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

<table>
<thead>
<tr>
<th>Technology Concept Description</th>
<th>Methane Activation</th>
<th>Intermediates</th>
<th>Process Inputs</th>
<th>Challenges</th>
<th>Benefits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic CH₄ (+/- CO₂)</td>
<td>Characterized pMMOs</td>
<td>CH₃OH</td>
<td>O₂, CH₄</td>
<td>Gas-phase fermentation/mass transfer</td>
<td>Low CapEx/power</td>
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<tr>
<td></td>
<td>Engineered/bio-mimetic MMOs and/or FDH</td>
<td>CH₂O</td>
<td></td>
<td>Decoupling growth from production</td>
<td>High selectivity</td>
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<tr>
<td></td>
<td>Alkyl hydroxylase</td>
<td>RuMP/serine cycle</td>
<td></td>
<td>Volumetric productivity</td>
<td>Low H₂O input</td>
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<tr>
<td></td>
<td></td>
<td>C₄ product</td>
<td></td>
<td>Genetics</td>
<td>pMMO enzyme (reasonably) well characterized</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PHB</td>
<td></td>
<td>Variable growth rates</td>
<td>Co-products value</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Carbon and energy efficiency</td>
<td>Endogenous PHB storage</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Heterologous MMO expression</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>CH₂O toxicity</td>
<td></td>
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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

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<tbody>
<tr>
<td>Isolated enzymes as biocatalysts</td>
<td>Routes to liquid intermediates</td>
<td>CH₃OH, CH₂O, HCOOH, Chemically derived C-C bond</td>
<td>O₂, CH₄</td>
<td>Need reductant such as H₂ or electrode</td>
<td>No cell maintenance, High productivity/high biocatalysts concentration, High intermediate concentration</td>
</tr>
</tbody>
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### Breakout Session 1 Output: Routes for Methane Conversion – Anaerobic conversion

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</thead>
<tbody>
<tr>
<td>Anaerobic/reverse methanogenesis (could involve consortia for CH$_4$ to H$_2$ to product)</td>
<td>Methyl CoM reductase</td>
<td>CH$_3$-H$_4$MPT, Other tightly bound C$_1$ molecules</td>
<td>CH$_4$, Oxidant such as SO$_4^{2-}$</td>
<td>Thermodynamics (need to drive reaction), Difficult to control intermediates, Management of mixed/syntropic communities, H$_2$ management, Currently no recombinant systems</td>
<td>Higher carbon and energy efficiency, Methanogens are robust organisms (engineer them to oxidize CH$_4$)</td>
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## Breakout Session 1 Output: Routes for Methane Conversion – Anaerobic conversion, Nitrite

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<tr>
<td>Anaerobic/nitrite</td>
<td>pMMO (uses O2 produced <em>in situ</em> from NO$_2^-$)</td>
<td>CH$_3$OH, CH$_2$O, RuMP-serine cycle, C$_4$ product</td>
<td>CH$_4$, NO$_2^-$</td>
<td>Extremely slow growth, Essentially the same as O$_2$ dependent MMO system</td>
<td>None identified</td>
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**Breakout Session 1 Output: Routes for Methane Conversion – Other *in situ* systems**

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</thead>
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<tr>
<td>P450</td>
<td>Metal cluster for C-H activation</td>
<td>CH₃OH, CH₂O, RuMP-serine cycle, C₄ product</td>
<td>O₂, CH₄</td>
<td>P450 low activity, Large active site, Low selectivity, Redox maintenance, Energy efficiency</td>
<td>Engineered enzyme could be envisioned with greater energy efficiency than MMO</td>
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<td>AMO</td>
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<td>Dioxygenase</td>
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<td>Active site engineering</td>
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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$_2$O sequestration and release to maintain non-toxic CH$_2$O 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$_3$OH, and then biologically convert CH$_3$OH to fuel product
Breakout Session 2 Output: Process – CH\textsubscript{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$_3$OH 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 $V_{\text{max}}$
- 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)