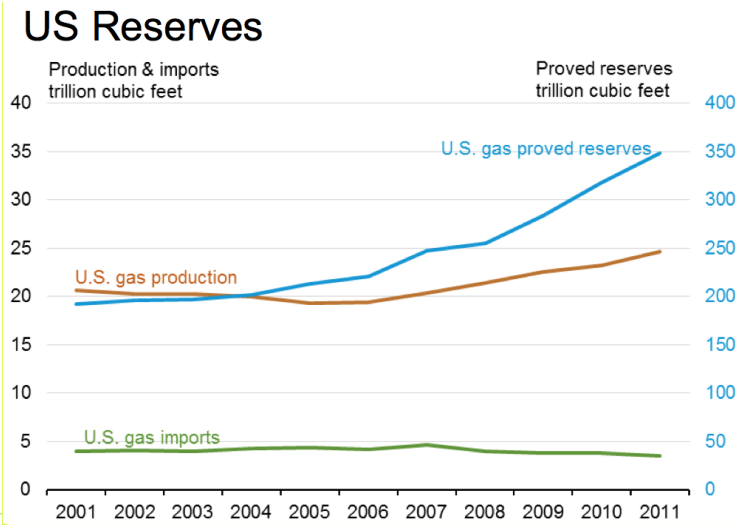
Workshop on Fundamental Science Needs to Address Waste to Energy Conversion

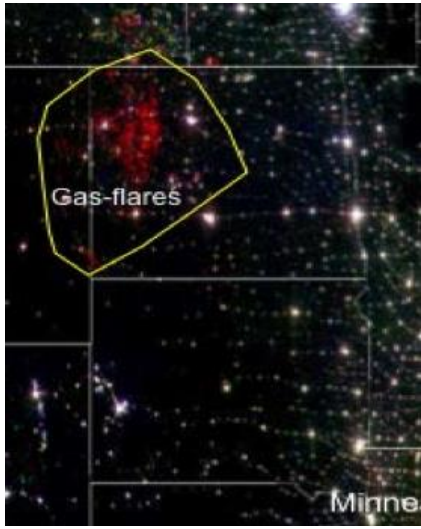
NREL is a national laboratory of the U.S. Department of Energy, Office of Energy Efficiency and Renewable Energy, operated by the Alliance for Sustainable Energy, LLC.

Methane Potential

- Methane is the primary component of natural gas and anaerobic digestion-derived biogas.
- >5 quadrillion BTU of fossil fuel energy is flared or vented globally on an annual basis.
- >9 quadrillion BTU can be generated via AD biogas.
- Massive market and energy value; notably equivalent to ~60% of US electricity production.



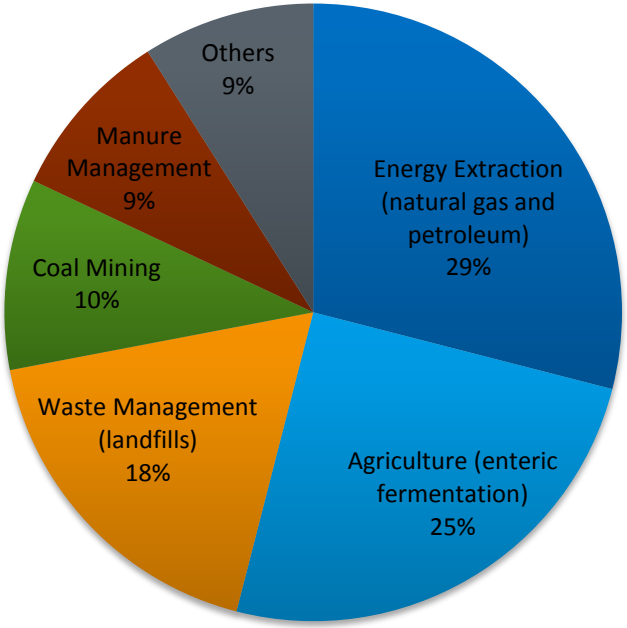


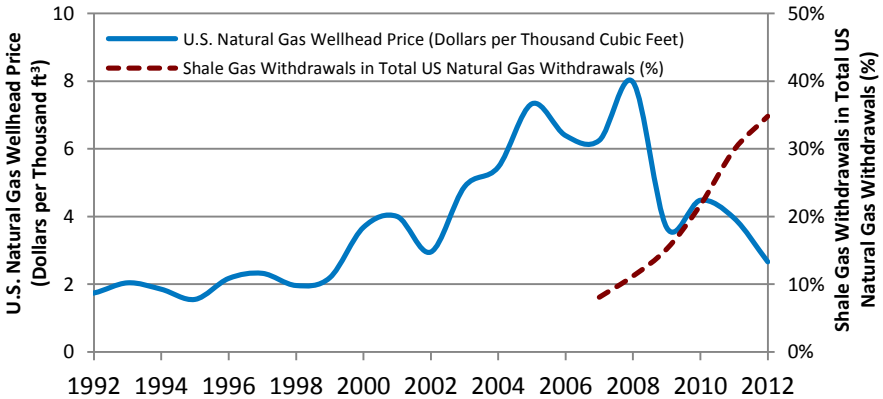


Methane Source

- Most energy dense alkane: 55 MJ/kg
1. Extraction: Natural gas (90% CH_4)
- Anaerobic digestion: Biogas (60% CH4)
- Global-warming potential (GWP) of CH4 :
  - CH4 lifetime is 12 years
  - 100-year GWP: 21 times of CO2
  - 20-year GWP: 72 times of CO2
- Methane Cost
  - Natural gas: pipeline NG vs. industrial waste stream (flaring gas)
  - Biogas: associates with plant size

U.S. Methane emissions by sources from 1990-2012 (US_EPA 2015)

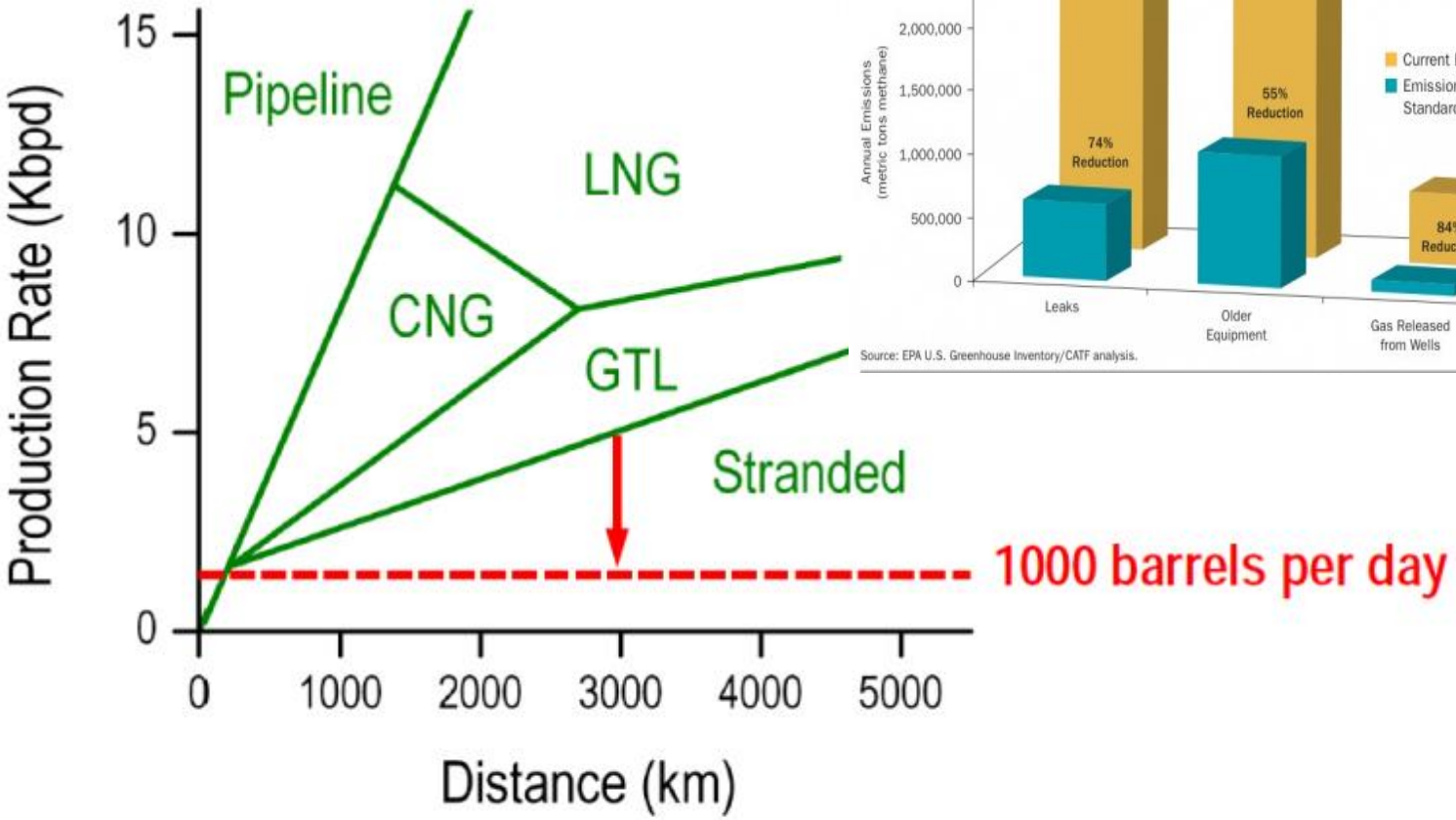


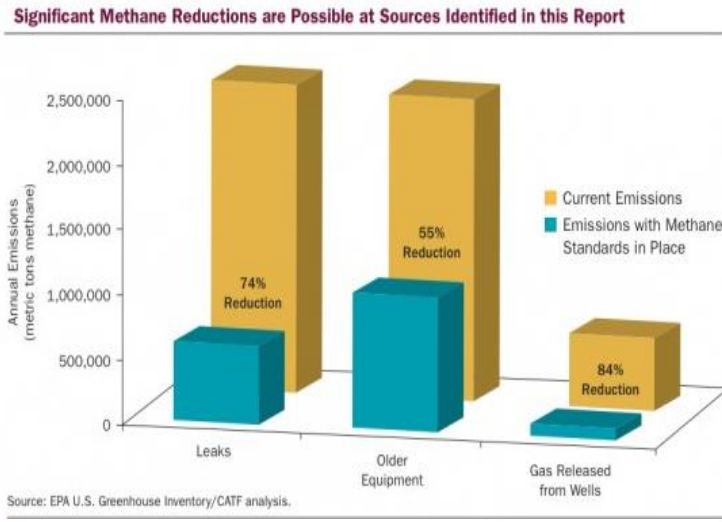


Source: U.S. Energy Information Administration.

Biogas source	$/MMBTU	Reference
Landfill	2-4	California Energy Commission, 2012
Livestock manure	6-10	CALSTART, 2010
Wastewater treatment plants	2-8	Nicholas Institute report,2014
Biomass	6-8	Nicholas Institute report,2014
Dairy farm	11	NRRL report, 2010

Yet profitability of bringing natural gas to market depends on both production rate and distance





Why methane bio-conversion to liquid transportation fuels?

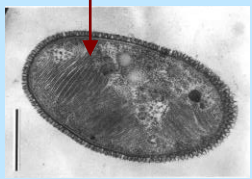
Efficiency	Carbon- and energy-efficient conversion • 66-100% carbon efficiency, 53-79% energy efficiency for conversion of methane to drop-in "designer" (C ≥ 4) products.
Opportunity	Decentralized model for technology deployment • Small-scale facilities allow access to remote/stranded gas
Process Integration	Reduction in conversion steps of natural gas to liquid fuels • Most/all of reactions takes place within engineered microorganism
Monetization	Cost-effective production of liquid transportation fuels from domestic energy source • Higher yield, lower pressure, lower temperature, lower CapEx

Methane Upgrading at NREL

- **ARPA-E project exploring biological upgrading of natural gas was initiated in FY13.**
  - Primarily targeting development of novel catalytic technology to directly convert methanotrophic lipids to liquid fuel.
  - Established baseline TEA models
  - Includes partnership with SDSU, UW, Lanzatech, and Johnson Matthey
- **Biogas-to-Liquid Fuels and Chemicals (Lactate) initiated in mid-FY14.**
  - Proof-of-concept co-production of lipids and lactic acid
  - Project currently pursuing CCE enhancements
  - Includes subcontract with SDSU
- **Biogas-to-Muconic Acid FOA Award Initiated in Q3-FY15**
  - Targets strain engineering, process intensification, and bioprocess integration
  - Includes partnership with Farmatic, Metabolon, NCSU, and SDSU

Bio-GTL: Novel Biocatalyst For Conversion Of Natural Gas Into Diesel Fuel

Natural Gas (CH_4)



