MICROBIAL CONVERSION OF METHANE TO METHANOL USING A PLUG FLOW REACTOR

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Opportunity

- Methane is a green-house gas produced as a byproduct in landfills
- Methanotrophic bacteria provide an opportunity to utilize low-value to produce a valuable commodity, methanol

Vehicle

- *Methylosinus trichosporium* (OB3b), a methanotrophic bacteria[1]
  - Metabolizes methane by the monooxygenase enzyme (MMO), producing methanol
  - Subsequently metabolizes the methanol to formaldehyde via MDH
  - Produces carbon dioxide as the final product upon further enzyme-induced reactions[1]
- MDH activity must be inhibited in order to achieve methanol product
- Cyclopropanol interacts with MDH, irreversibly inhibiting its activity, shown below. Cyclopropanol is expected to be produced via oxidation of cyclopropane by MMO[2]

$$MDH_{ox} + cyclopropanol \overset{K_d}{\leftrightarrow} MDH_{ox} * cyclopropanol \overset{k_i}{\rightarrow} MDH_{inactive}$$

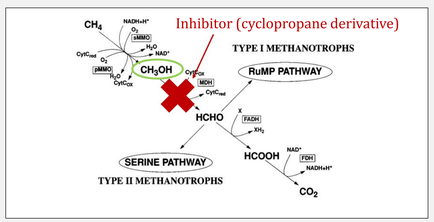

Figure. The metabolic pathway of OB3b. The consumption of methanol by the MDH enzyme must be inhibited in order to accumulate MeOH product. Figure from Hansen, R.S.[1]

Objective

- Immobilize OB3b culture in alginate beads
- Design a reactor system to investigate performance of immobilized OB3b
- Evaluate methane consumption rate of immobilized OB3b cells in a batch setting
- Investigate methane conversion in an immobilized-cell packed bed plug flow reactor
- Evaluate methanol selectivity of reactor in the presence of an MDH inhibitor

[1] Hansen, R.S., Hansen, T.E., *Methanotrophic bacteria.*,(Microbiology Reviews: 1996), 60(2):439, Accessed from http://mmbr.asm.org/ on January 10, 2014.
[2] Frank, Johannes, et al., *On the mechanism of inhibition of methanol dehydrogenase by cyclopropane-derived inhibitors*, (Eur. J. Biochem. *184*, 187-195: 1989).
Landfill cover Soil, Appl Biochem Biotechnol (2013) 171:1487-1499
http://en.wikipedia.org/wiki/File:Methane-CRC-MW-3D-balls.png
http://en.wikipedia.org/wiki/File:Methanol-3D-balls.png

Design

Reactor Packing

OB3b cells immobilized within alginate hydrogel beads. Alginate solution made with 2 wt% sodium alginate and cell concentration of ~1 g/L. Sodium alginate beads of 2.5 mm diameter were made under critical drop conditions, determined by Weber and Bond numbers.

$$Bo = \frac{D_c^2 \rho g}{\sigma}$$

$$We = \frac{16 \rho Q^2}{\pi^2 \sigma D_c^3}$$

- D_c, capillary ID (m)
- ρ, fluid density (kg/m^3)
- σ, surface tension (N/m)
- Q, flow (m^3/s)

Bead Formation Process

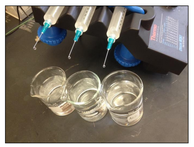

Figure. Bead production via culture-alginate dropping into 0.1 M $CaCl_2$.

- Concentrate OB3b culture via centrifugation
- Add 2 wt% alginate to concentrated culture
- Drop culture-alginate into 0.1 M $CaCl_2$ solution using a 23 gauge stainless steel syringe needle
- Allow 30 min in solution for adequate cross-linking

Reactor Column

Reactor dimensions evaluated based on bead size and pressure drop across the packed column

Pressure drop estimated using the Ergun equation

$$\Delta P = \frac{150 \mu u_0 (1-\epsilon)^2 L}{\epsilon^3 D_p^2} + \frac{1.75\, u_0^2 (1-\epsilon) \rho L}{\epsilon^3 D_p}$$

- μ, dynamic viscosity of the fluid (Pa-s)
- ρ, fluid density (kg/m^3)
- ϵ, packing void fraction
- L, packing height (m)
- D_p, diameter of the alginate beads (m)
- u_0, superficial fluid velocity (m/s)

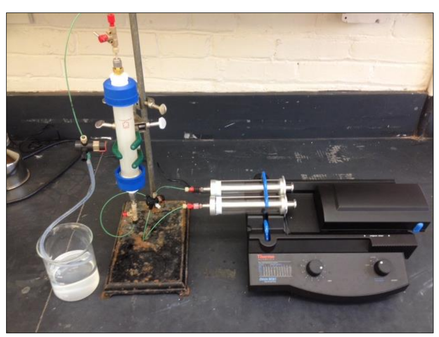

Figure. Reactor set-up. Orion syringe pump driving two 100 mL Hamilton gas-tight syringes. Syringes filled with growth media containing dissolved CH_4 and excess O_2. The ratio of column to bead diameter was maintained above 10 to avoid wall effects. Final reactor dimensions: 2.5 cm ID x 10 cm height.

