CHANGING WHAT'S POSSIBLE

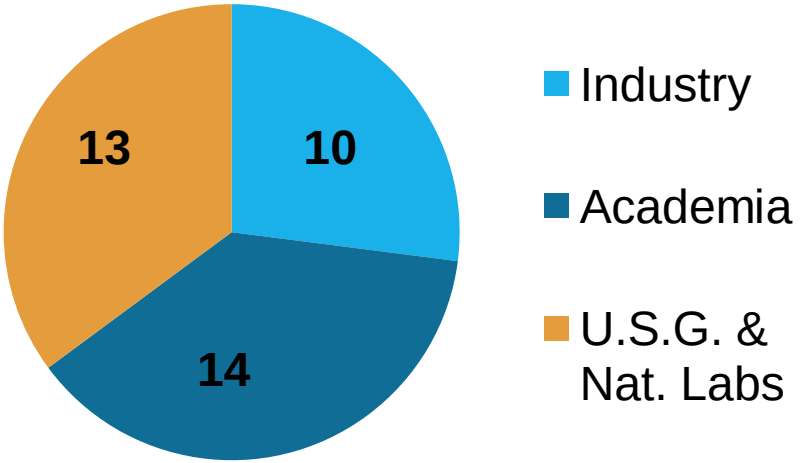
Biological Technologies for Methane-to-Liquid Fuels Workshop

Breakout Session Report-out Summary

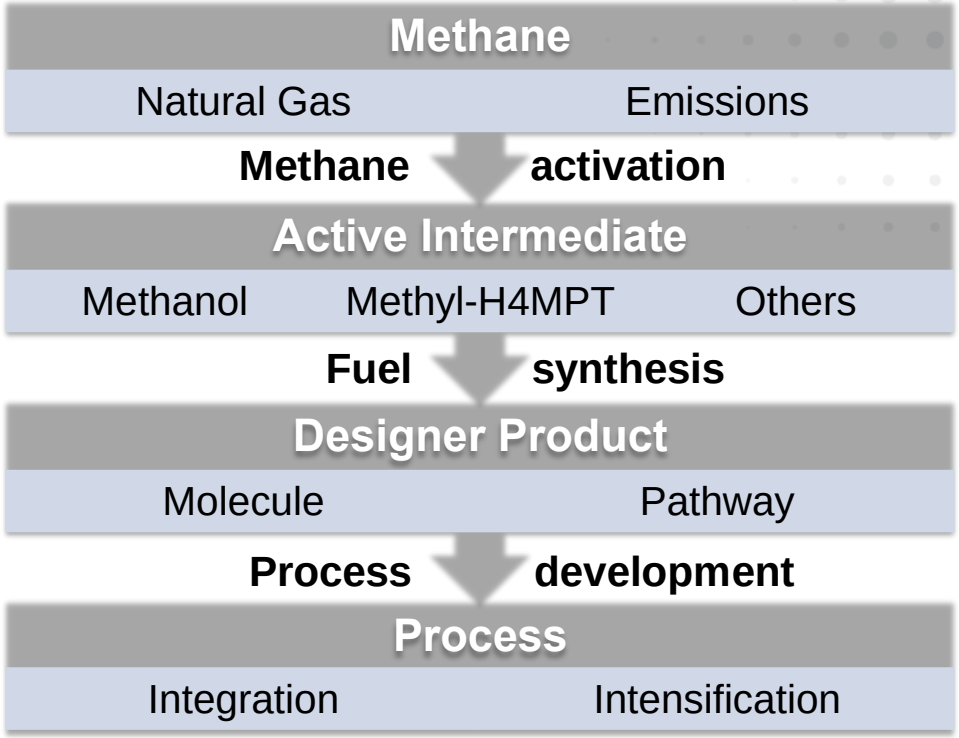
July 17, 2013

Primary outcome is to identify and discuss new bio-based technologies for methane to liquids

Workshop Participants



- 37 individuals participated in the workshop representing Industry, Academia, and the U.S.G. in roughly equal numbers.
- Representative expertise included methanogenesis, aerobic methanotrophs, anaerobic & C1 metabolism, electrosynthesis, synthetic biology & protein engineering, and industrial processing.



Methane activation and fuel synthesis flow-diagram presented to workshop participants for additional context.

Representative goals and discussion questions presented to participants by PD Gonzalez

Goals

- Discuss the feasibility of biological conversion of methane to liquid fuels:
  - Representative technologies
  - Prior experience/lessons learned
    - Data
    - TEA
  - Prioritization of technologies
  - Increased understanding
- Community building
- Metrics
  - What metrics should we use?
  - What should be their value (roughly)?