Methylomicrobium buryatense 5GB1

Lipids

Extraction

Fuel precursors

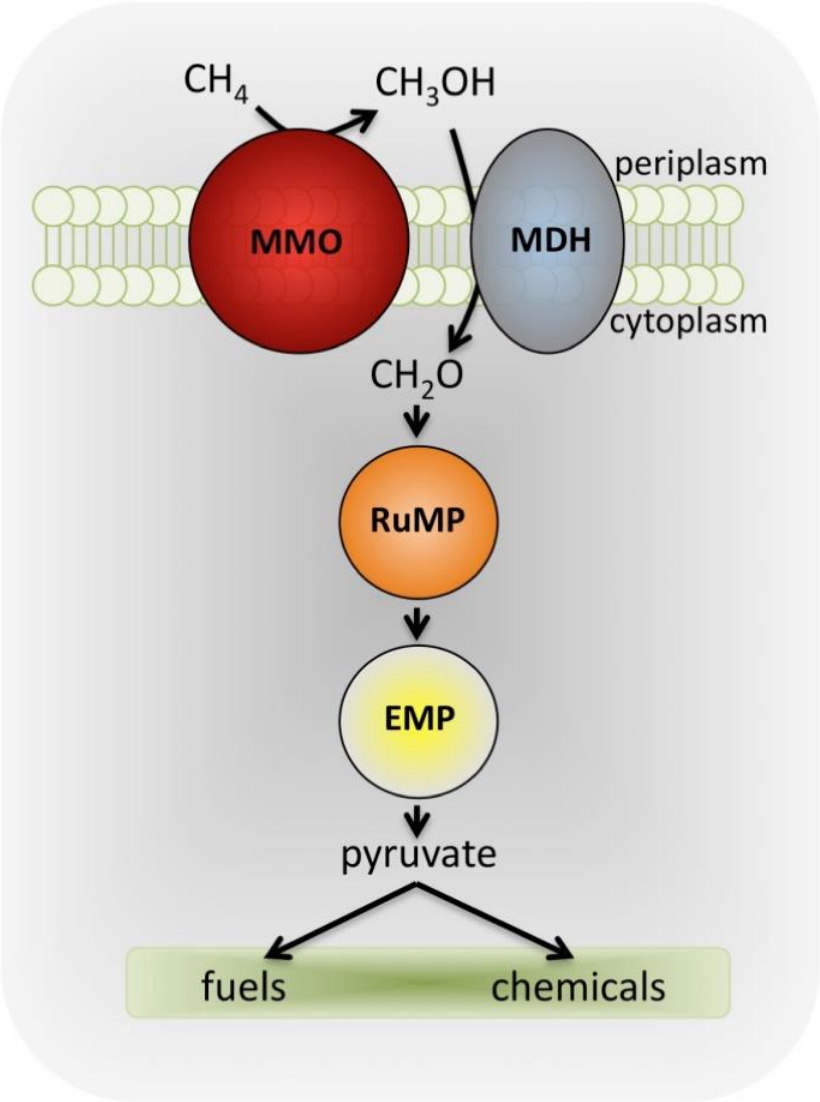
Catalytic upgrading

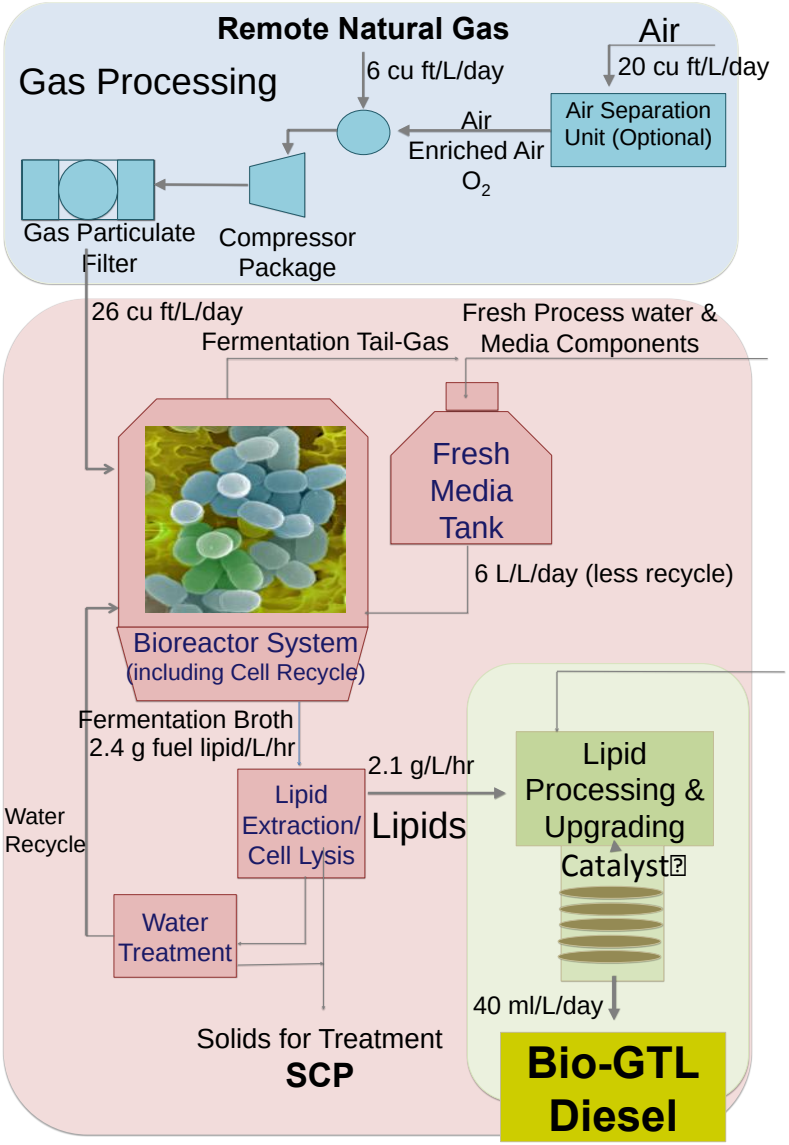
BioGTL Diesel

Overall goal: Convert methane to an alkane mixture using the methanotroph *Methylomicrobium buryatense* 5GB1 to generate membrane lipids from methane, extracting the lipids as fatty acids and converting them to alkanes

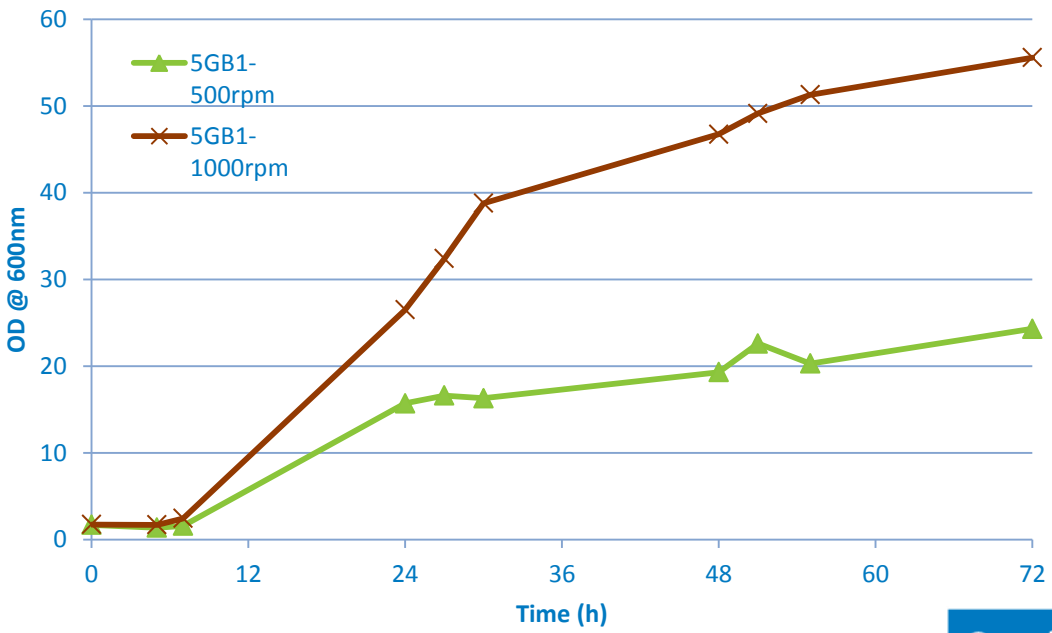
Challenges: Increase lipid productivity via reactor design and metabolic engineering; optimize lipid extraction and develop high efficiency and robust catalyst

Bio-GTL Project





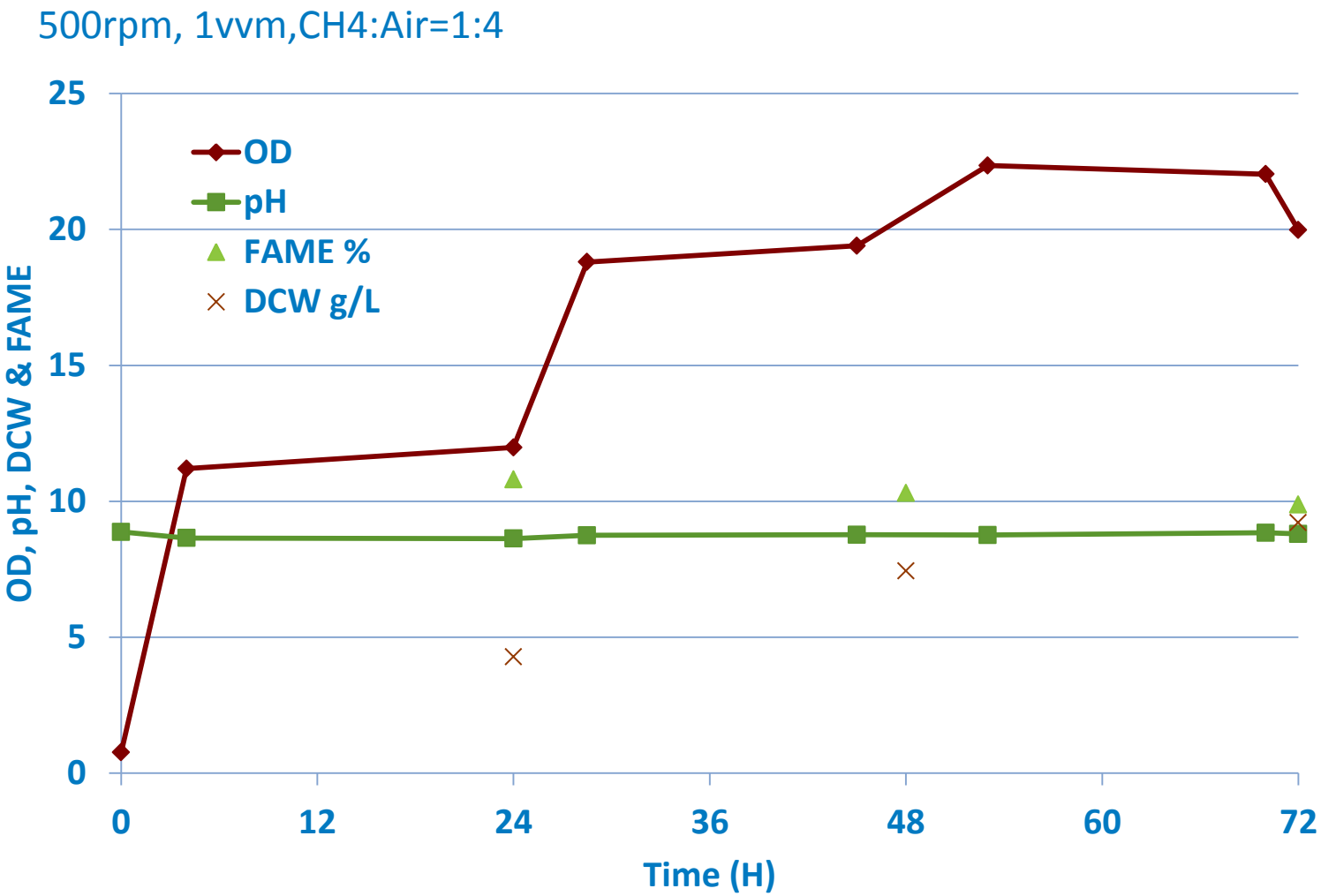
Effect of Agitation Rate on Growth and Composition



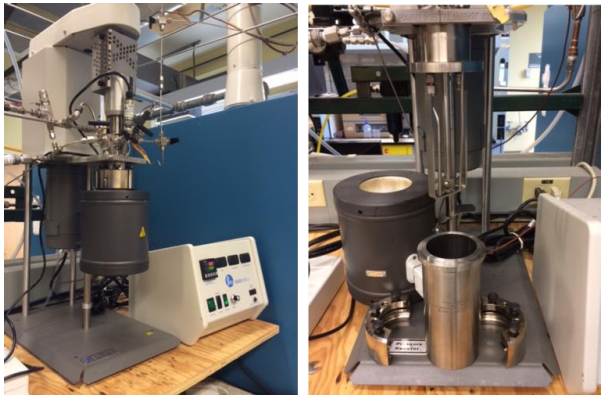
- Medium : 8X nitrate, 2X phosphate, and 4X TE
- CH4: Air=1:4

Strain	5GB1			
	500rpm		1000rpm	
	FAME	Glycogen	FAME	Glycogen
24	9.84%	10.1%	7.94%	12.3%
48	9.59%	9.8%	6.10%	18.7%
72	8.54%	8.8%	5.49%	23.1%

Batch Fermentation for Biomass Production

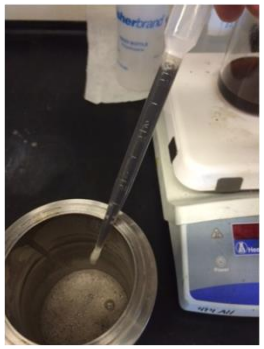


Oil Extraction for Upgrading



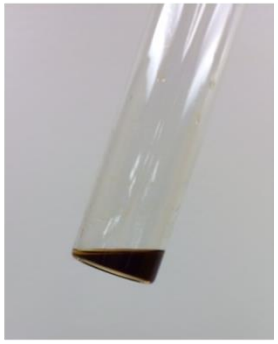
Parr reactor (Colorado School of Mines)
300 mL working volume