Experiments

Batch Kinetics

- Made 12 identical batches in septum vials containing 10 mL immobilized cell beads (OD~1) submerged in 8 mL growth media
- CH_4 volumes of in the range of 0.1-1 mL were added to 9 mL batch headspace
- Batches were shaken at 300 RPM and analyzed for CH_4 every 20 min for 80 min using HP 6890 GC

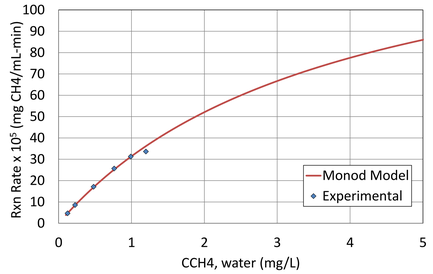

Figure. Reaction rate of immobilized cells at given initial dissolved methane concentrations. Experimental data was fit to Monod kinetics using the Lineweaver-Burk method ($K_s = 3.8 \frac{\text{mg}}{\text{L}}$ and $r_{max} * 10^5 = 152\ \frac{mg\ CH_4}{mL\ beads - min}$). Data points are averages of duplicate trials.

Investigation of Bead Degradation

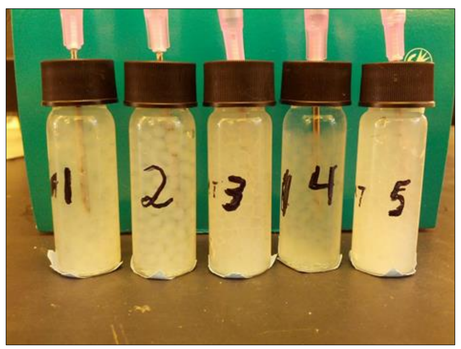

Figure. Investigation of bead deformation by growth media (1), 10 times dilution of growth media (2), growth media without trace elements and EDTA (3), HEPES buffer (4), and phosphate buffer (5) after 15 flushes.

- Beads began deteriorating after ~12 τ in PFR
- A media component was assumed to be interacting with the alginate-Ca^{2+} cross-linking
- Vials of beads were flushed 15 times with various media components in 3 mL (~ 2 void volume) increments
- Effects on bead integrity were visually monitored

Plug Flow Experiments

- Fed dissolved methane (S_0 = ~0.08 mg/L) and oxygen (in excess) to reactor with and without MDH inhibitor (15.6 mg/L cyclopropane)
- Sampled inlet and outlet for methane, cyclopropane, and methanol to determine conversion (X) of methane and cyclopropane and methanol selectivity
- Reactor performance equation:

$$r = \frac{r_{max} S}{K_s + S} \rightarrow \frac{1}{\eta r_{max}}(XS_0) - \frac{K_s}{\eta r_{max}} ln(1-X) = \tau$$

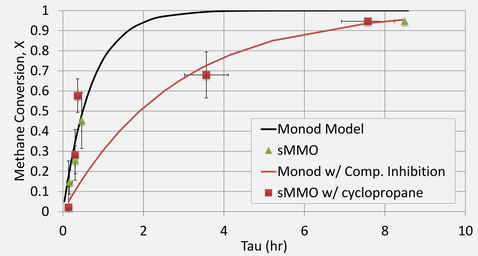

Figure. Plug flow reactor model, based on batch Monod kinetics. Error bars represent 95% confidence interval of 3 trials.

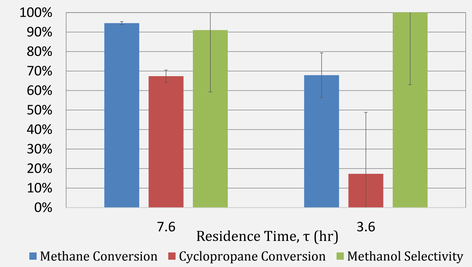

Figure. Reactor performance metrics at the two largest residence times measured in the PBR. Error bars are 95% confidence intervals.

Commercialization Potential

Biolamina-plate Bioreactor

- Stacking for scalability
- Increased mass and heat transfer

Team Project Contributions

- Analysis of alginate integrity in presence of inhibitor
- Experimental platform for immobilized cells
- Proof of concept for methane to methanol conversion of OB3b within alginate

Acknowledgements

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