Representative discussion questions

- What is the resource potential for “wet”/“sour” gas?
- Are there ways around inefficiencies w/ methane conversion?
- Is it advantageous and possible to divert carbon away from CO_2 towards fuel production in the anaerobic pathway?
- What synthetic biological routes could/should be considered?
- What are possible bio-process intensification & integration strategies?

Morning and afternoon breakouts focused on routes for methane conversion & process

- 1st breakout session –
  - What are the possible routes to convert CH_4 to liquid fuels?
    - Mechanism for methane activation
    - Intermediates
    - Process inputs
    - Limitations
    - Challenges
    - Benefits

- 2nd breakout session –
  - What processes are needed to economically produce CH_4 to liquid fuels for a given route?
    - Impact of scale and feedstock
    - Process intensification & integration

BREAKOUT SESSION 1: ROUTES FOR METHANE CONVERSION

Breakout Session 1 Output: Routes for Methane Conversion – Aerobic conversion

Technology Concept Description	Methane Activation	Intermedi-ates	Process Inputs	Challenges	Benefits
▪ Aerobic CH_4 (+/- CO_2)	▪ Characterized pMMOs ▪ Engineered/ bio-mimetic MMOs and/or FDH ▪ Alkyl hydroxylase	▪ CH_3OH ▪ CH_2O ▪ RuMP/serine cycle ▪ C_4 product ▪ PHB	▪ O_2 ▪ CH_4	▪ Gas-phase fermentation/ mass transfer ▪ Decoupling growth from production ▪ Volumetric productivity ▪ Genetics ▪ Variable growth rates ▪ Carbon and energy efficiency ▪ Heterologous MMO expression ▪ CH_2O toxicity	▪ Low CapEx/power ▪ High selectivity ▪ Low H_2O input ▪ pMMO enzyme (reasonably) well characterized ▪ Co-products value ▪ Endogenous PHB storage

Order of "Technology Concept Description" is not indicative of prioritization by workshop participants or ARPA-E

Breakout Session 1 Output: Routes for Methane Conversion – Isolated biocatalysts

Technology Concept Description	Methane Activation	Intermedi-ates	Process Inputs	Challenges	Benefits
▪ Isolated enzymes as biocatalysts	▪ Routes to liquid intermediates	▪ CH_3OH ▪ CH_2O ▪ HCOOH ▪ Chemically derived C-C bond	▪ O_2 ▪ CH_4	▪ Need reductant such as H_2 or electrode	▪ No cell maintenance ▪ High productivity/ high biocatalysts concentration ▪ High intermediate concentration

Order of "Technology Concept Description" is not indicative of prioritization by workshop participants or ARPA-E

Breakout Session 1 Output: Routes for Methane Conversion – Anaerobic conversion

Technology Concept Description	Methane Activation	Intermedi-ates	Process Inputs	Challenges	Benefits
▪ Anaerobic/ reverse methano-genesis (could involve consortia for CH_4 to H_2 to product)	▪ Methyl CoM reductase	▪ CH_3-H_4MPT ▪ Other tightly bound C_1 molecules	▪ CH_4 ▪ Oxidant such as SO_4^{2-}	▪ Thermodynamics (need to drive reaction) ▪ Difficult to control intermediates ▪ Management of mixed/syntropic communities ▪ H_2 management ▪ Currently no recombinant systems	▪ Higher carbon and energy efficiency ▪ Methanogens are robust organisms (engineer them to oxidize CH_4)

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Breakout Session 1 Output: Routes for Methane Conversion – Anaerobic conversion, Nitrite

Technology Concept Description	Methane Activation	Intermedi-ates	Process Inputs	Challenges	Benefits
▪ Anaerobic/ nitrite	▪ pMMO (uses O2 produced *in situ* from NO_2^-	▪ CH_3OH ▪ CH_2O ▪ RuMP/serine cycle ▪ C_4 product	▪ CH_4 ▪ NO_2^-	▪ Extremely slow growth ▪ Essentially the same as O_2 dependent MMO system	▪ None identified

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Breakout Session 1 Output: Routes for Methane Conversion – Other *in situ* systems