Pelleted biomass
16.6 g DCW
FAME=9.88% DCW



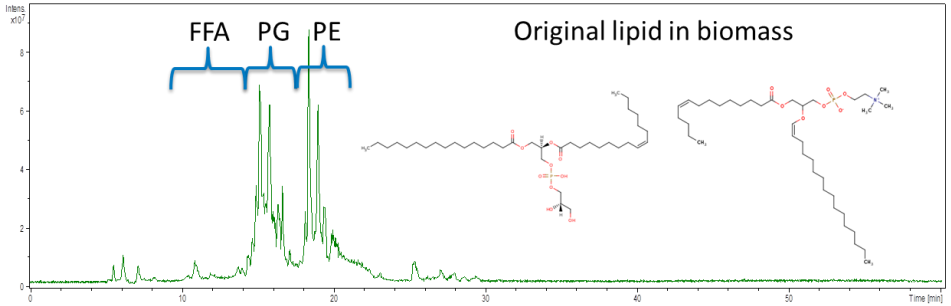
After alkaline treatment

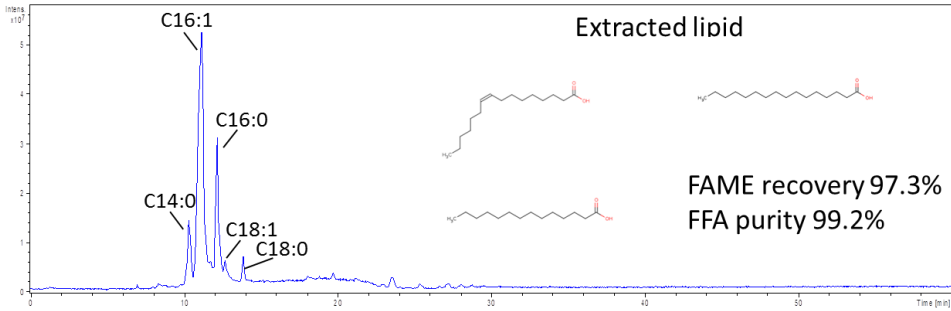
Phase separation



Oil = 1.53 g
Recovery = 92.3%

LC-MS Ion Trap analysis



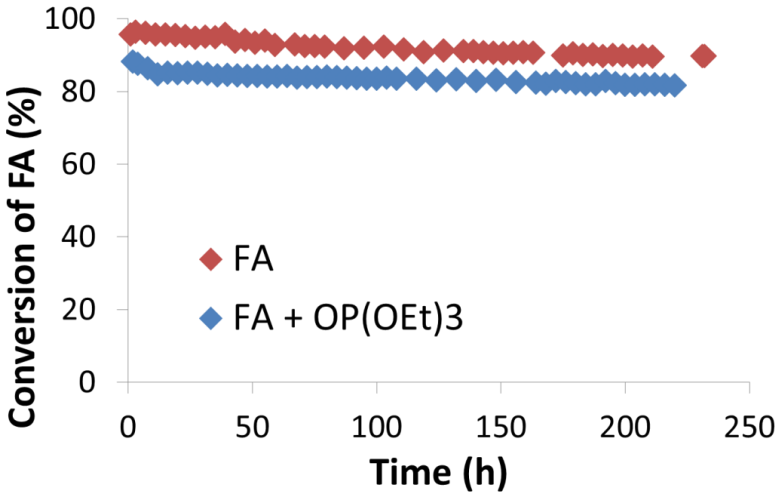


Upgrading Model Lipids

Dodecanoic acid

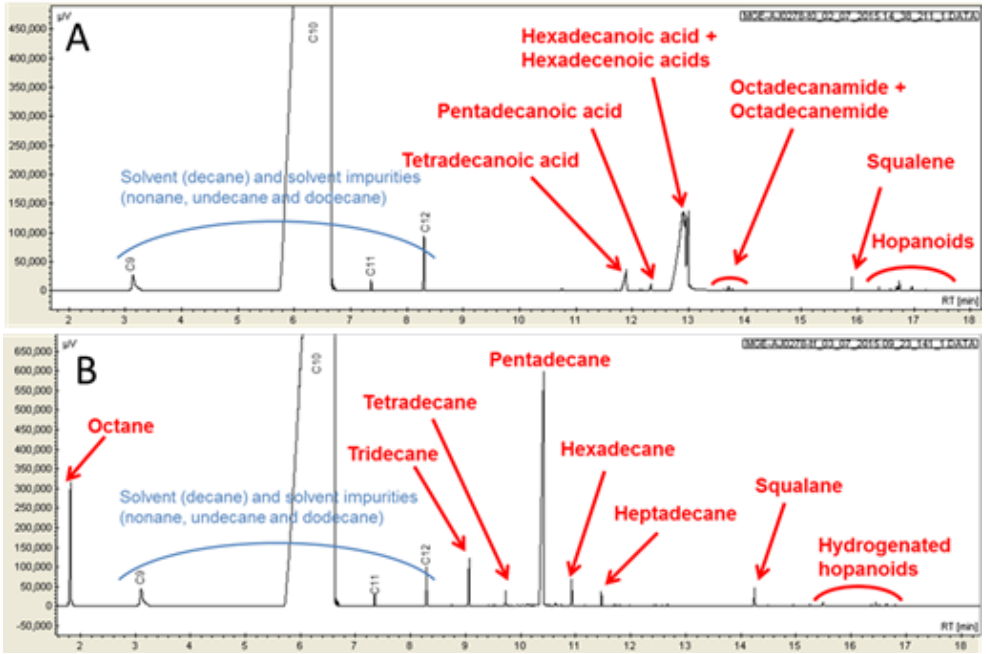
Tri(ethyl)phosphate

- ⇨ Fatty acid with slightly shorter chain length compared to real feed (improved solubility for lab scale tests)
  - ✓ but effect of C10 *versus* C16 is minimal on catalyst deactivation
- ⇨ Tri(ethyl)phosphate used as phosphorus source
  - ✓ same type of phosphorus as expected from the bacteria (phosphate)
- ⇨ Phosphorus level: 200ppm compared to fatty acid
  - ✓ higher level of phosphorus compared to real feed to simulate long term effects



- ⇨ Tests conducted allow full conversion to allow rate of deactivation to be quantified
- ⇨ Catalyst shows good performance in extended operation time
- ⇨ No difference in the presence of 200ppm of phosphorus

Methanotroph Lipid Upgrading

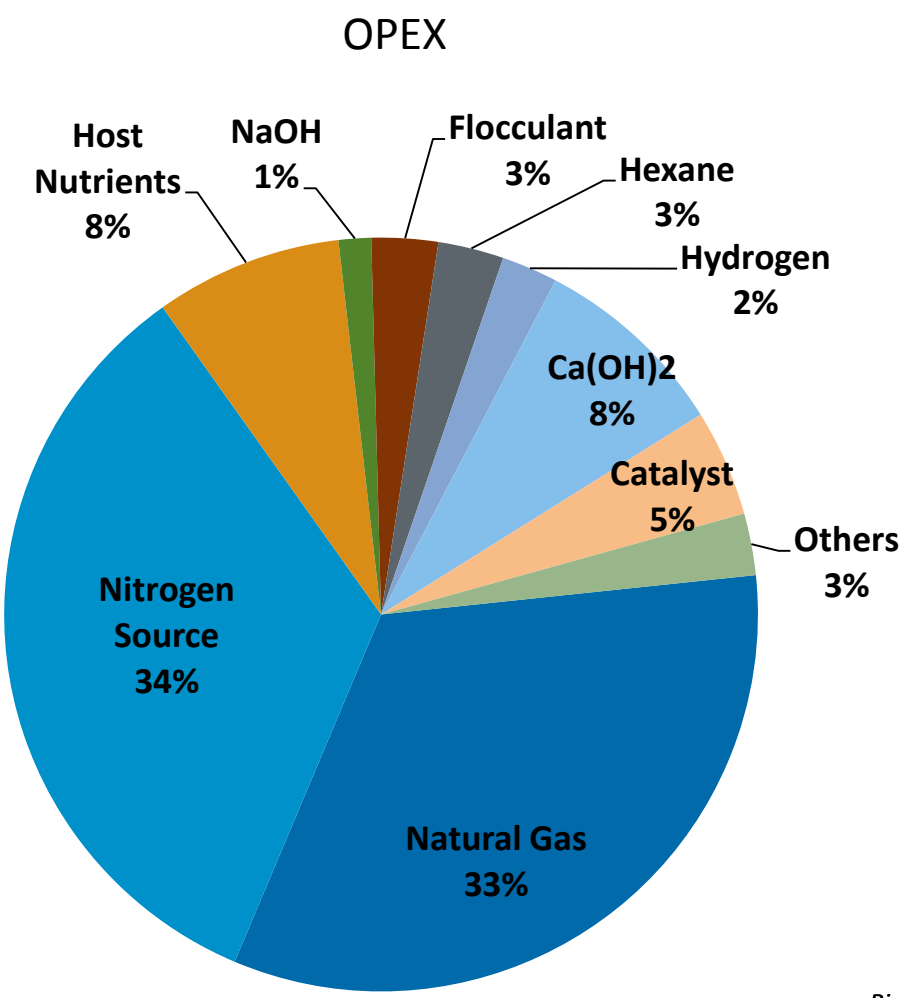


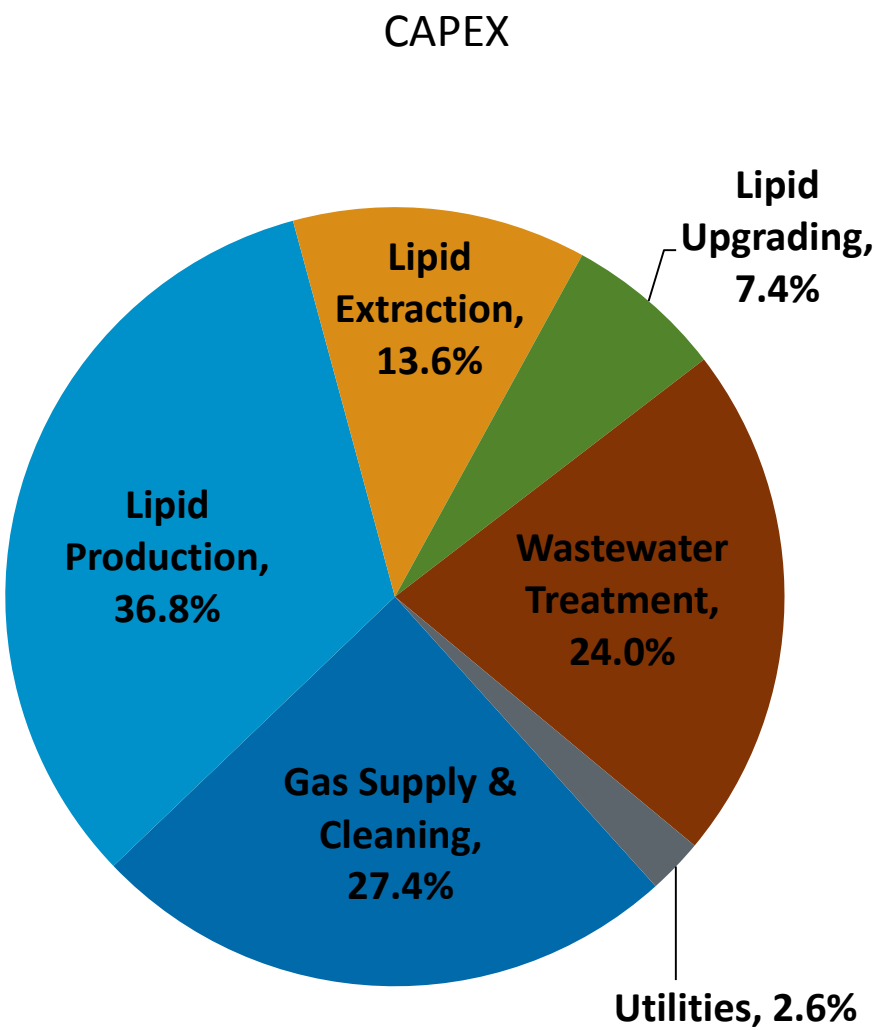
GC traces of the reaction medium before (A) and after (B) the upgrading of the extracted bacterial oil. Reaction conditions: bacterial oil (0.5mL), decane (25mL), catalyst (5% Pd on silica, 100mg), 40bar H_2, 360°C, 6h.

Fatty acid	Content %	Hydrocarbon	Content %
C14:0	6.1	C13	6.2
C15:0	1.1	C14	1.8
C16:0	18.2	C15	87.9
C16:1	73.6	C16	2.8
C18:0	0.5	C17	1.3
C18:1	0.3		

Fatty acid profile in extracted oil and hydrocarbon profile in finished biofuel product

