MICROBIAL CONVERSION OF METHANE TO METHANOL USING A TWO-PHASE FLOW REACTOR

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BACKGROUND

OPPORTUNITY

- Methane (CH_4), a landfill byproduct and greenhouse gas, has limited value as a fuel
- Liquid methanol (CH_3OH) has a higher energy density than methane, making it more valuable and versatile
- Biocatalysts, such as methanotrophic bacteria, provide an energy efficient means to convert methane to methanol

BIOCATALYST

- *Methylosinus trichosporium* (OB3b) is a methanotrophic bacteria that:
  - Degrades methane via action of monooxygenase enzyme (MMO)
  - Produces methanol as intermediate in its metabolic conversion of CH_4 to CO_2
  - Can be inhibited with cyclopropanol, allowing methanol product to accumulate (Figure 1)

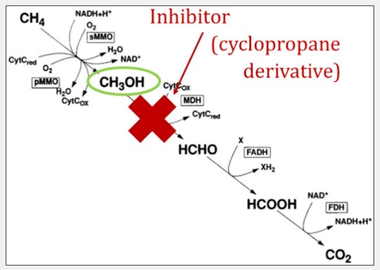


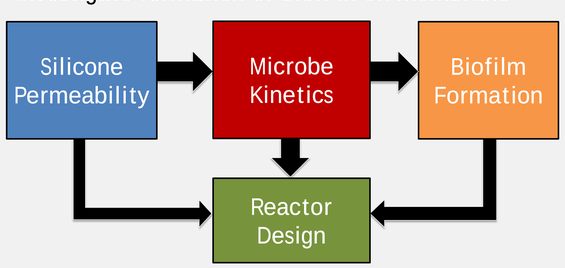
Figure 1. Metabolic pathway of OB3b. The inhibition of MDH prevents the formation of CO_2, yielding methanol product (green circle).[1]

TWO-PHASE FLOW REACTOR OPPORTUNITY

- Methane's low solubility in water makes it difficult to contact with microbes
- Interfacing gas and liquid feed streams using a silicone membrane passively supplies methane and oxygen to aqueous microbes

OBJECTIVES

- Construct dynamic testing environment
- Characterize gas transfer across membrane
- Analyze microbial activity in membrane system
- Investigate formation of biofilm on membrane



DESIGN

PERMEABILITY

Confirming the transport properties of oxygen and methane across silicone membranes was necessary to gauge the capacity for microbial growth in a two-phase flow system. Permeability of silicone tubing was evaluated using a PermSelect hollow fiber module (Figure 2). Data was compared to expected flux with equations at right.

Figure 2. Membrane module with 10 cm^2 of interfacial surface area [2] was ran for 40 min, with pure oxygen at ~2 psig flowing at 10 mL/min on tube side of the unit, saturating ~200 mL of water recirculating through shell side.

MICROBIAL GROWTH

Semi-batch reactors were made using 23 mL crimp bottles, 16G needles and 40 cm sections of silicone tubing (0.58 ID, 0.008 wall thickness). Reactors were run with 3% methane in air, fed through silicone tubing at ~2 psig and ~1 mL/min. One mL samples were taken daily to measure cellular activity. Ethylene to ETO activity tests were used to measure pMMO* expression of cells (Figure 3).

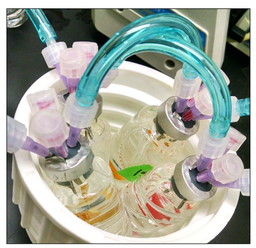


Figure 3. Semi-batch reactors growing MOT OB3b in stagnant media. Reactors were inoculated with 0.1, 1, and 10 mg/L cell concentration, and were ran for ~4 days.

BIOFILM FORMATION

Gas comprised of 29% methane and 71% air was fed through reactors membranes at 21 mL/min. A culture solution recirculated continuously through two membrane modules while crimp vials housed 23 mL of stagnant culture of high and low cell density. SOUR** tests were conducted daily for 7 days to evaluate microbe growth. Ethylene to ETO tests were performed at the end of experimentation to test for presence of membrane biofilm.