Technology Concept Description	Methane Activation	Intermedi-ates	Process Inputs	Challenges	Benefits
▪ P450 ▪ AMO ▪ Dioxygenase ▪ Active site engineering	▪ Metal cluster for C-H activation	▪ CH_3OH ▪ CH_2O ▪ RuMP/serine cycle ▪ C_4 product	▪ O_2 ▪ CH_4	▪ P450 low activity ▪ Large active site ▪ Low selectivity ▪ Redox maintenance ▪ Energy efficiency	▪ Engineered enzyme could be envisioned with greater energy efficiency than MMO

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Breakout Session 1 Output: Other discussion points shared by workshop participants

- Heterologous expression of sMMO – need protein expression toolkit
- Protein engineering of alkane processing enzymes
- Chemical/ Photocatalysis w/ bioconversion of methyl radical
- Electrochemical coupling as electron source or sink
- Engineer MCR from methanogenesis for methane oxidation

- Process Ideas
  - Facultative methanotrophy to utilize CH_4 and > C2 compounds (e.g. ethane)
  - Separate biocatalyst production from use (ship as freeze dried)
  - Non-aqueous media to increase CH_4 solubility
  - High pressure systems to increase driving force for CH_4
  - Thin film/fiber support for process intensification
  - CH_2O sequestration and release to maintain non-toxic CH_2O conc.
  - Keep H_2/other products @ very low conc. to drive reverse methanogenesis
  - CH_4-hydrates as a way to get very high CH_4 concentrations in solution
  - Dealing with process water
  - Co-metabolism with methylotrophic yeast

BREAKOUT SESSION 2: PROCESS

Breakout Session 2 Output: Cross-cutting process challenges

- Maintenance of operational parameters – inputs
- Genetic engineering – protein expression, control
- Mass transfer for scale-up
- High productivity – has been commercially demonstrated at 10 g/L/day (fish food); 0.5 g/L/hr was suggested as the minimum for a commercial process
- Heat removal
- Water removal & product separations

Breakout Session 2 Output: New technologies required for aerobic process improvements

- Continuous or semi-continuous system
- High methane per pass capture
- Low pressure reactors
- Use air (instead of pure oxygen) and low pressure to achieve g/L/h productivities
- Feed components including ethane and propane
  - Mitigate toxicity by co-culture implementation or expression of alcohol dehydrogenase
- Considerations for catalytic methane oxidation to improve overall energy efficiency

Breakout Session 2 Output: Discussion points shared by workshop participants

- Difficult to decouple growth from fuel production, but possible in methanotrophs:
  - Starve of N,P: produce PHBs
  - Starve of CH_4, O_2: produce lipids
- Is it possible to do better than MMO? One idea:
  - Create/find a dioxygenase that only uses 1 NADH for 2 CH_4 molecules
- Aerobic concepts that were explored:
  - Accumulate or secrete products from CH_4 and O_2
  - Convert CH_4 to biomass, then hydrotreat biomass to produce fuels
  - Isolated enzymes as biocatalysts
  - Chemically convert CH_4 to CH_3OH, and then biologically convert CH_3OH to fuel product

Breakout Session 2 Output: Process – CH_4 to biomass followed by hydrotreating, other

- Typical biomass accumulation is 15 g/L titers
- Produce onsite biomass and then ship to processing facility
- Convert proteins in biomass to ketoacids and then convert to alcohols
- This process probably requires onsite use of all products and recycle all nutrients
  - Is there value to the co-products from this process?

Breakout Session 2 Output: Process – Isolated enzymes as biocatalysts

- Potentially more amenable to optimization
- Can produce CH_3OH in cell free systems now
- pMMO is difficult to handle/use in a cell free system
- Explore and use sMMO in cell free systems; sMMO has higher V_{max}
- Where will the reducing equivalents come from?
- What is the cost of the catalysts?

Breakout Session 2 Output: Process – Other thoughts

- Means to increase CH_4 solubility:
  - Technology for super-saturating with CH_4
  - Product accumulation
  - High pressure (may limit CO_2 removal)
- Thermophile systems will reduce CH_4 solubility (slow growth?)
- Some methanotrophs accumulate PHB…could this carbon be redirected to TAGs?

Breakout Session 2 Output: Process – Other considerations for scale-up

- Small scale systems are challenging (e.g. offshore, emission sites); is the product transportable?
- Technologies for thin-film/fiber support for biocatalysts needed
- Safety
- Need to utilize low value methane sources
- Capable of accessing geographically dispersed sources and low methane productivities (e.g. landfill gas)
- Skid-mounted (modular) systems to reduce and integrate unit operations
- Automation to reduce labor costs (considerable at small scale)