Distribution of raw material cost and capital cost

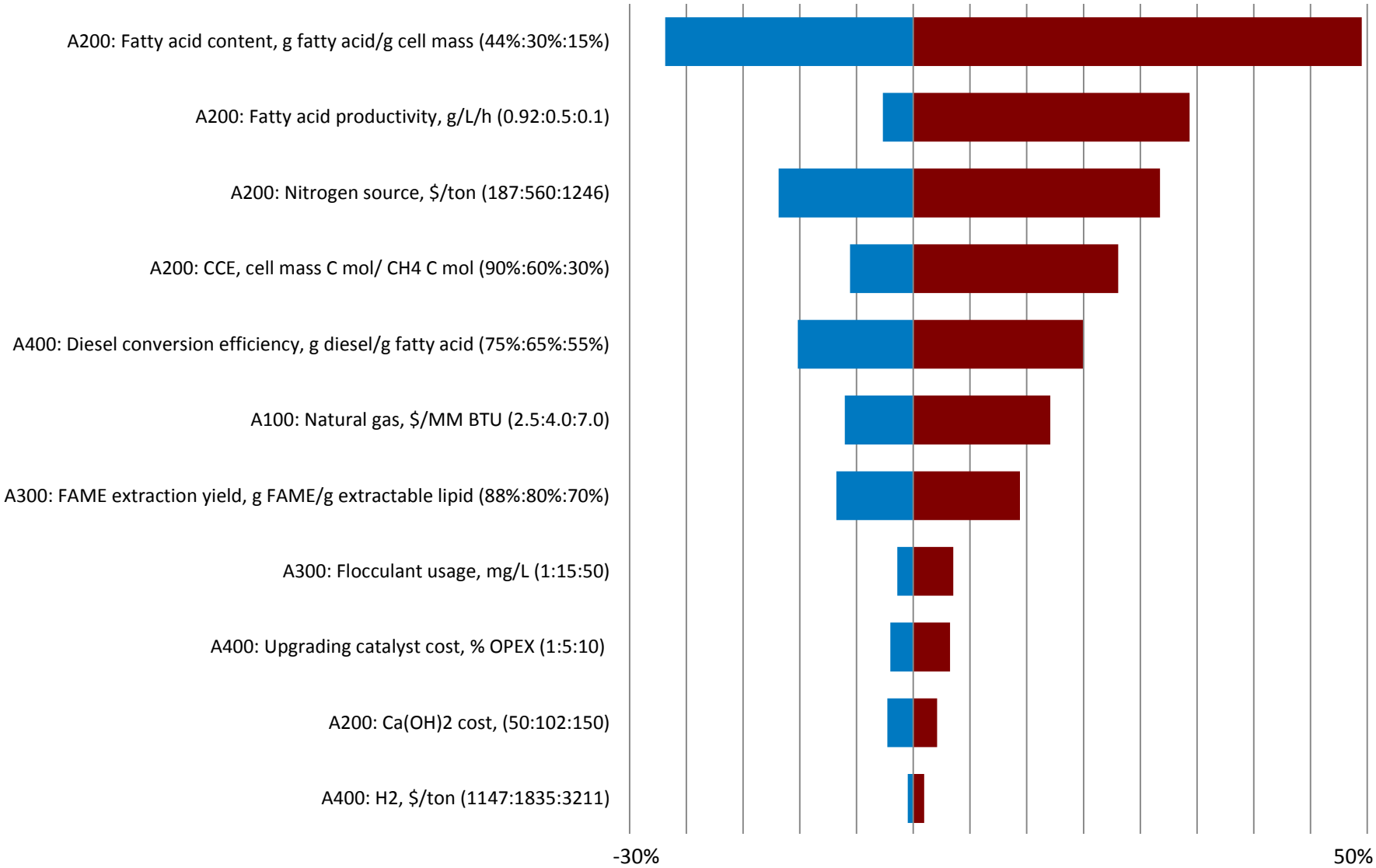




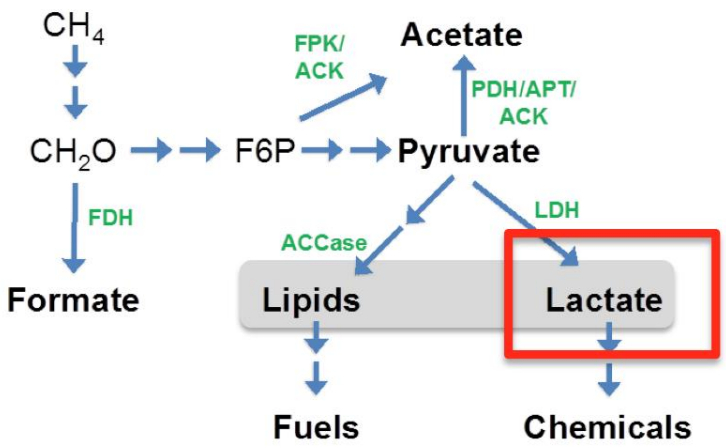
BioGTL

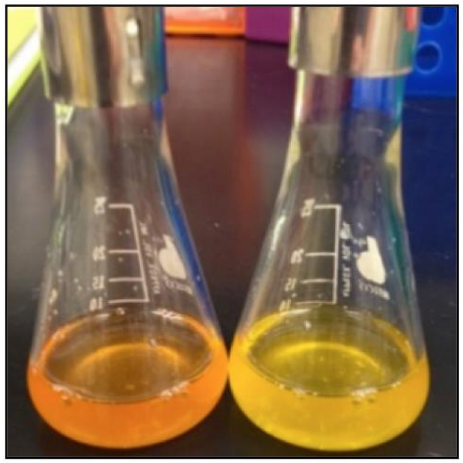
* Host nutrients include MgSO4 and phosphate. $Ca(OH)_2$ for recovery of NaOH from Na2CO3. Chitosan as flocculant agent

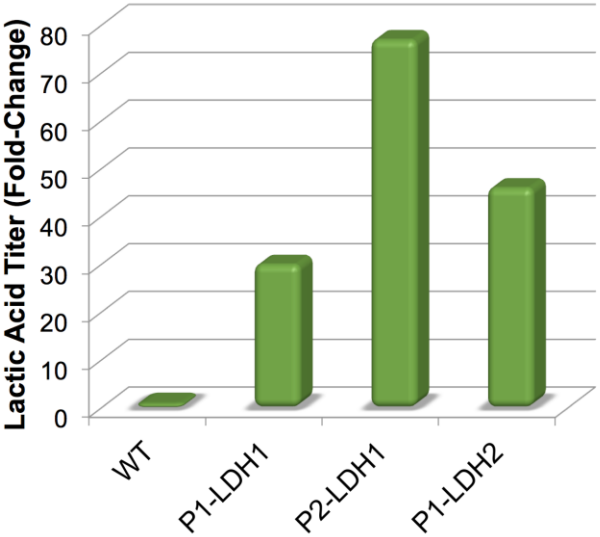
Single-point sensitivity analysis – tornado chart



Methane Biocatalysis to Lactic Acid



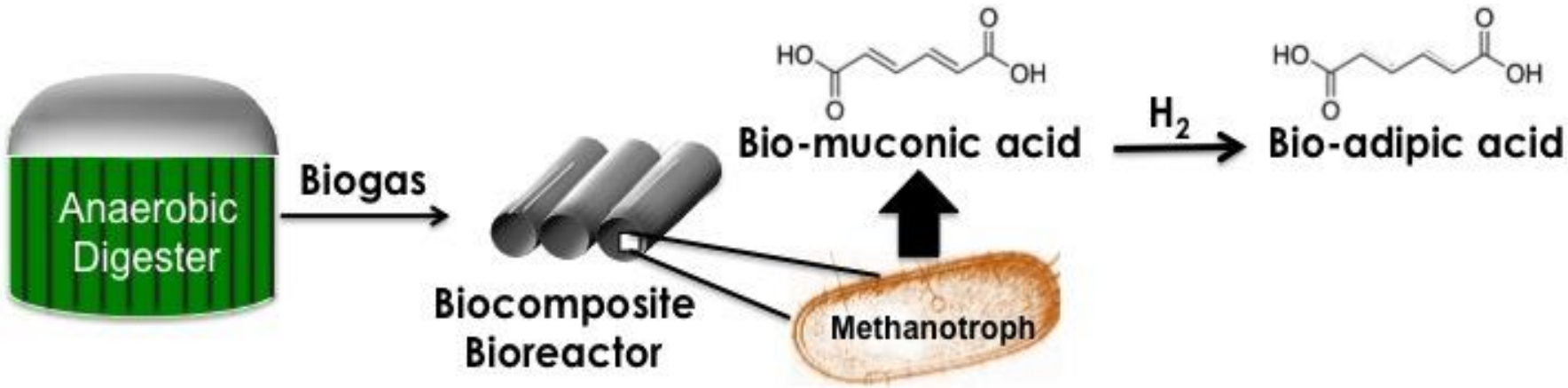




- Effective implementation of genetic engineering strategy targeting lactic acid biosynthesis
  - Titers >1g/L
  - No impact on lipid titers
- Preliminary TEA models generated; will serve as baseline for muconate models
- Current focus is on improvement of CCE

BCU FOA: CH_4-to-Muconic Acid

- Development of a biological route for conversion of biogas to MA
- Critical success factors will include enhanced i) strain, ii) bioreactor biocatalyst intensification, and iii) integration development.



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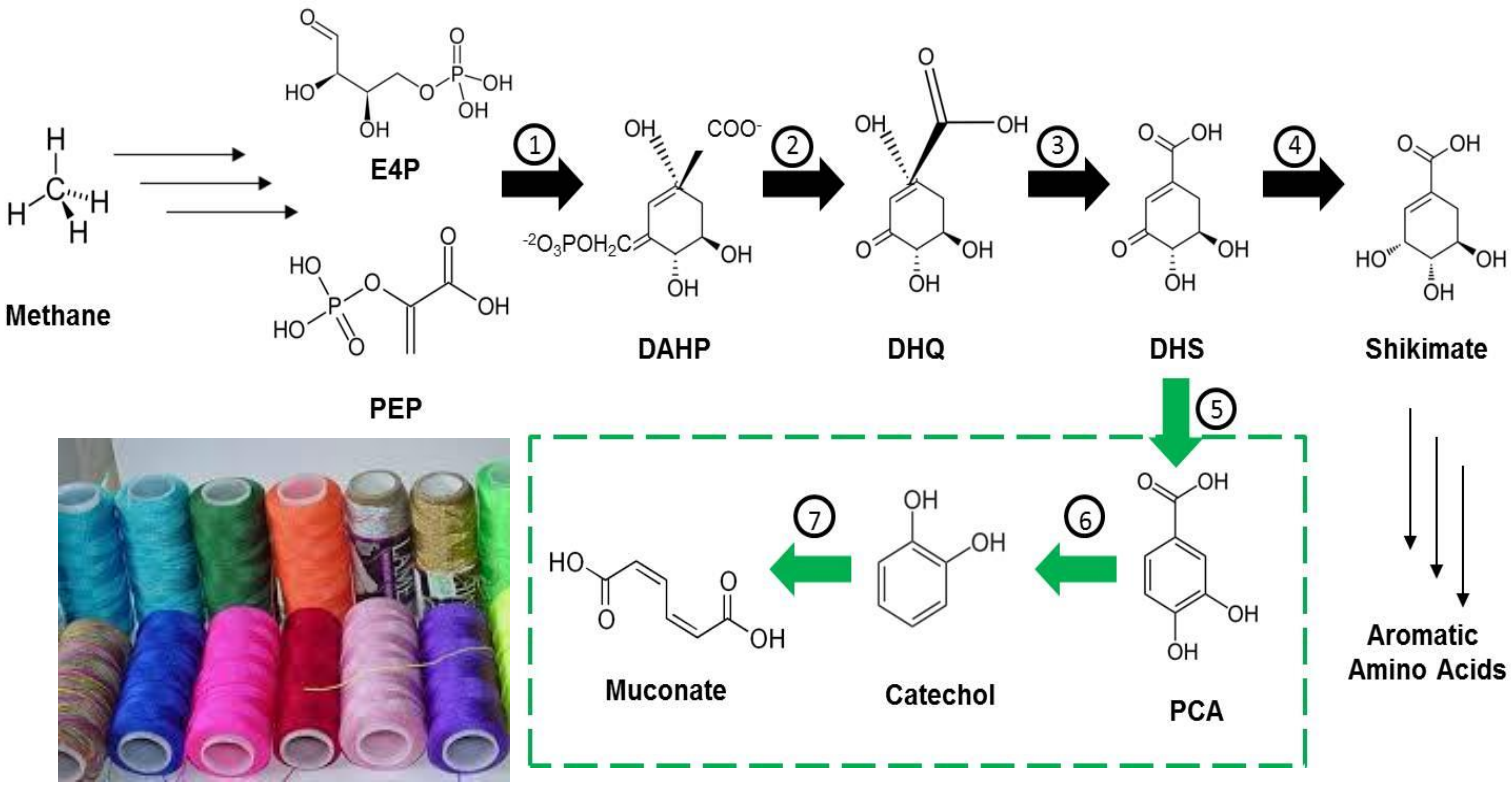
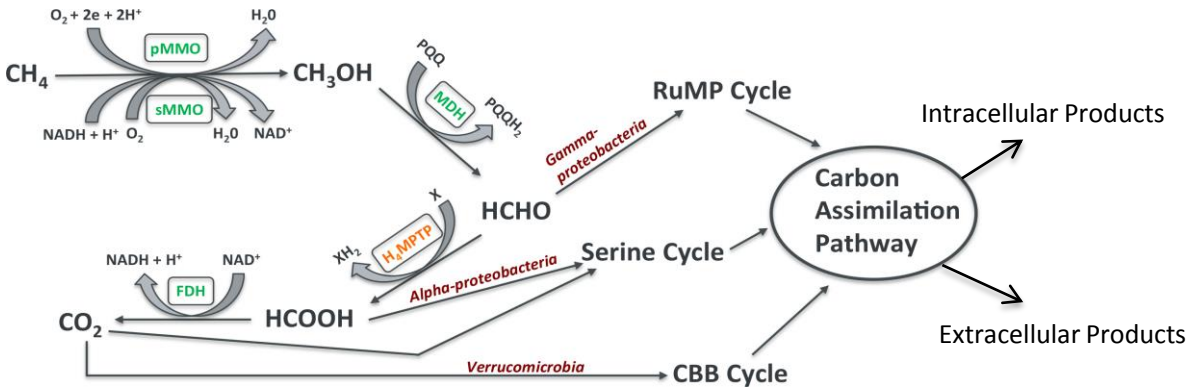
Methane Biocatalysis to Muconic Acid



- Examining process economics within the context of stand-alone AD, stranded natural gas, and as a bolt-on technology for a biorefinery.
- Process intensification dramatically enhances mass transfer and productivity…and techno-economics.

Biological Conversion of Methane

❖ Methanotrophic bacteria (Aerobic cultivation)

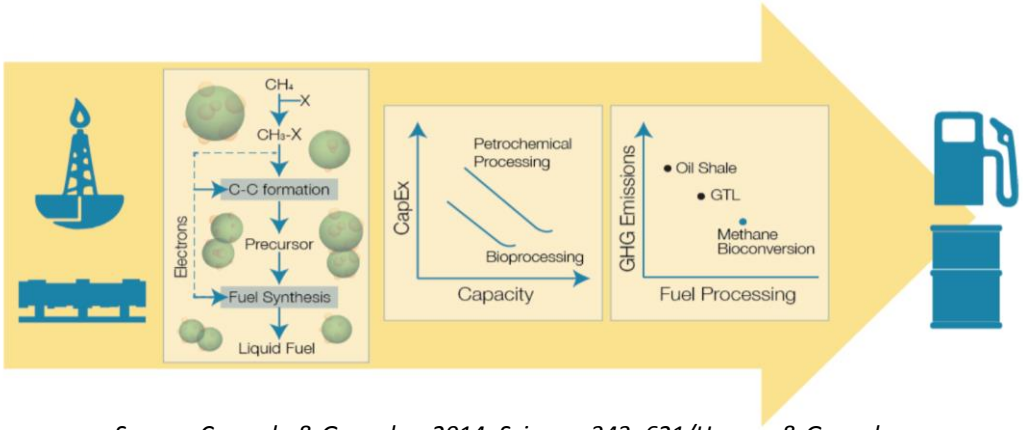


❖ Selected products

Product	Formula	Application
Formate	CH_2O_2	Chemical
Glycogen	$C_6H_{12}O_6$	Chemical
Ectoine	$C_6H_{10}N_2O_2$	Health care
Astaxanthin	$C_{40}H_{52}O_4$	Health care
Sucrose	$C_{12}H_{22}O_{11}$	Chemical
Isoprene	C_5H_8	Fuel/Rubber
Lactic acid	$C_3H_6O_3$	Chemical
Lipid	C_{15}-C_{18}	Fuel
PHB	$C_4H_8O_2$	Fuel/plastic
Single cell protein*		Food/feed

*, including glutamic acid, aspartic acid, alanine, leucine, valine, glycine, lysine, isoleucine, threonine, serine etc.