Figure 4. Biofilm observed after a week of growth in in stagnant media.

*pMMO= Particulate Methane Monooxygenase
**SOUR= Specific Oxygen Uptake Rate

MODELS

MOLAR FLUX EQUATION

$$N_A = p_A A \frac{\Delta P_A}{t}$$

- N_A = molar flux of A from the gas to the liquid **[mmol/min]**
- p_A = permeability of A **[mmol-mm/cm²-psi-min]**
- A = interfacial area **[cm²]**
- ΔP_A = partial pressure driving force **[psi]**
- t = membrane thickness **[mm]**

HENRY'S LAW

$$\Delta P_A = y_A P_g - H C_A$$

$$H = \frac{p_A}{C_A}$$

- ΔP_A = partial pressure driving force **[psi]**
- y_A = gaseous mole fraction of A
- P_g = total pressure of gas **[psi]**
- H = Henry's constant **[psi-L/mg]**
- C_A = concentration of A in liquid **[mg/L]**

RESULTS

OXYGEN ACCUMULATION VS. TIME

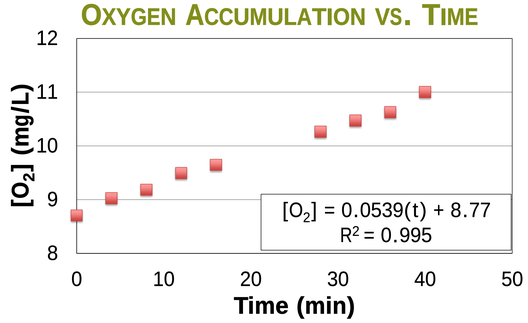


Figure 5. Oxygen diffusion tests using the PermSelect membrane module. Pure oxygen was ran counter current to a recirculating liquid flow. Liquid samples were periodically taken from liquid reservoir and analyzed for dissolved oxygen concentration.

RELATIVE MICROBIAL ACTIVITY VS. TIME

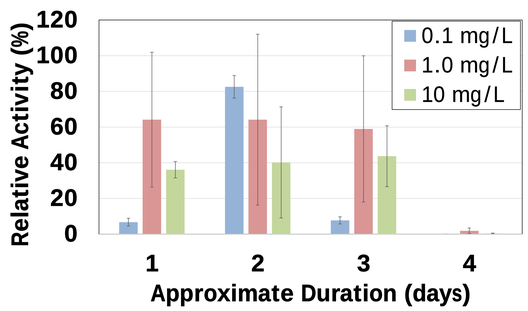


Figure 6. Relative ethylene oxide concentrations for 3 cell inoculum densities are compared over 4 days. Cell inoculum of 1 mg/L exhibited the most stable activity over the duration of the experiment Error bars represent 95% confidence intervals for each sample.

COMMERCIALIZATION POTENTIAL

BIOLAMINA PLATE REACTOR

- Potential platform for biofilm formation
- Increased rate of methane gas transferred to biocatalysts

TEAM PROJECT CONTRIBUTIONS

- Silicone is a feasible material for sufficiently delivering necessary gases to microbes
- Microbial activity indicates that biocatalysts can be sustained in a membrane system
- Biofilm was present in stagnant media, however it degraded rapidly due to shearing

Figure 7. Separate cell culture solutions were pumped through two membrane units. The solutions are contracted with 29% methane and 71% air via silicone membrane module to stimulate cell growth. Liquid flowrate was kept low to prevent shearing of the biofilm.

LOOKING FORWARD

FUTURE WORK

- Investigate modification of silicone or alternative immobilization mediums
- Characterize impact of shear on biofilm formation and integrity
- Evaluate alternative methods of quantifying activity of system

ACKNOWLEDGEMENTS

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[1] Mihailovic, M., S. Johnson, and T. Hollenberg. *Proposal for Bio-Lamina-Plates Bioreactor for Enhanced Mass and Heat Transfer,* Oregon State University. *ChE 406* (2014): Print.
[2] "PDMSXA - 10 Tiny Data Sheet." MedArray, Inc. MedArray, Inc. Web. 12 May 2015.