❖ Biological routes chemical route for fuel



Source: Conrado & Gonzalez, 2014. Science, 343: 621/Haynes & Gonzalez, 2014. Nat. Chem. Biol. 10: 331

Safe Use of Methane in Air

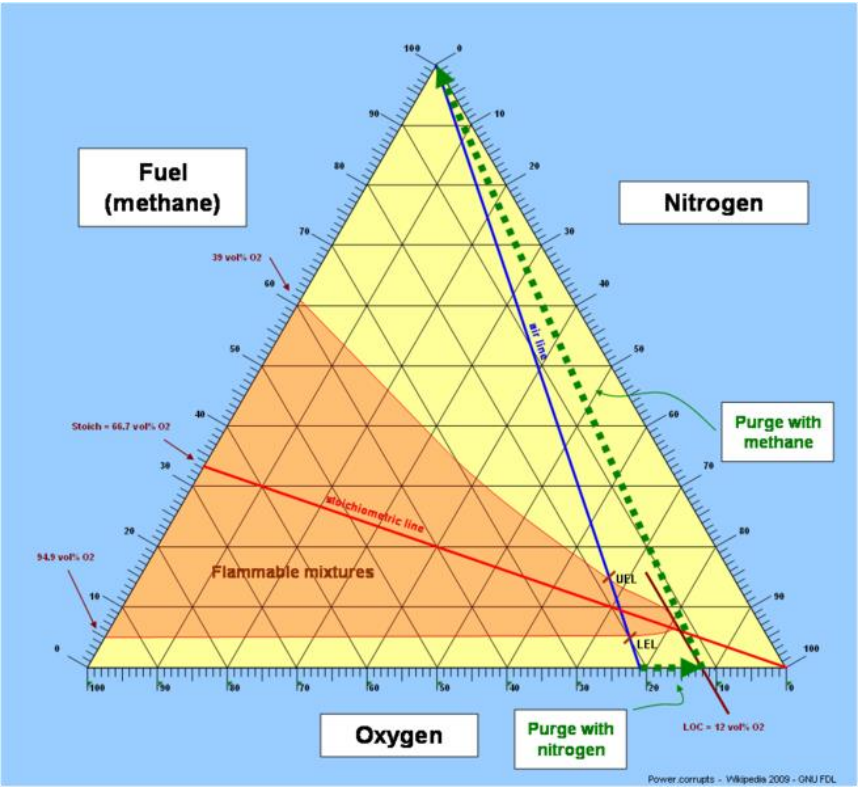
❖ **Safety consideration**

Limiting Oxygen Concentration (LOC) = 12 vol % O_2

UEL of CH_4: 15% in Air (v/v)

LEL of CH_4: 5% in Air (v/v)

CH_4 Flammability Diagram

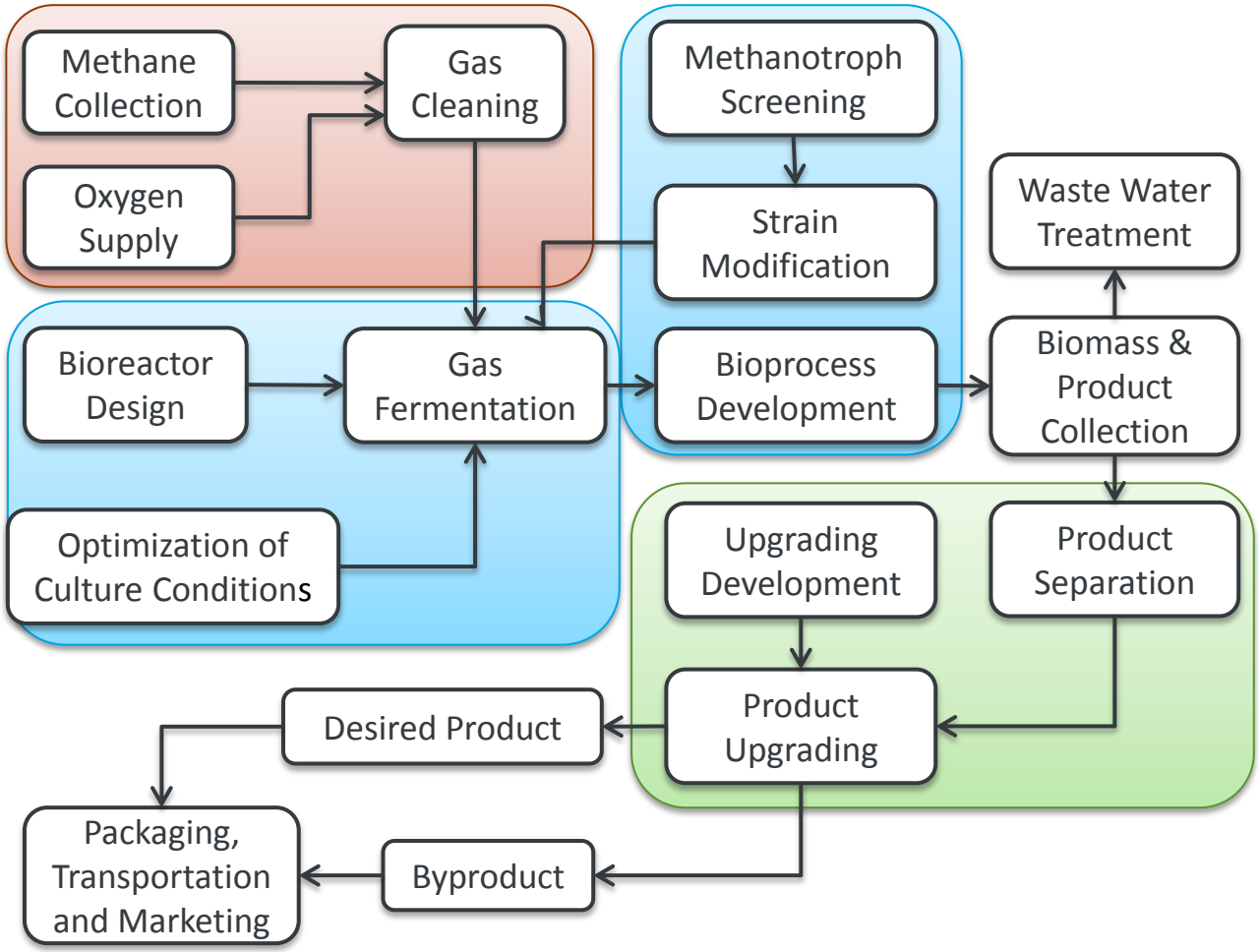


❖ *Source: wikipedia.com*

- Established cultivation system in baby steps
  - Vial-based batch culture
  - STR in hood batch and fed-batch
  - STRs on bench for continuous sparging
- Engineering safety features
  - Restriction of methane storage based on lab air flow
  - Restricted orifices on cylinders to reduce gas escape rate in event of regulator failure
  - Methane monitoring system
  - Automatic gas shut off in event of power failure or detection of methane in lab
- System safety features
  - Only work above UEL or below LEL to eliminate possibility of explosions
  - Approved safe operating procedure documentation in place
  - Access to equipment limited to trained personnel.
  - Warning signs to alert lab personnel of use of methane

Hurdles of Bioconversion of Methane

- **Technical hurdles (R&D Map for Bioconversion of Methane)**
  - Gas Supply & Cleaning
  - Bioprocess Development
  - Product Separation & Upgrading



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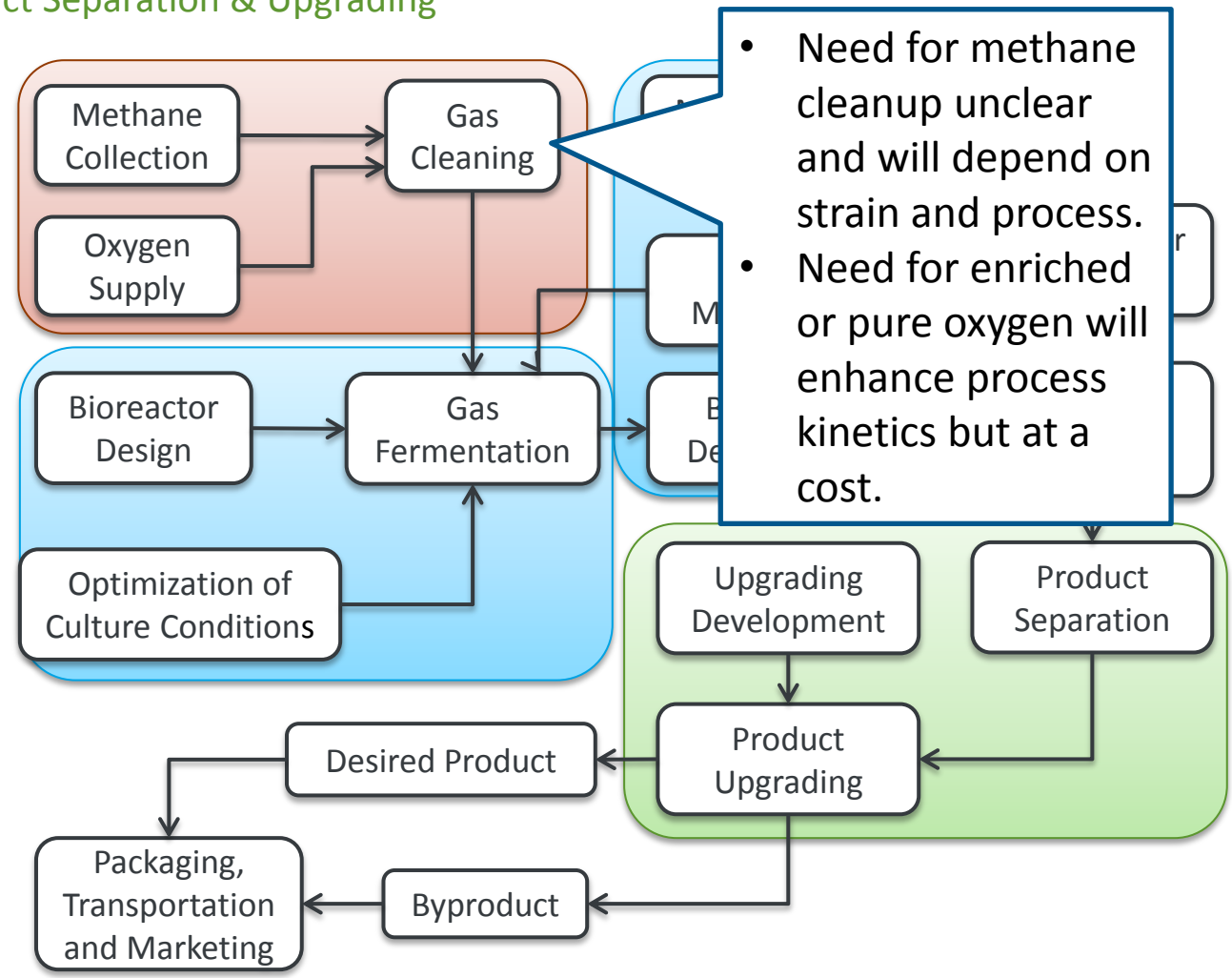

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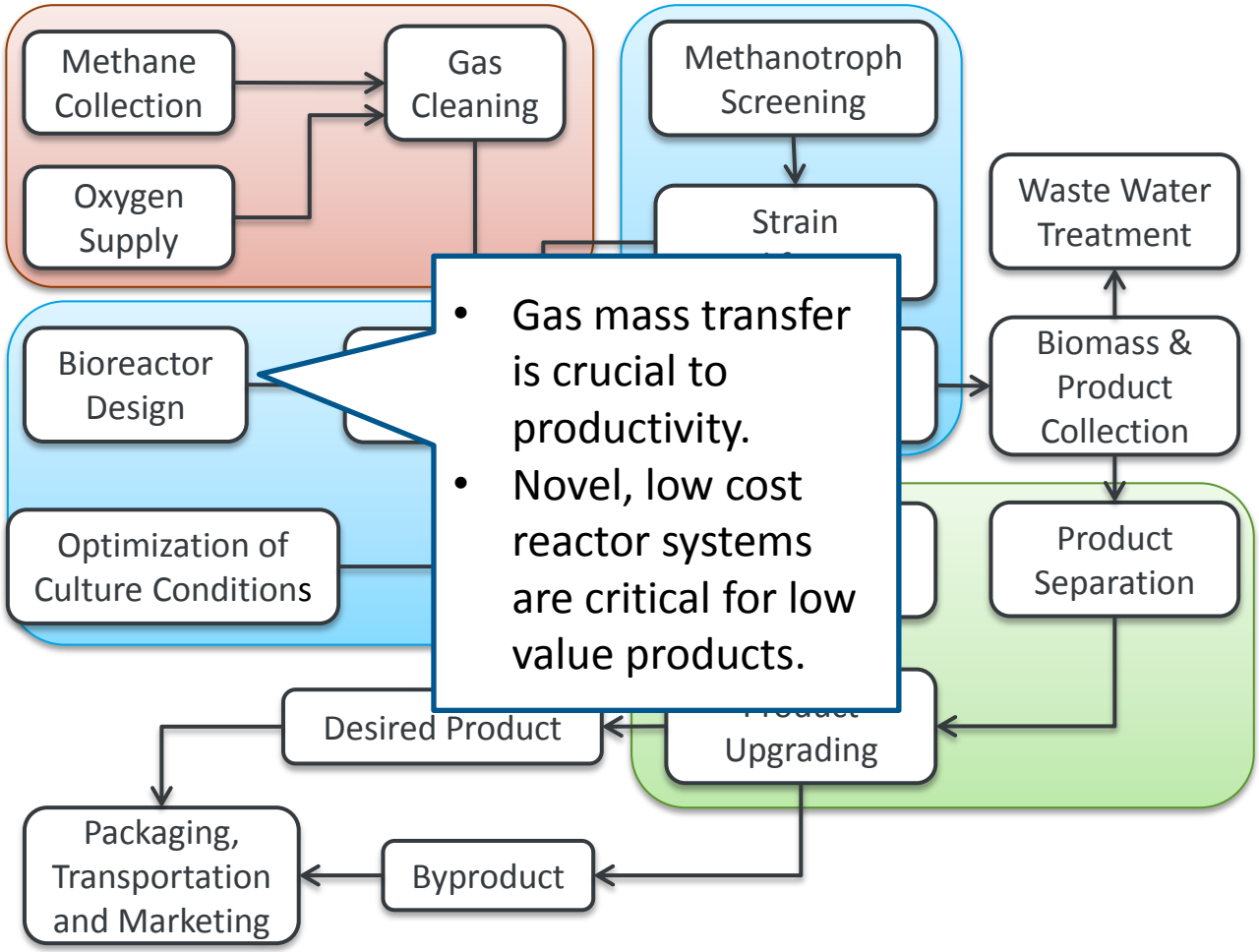

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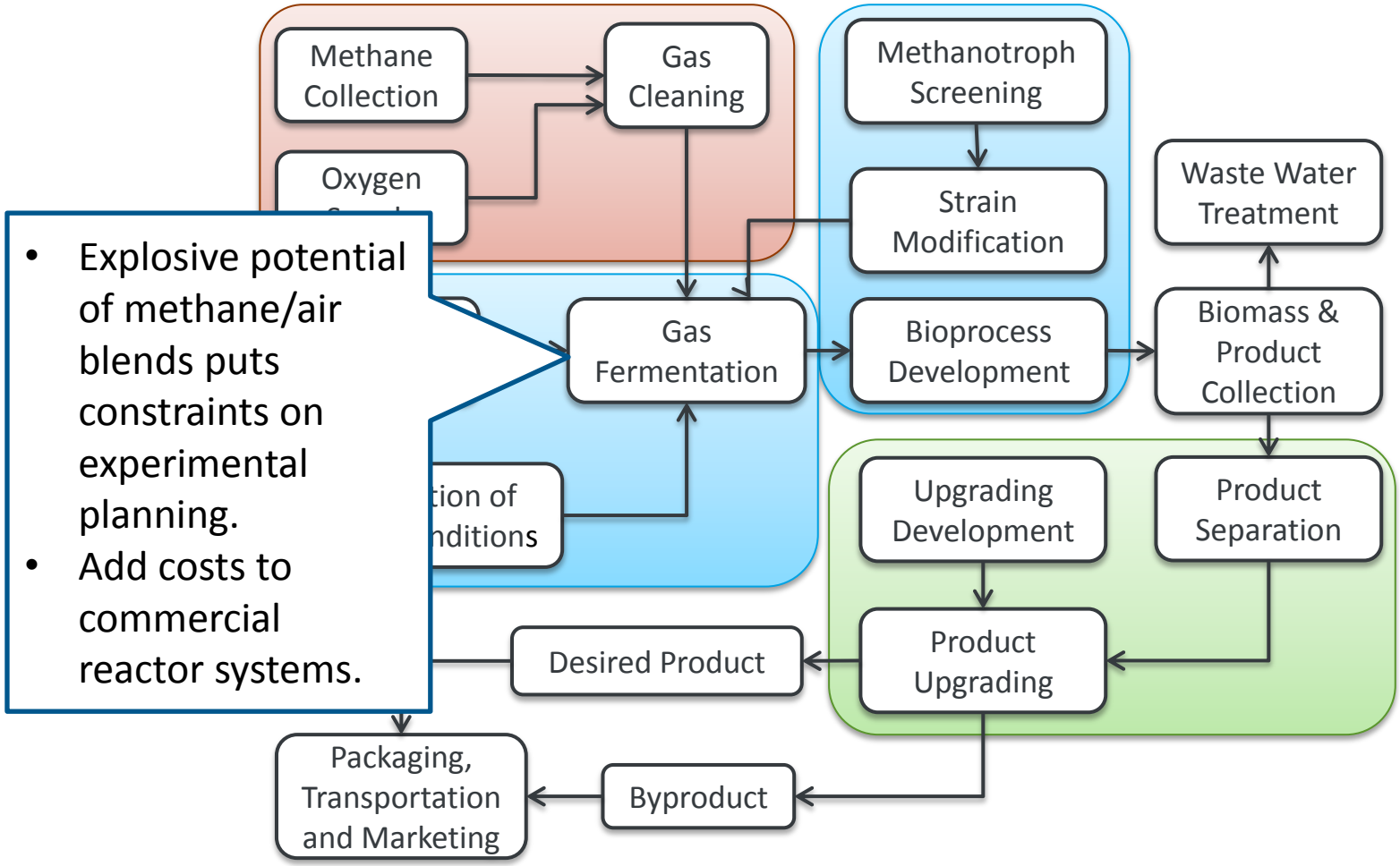


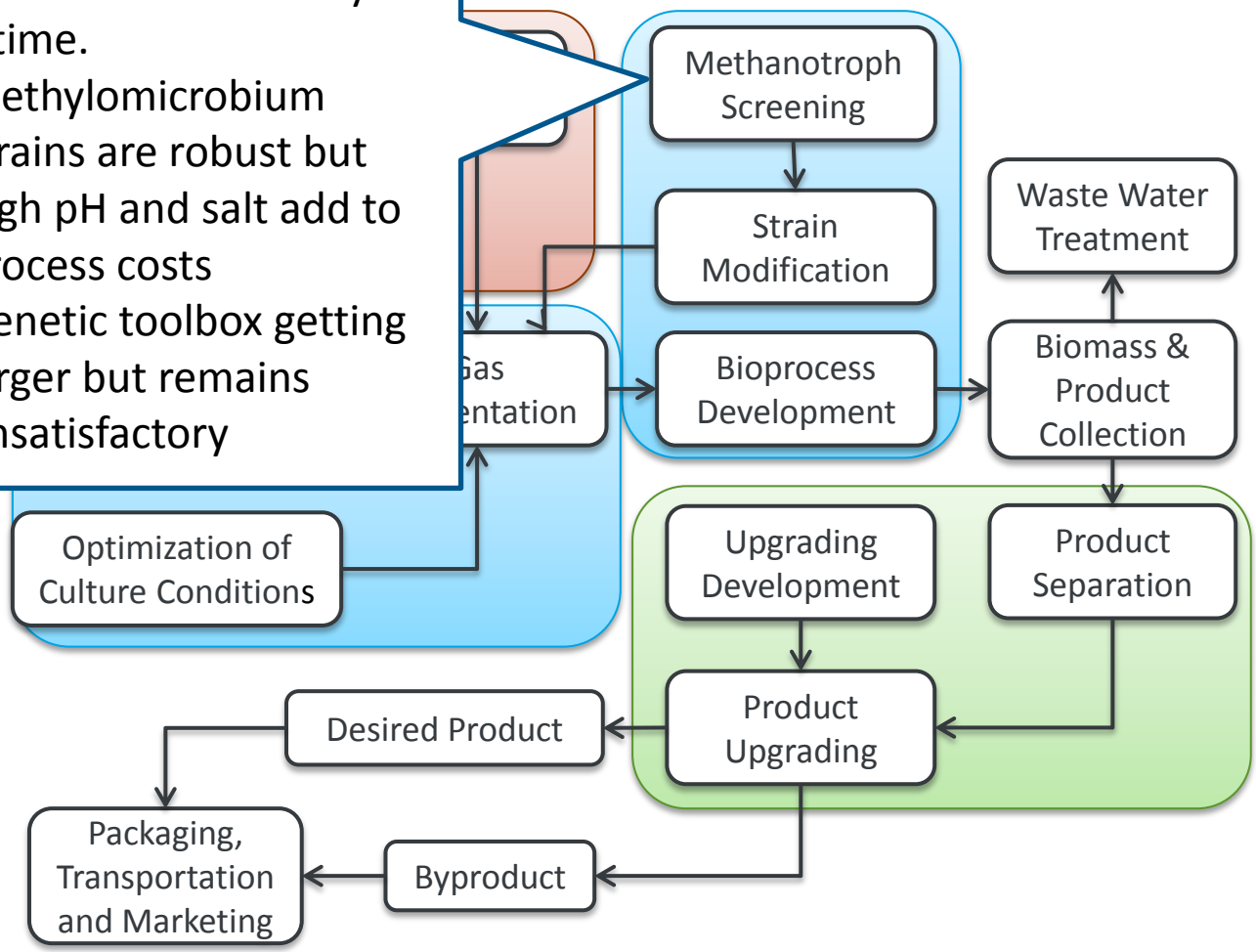
- Explosive potential of methane/air blends puts constraints on experimental planning.
- Add costs to commercial reactor systems.

Hurdles of Bioconversion of Methane

❖ **Technical hurdles (R&D Map for Bioconversion of Methane)**

- ➢ Gas Supply & Cleaning
- ➢ Bioprocess Development

- Available strains not ready for prime time.
  - Methylomicrobium strains are robust but high pH and salt add to process costs
  - Genetic toolbox getting larger but remains unsatisfactory



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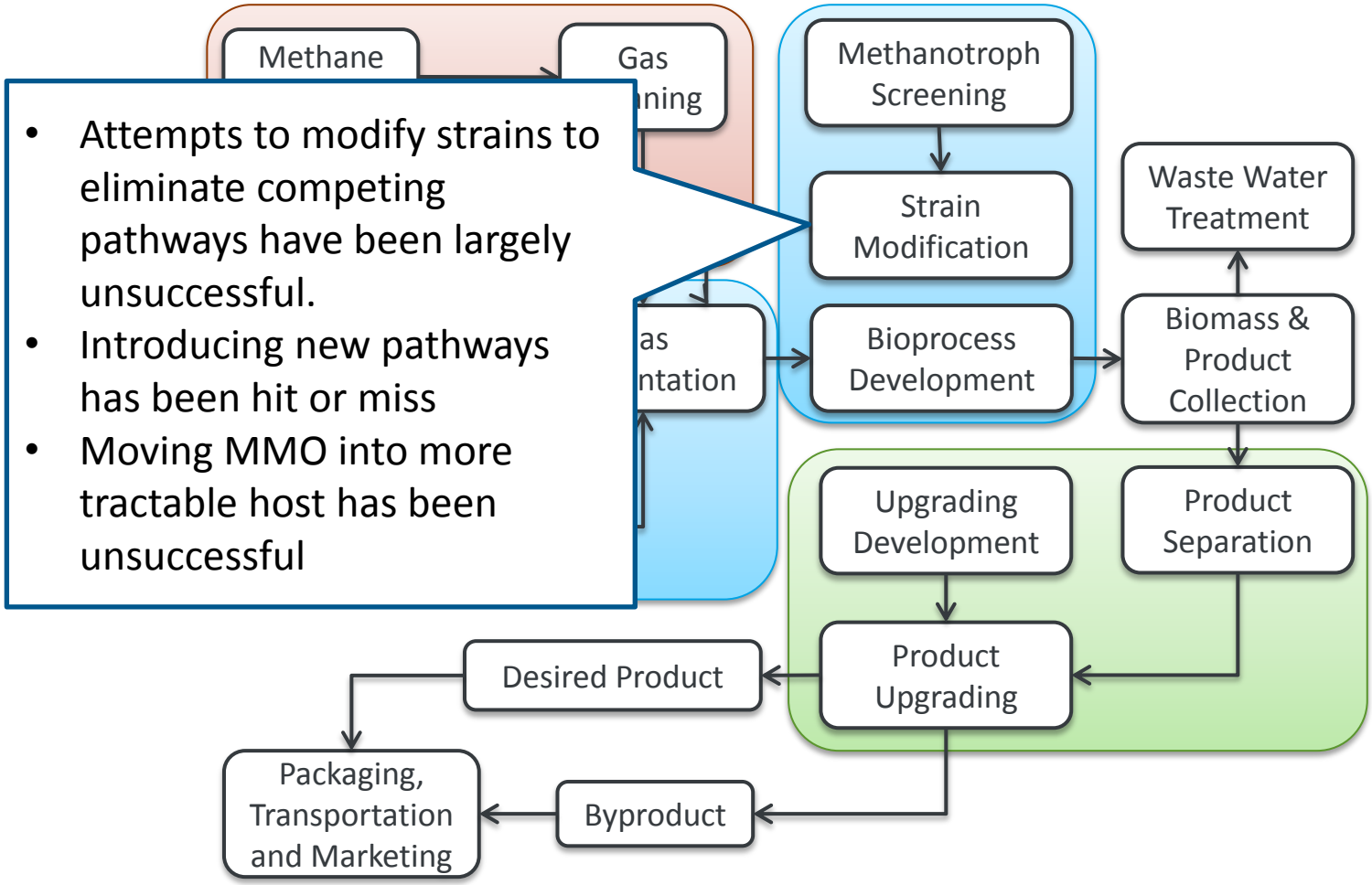

- Attempts to modify strains to eliminate competing pathways have been largely unsuccessful.
- Introducing new pathways has been hit or miss
- Moving MMO into more tractable host has been unsuccessful

Hurdles of Bioconversion of Methane

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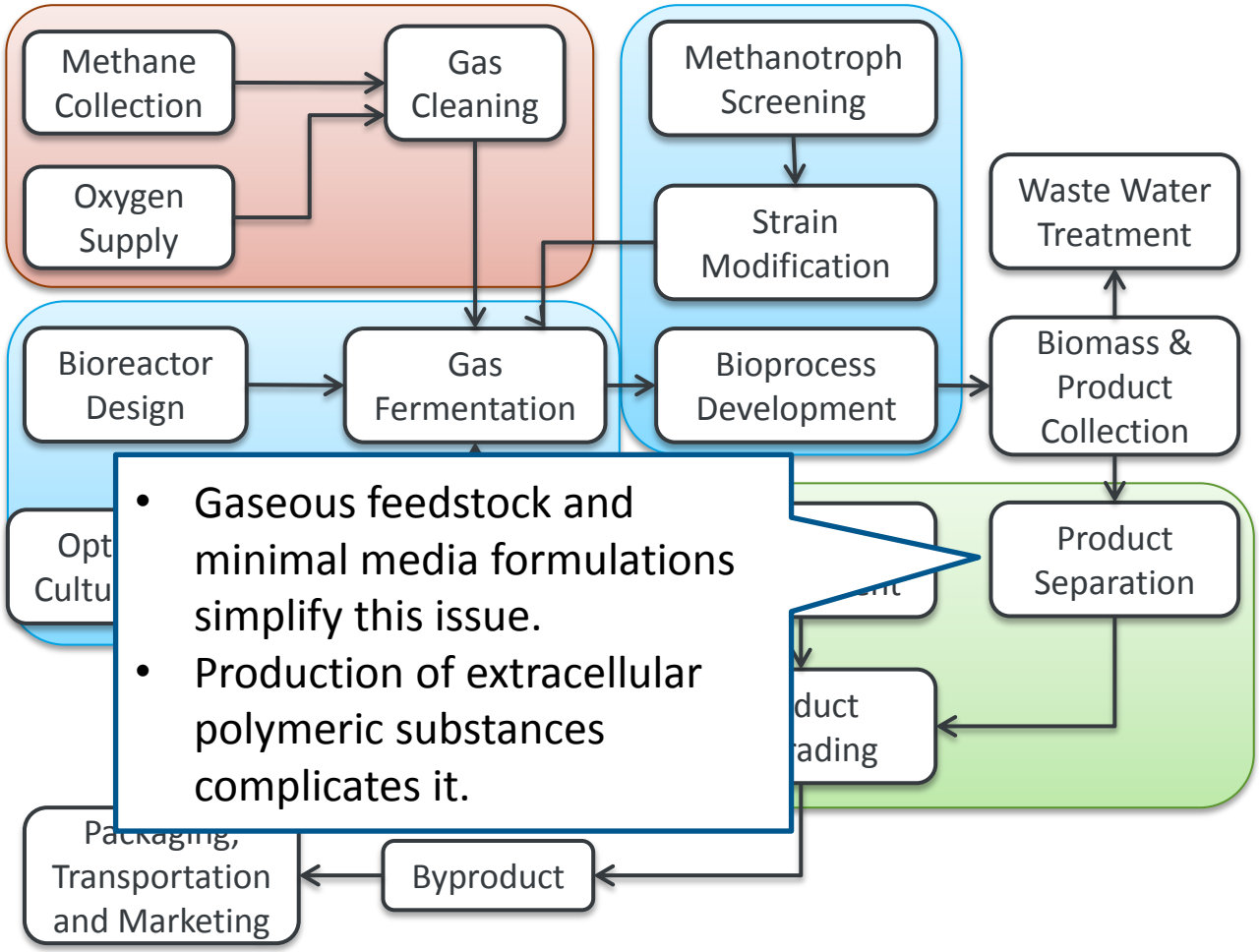
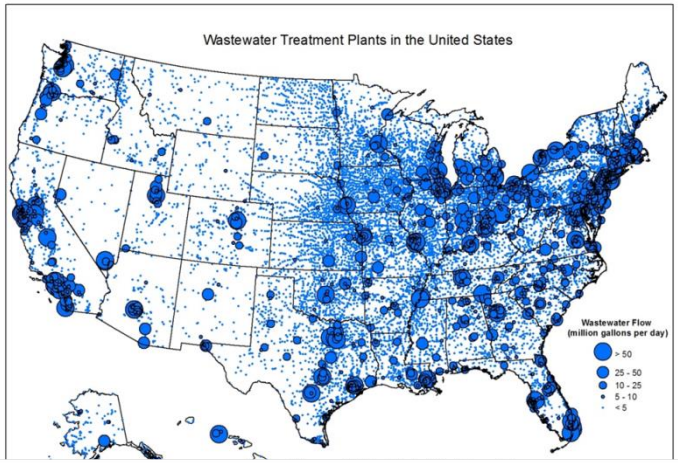
  - Gas Supply & Cleaning
  - Bioprocess Development
  - Product Separation & Upgrading

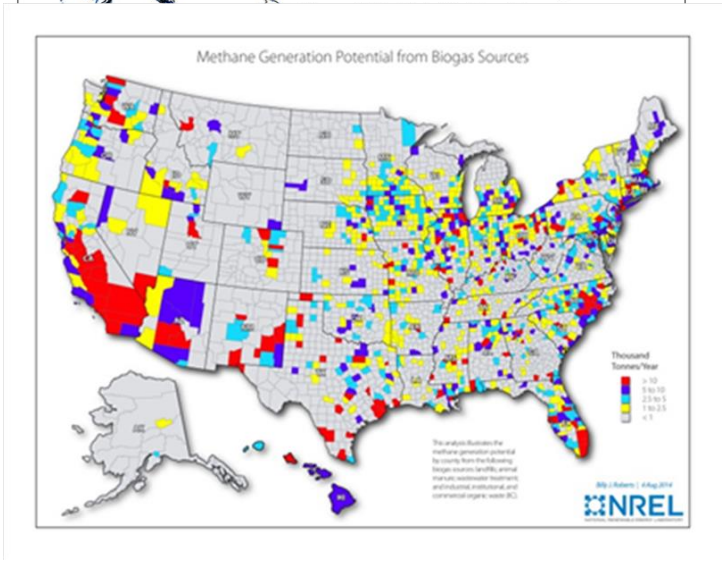


- Gaseous feedstock and minimal media formulations simplify this issue.
- Production of extracellular polymeric substances complicates it.

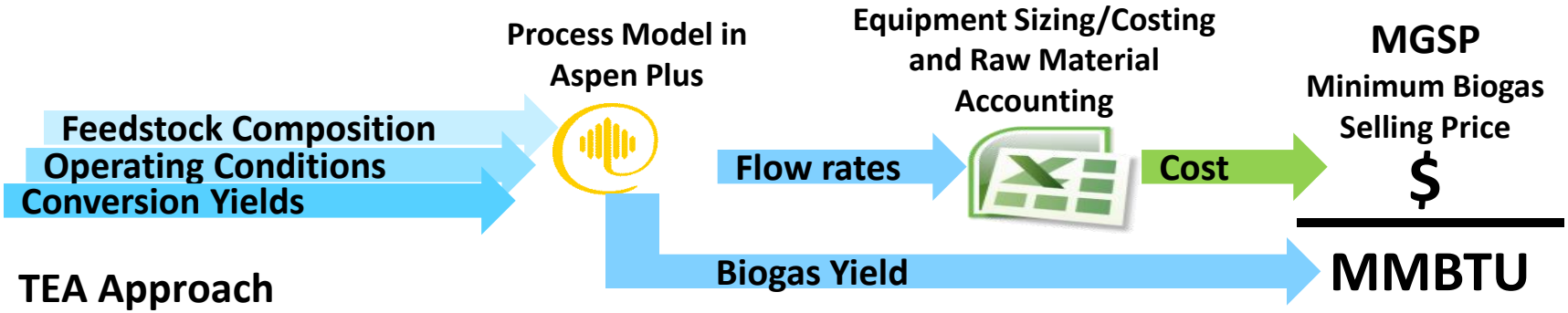
Waste Feedstock: Joint AOP with PNNL

Inventory WTE feedstocks — waste fat, oil, and greases; municipal solid wastes; biosolids (from wastewater treatment facilities); biorefinery residues and biogas—and work to address feedstock data gaps and quality issues, inventory feedstock characteristics, and estimate the biofuels potential from these resources.



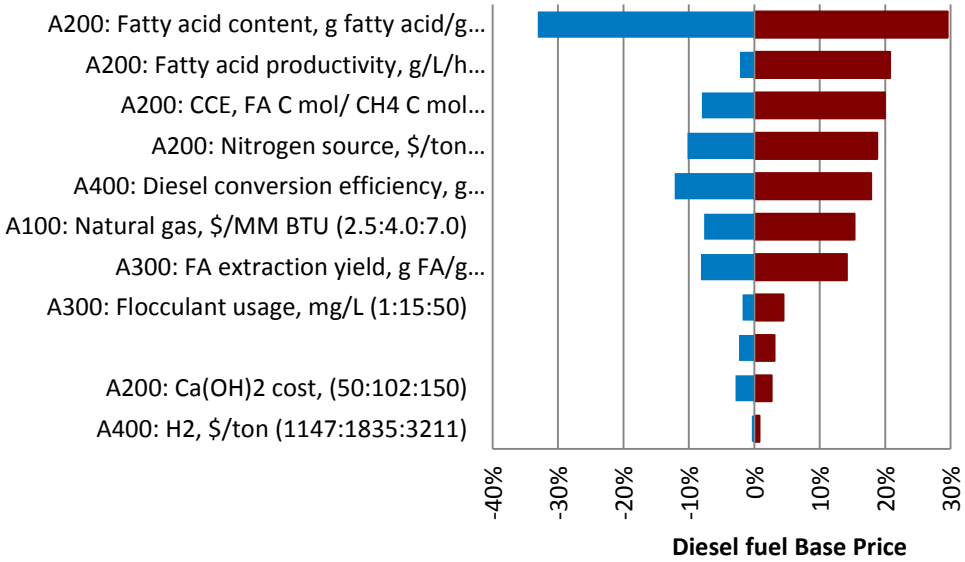


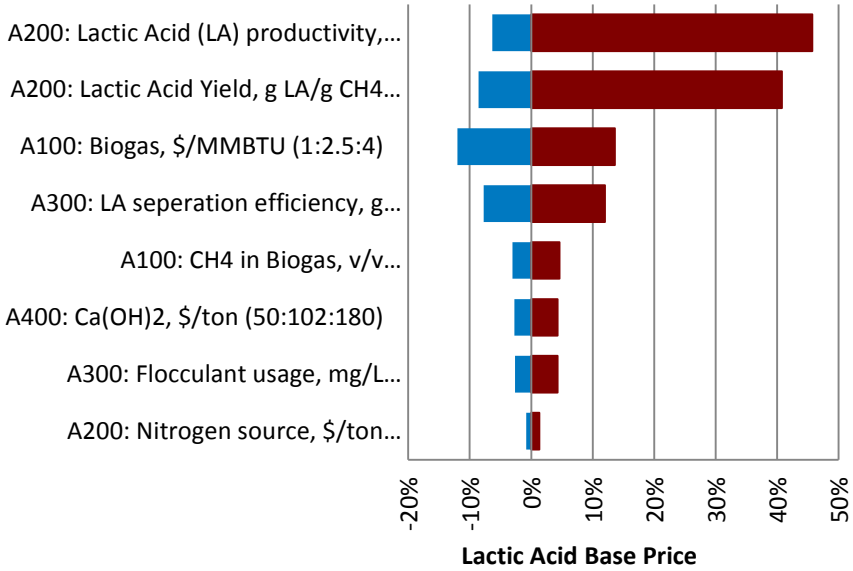
WTE TEA: Subtask on AOP Project



TEA Approach

- Modeling is rigorous and detailed with transparent assumptions
- Discounted cash-flow rate of return on investment, equity payback, and taxes
- Provide strategic, comparative cost analysis for various conversion technologies
- Iterative analysis process among R&D, DOE goals, LCA on key technology targets



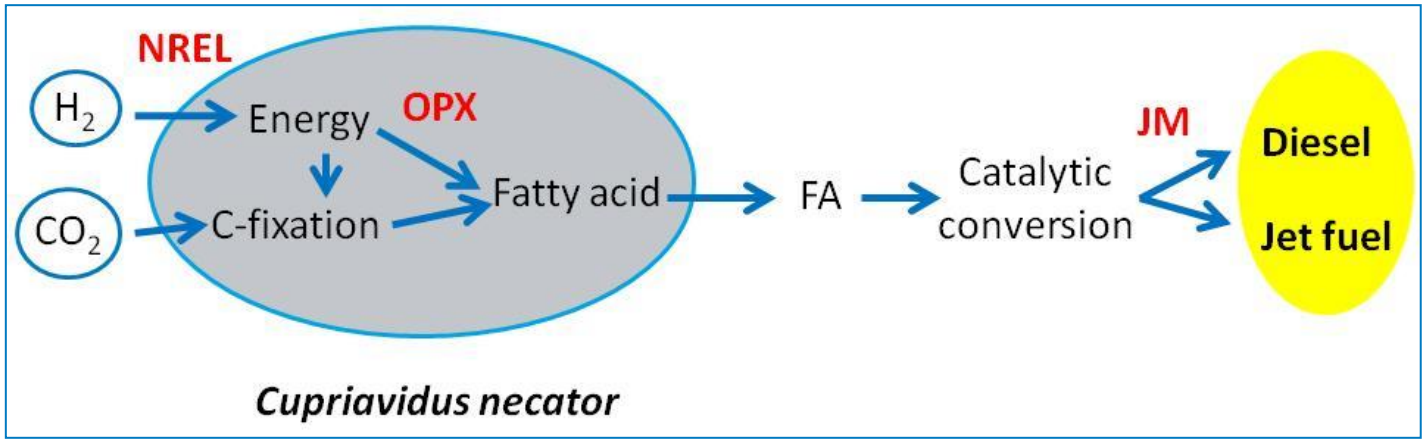


Waste-to-Energy Systems Model (WESyS): AOP Project

- **Objectives**
  - To develop and analyze scenarios that explore evolution of the waste-to-energy (WtE) industry
    - How can WtE fuel technologies be deployed to make a significant contribution to the country's energy system?
  - Developing insights into WtE industry growth and market penetration.
    - Policies, incentives, technological advances, related and/or competing markets, demand for petroleum-based fuels, and competing uses of feedstock.
- **WESyS Model Now**
  - The modular structure of landfills can be parameterized for a region.
  - Accounts for landfills in EPA's LMOP dataset
  - Capital costs associated with biogas to electricity, CNG, and flaring.
  - Model calibration against historic data.
- **Next Steps**
  - Assess biogas potential from additional resources:
    - Municipal wastewater treatment plants
    - Concentrated animal feeding operation (CAFO)
  - Analysis of the economic tradeoffs of WtE options
  - Assess additional technology options

Novel Biological Conversion of H_2 and CO_2 Directly into Biodiesel: ARPA-E Electrofuels

Improving Rubisco activity for efficient CO_2 fixation in *Cupriavidus necator* for biodiesel production



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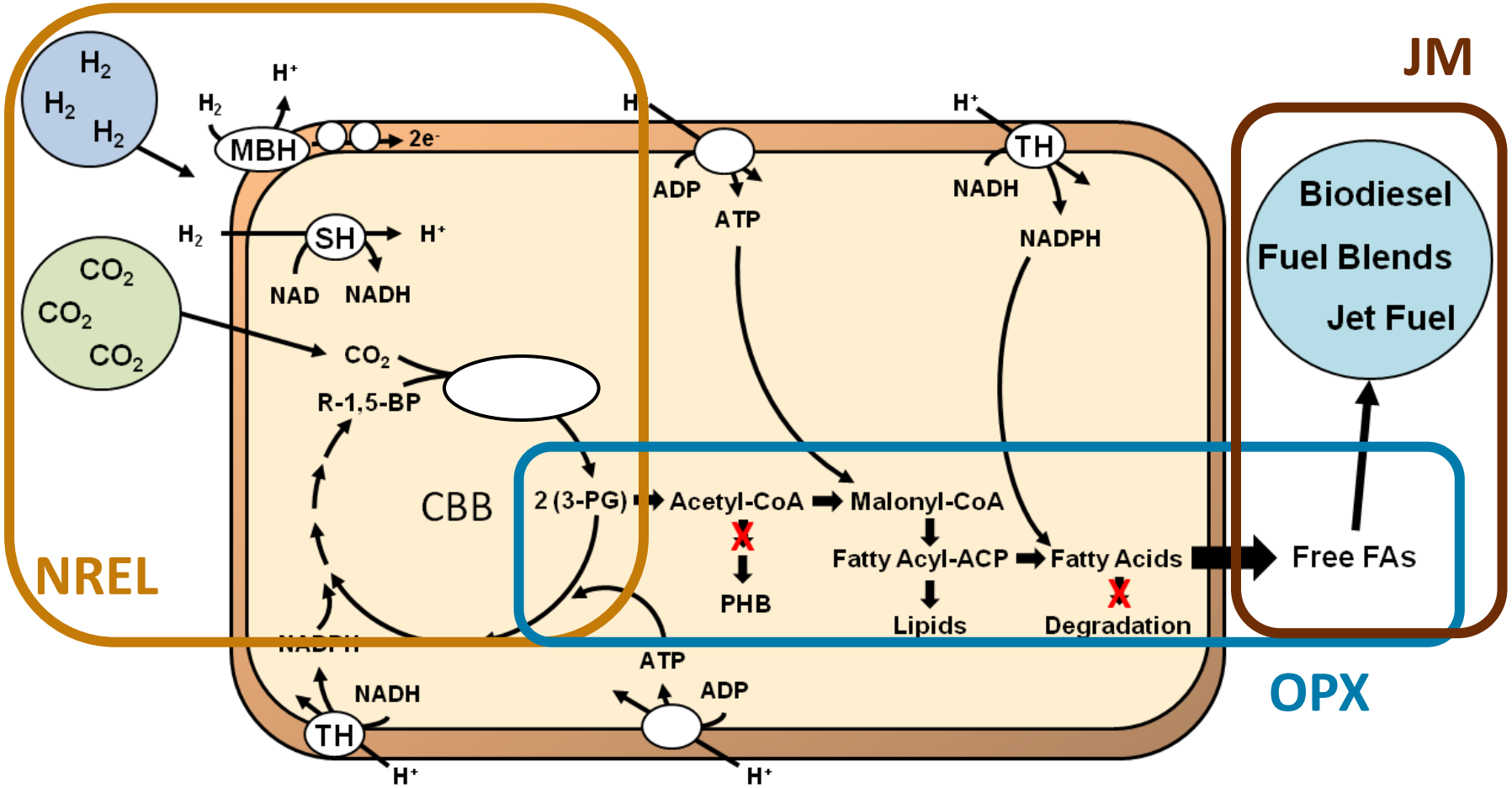
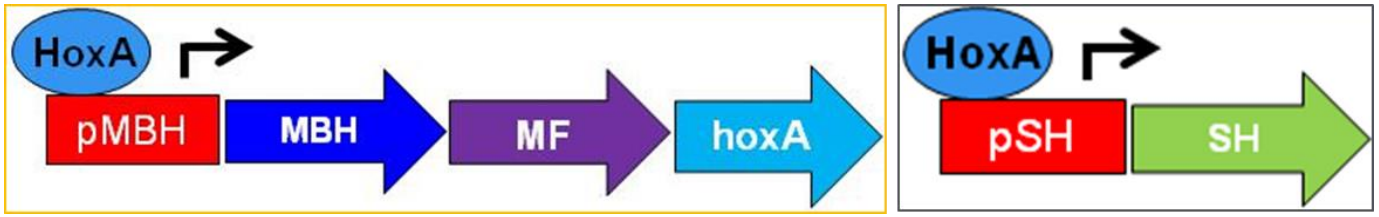
arpa·e CHANGING WHAT'S POSSIBLE

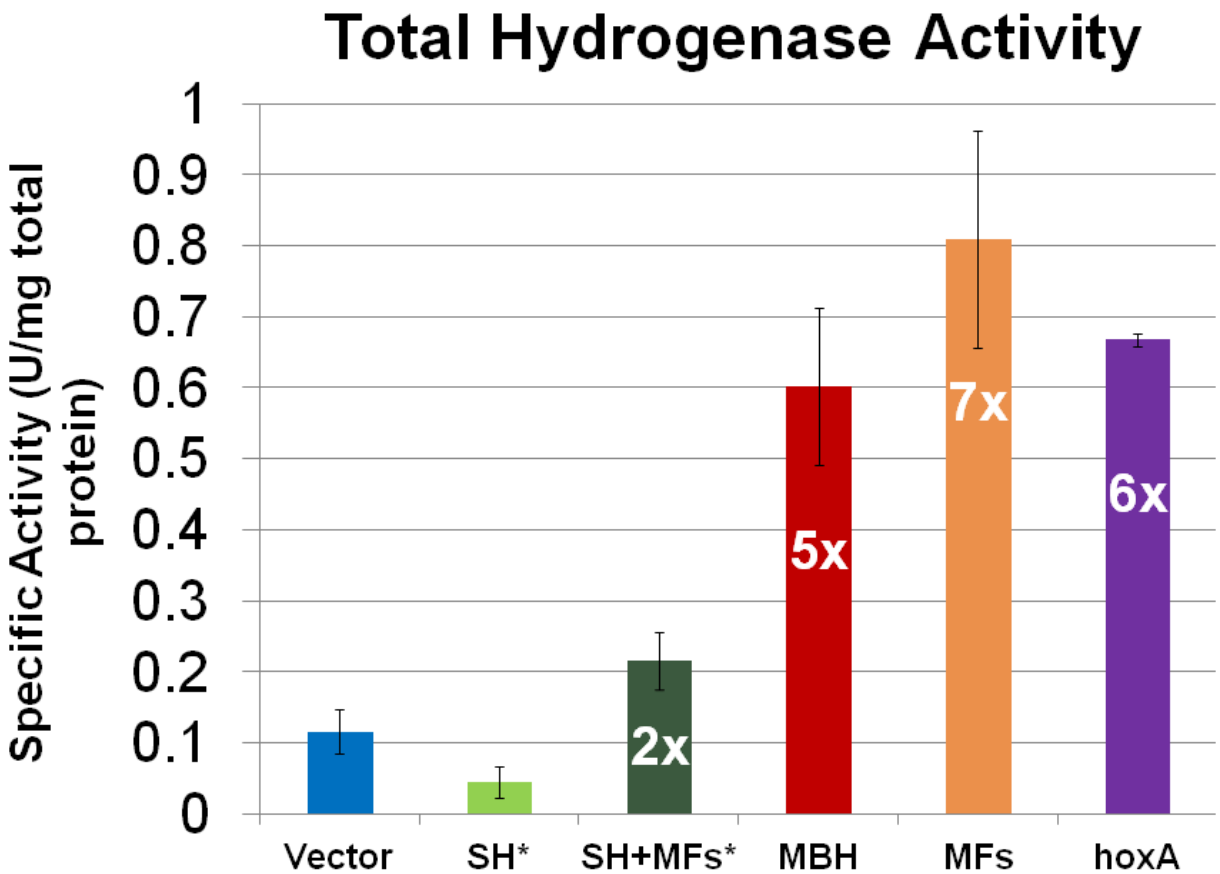
ARPA-E Electrofuels - Novel Biological Conversion of H_2 and CO_2 to Biofuels



Project Goal: Develop and optimize *Ralstonia eutropha* to produce a biodiesel-equivalent fuel from renewable H_2 and CO_2, at costs of less than $2.50 per gallon

H_2 Utilization: Hydrogenase Improvements

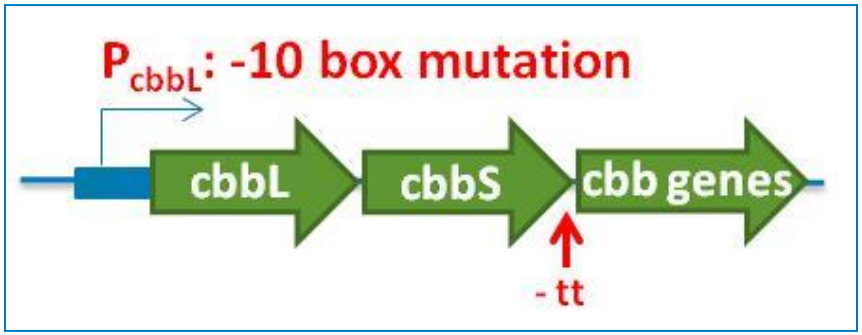




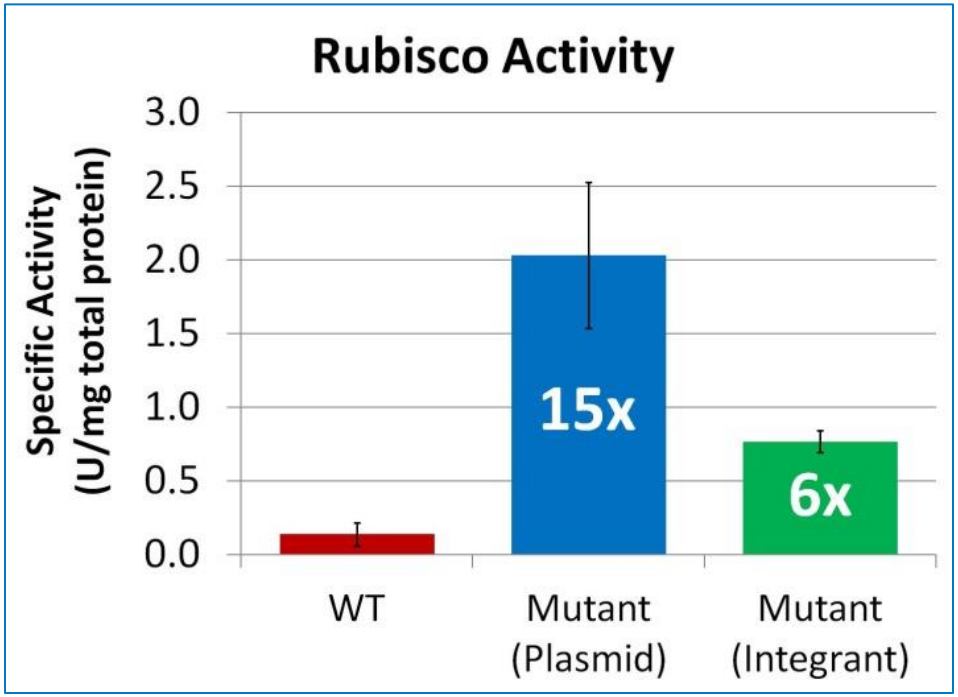
- SH: Soluble Hydrogenase
- MBH: Membrane-bound Hydrogenase
- MF: Maturation Factors
- hoxA: Response Regulator (regulatory)

- Hydrogenase promoter engineering improves activity by up to 7 folds.

CO_2 Utilization: Rubisco Improvements

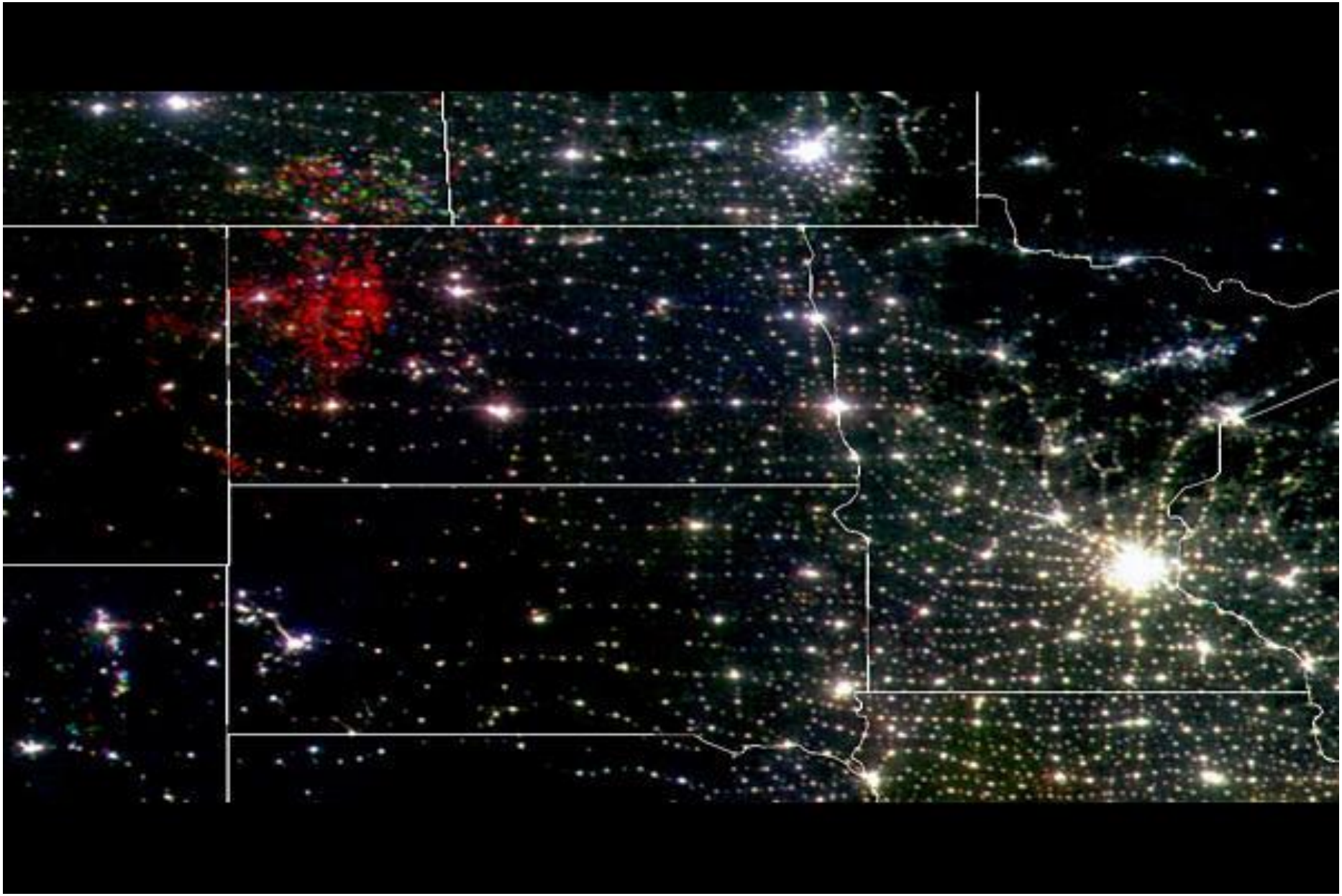


- RubisCO promoter and terminator engineering improves activity by up to 15 folds.



- **Summary**
  - Generated strains with improved hydrogenase and Rubisco activities.
  - Both modifications were combined in the same strain.
  - The engineered strain could serve as the host to over-express fatty acid pathway to boost its titer.
  - ***R. eutropha* could convert waste organic acids/CO_2 to high value products.**

Acknowledgments

- **Biogas Upgrading: Mike Guarnieri**
- **Waste Feedstock: Anelia Milbrandt**
- **WTE TEA: Ling Tao**
- **WESyS: Danny Inman**
- **CO2 Upgrading: Pinching Maness**

Any Questions?