Attachment of Methanotrophic Bacteria to Materials for Methane Emission Reduction

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University of Minnesota Twin Cities, College of Science and Engineering, Spring 2018

Introduction

Agriculture is one of the main contributors of greenhouse gasses (GHGs) to the Earth's atmosphere, accounting for nearly 30% of GHG emissions in the US [1]. Methane production from ruminants accounts for 25-30% of agricultural GHG emissions by some estimates [2]. As a GHG, methane is about 25 times more potent than carbon dioxide as a contributor to global warming [3]. Methanotrophic bacteria have been explored as a possibility for reducing methane emissions. These bacteria oxidize methane to carbon dioxide at a one to one ratio and occur naturally in wet environments with high methane content [4].

Purpose

The goal of this study was to explore the attachment of methanotrophic bacteria to different materials via bacterial biofilm formation. This information could be used in future studies for cultivation and harvesting of methanotrophic bacteria for application in agricultural settings for reduction of methane emissions.

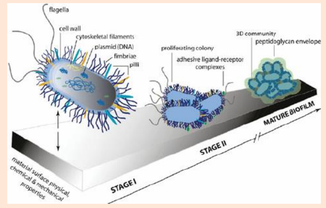

Figure 1: The process of bacterial biofilm formation [5].

Procedures

For all procedures described, NMS media was used either in liquid or agar form with 0.74% methanol as the sole carbon source. Liquid cultures were cultivated in sealed serum bottles.

Part 1: The CO_2 production kinetics and methanol consumption of an anaerobic digester sample were tested using gas chromatography and high-performance liquid chromatography. This was used to determine necessary cultivation time.

Figure 2: Samples before (left) and after (right) cultivation with the anaerobic digester inoculate for the CO_2 production kinetics tests.

Procedures Continued

Part 2: The anaerobic digestor sample was tested for wild-type methanotrophic attachment to wood, polypropylene, and hemp rope with one and two-week cultures. Attached mass was analyzed after oven drying using mass balance procedures.

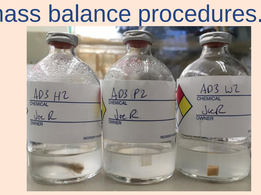

Figure 3: Attachment samples inoculated with the anaerobic digester sample after cultivation.

Part 3: A pure strain of *Methylosarcina fibrata* was purchased and tested for attachment to wood, polypropylene, hemp rope, and nylon fabric with two week cultures. Samples were inoculated with $8.375*10^6$ cells.

Figure 4: Attachment samples after drying in oven for mass balance.

Part 4: Bacterial strains were isolated from the anaerobic digester sample by plate streaking for later use and analysis.

Results

Part 1: CO_2 Production Kinetics and Methanol Consumption

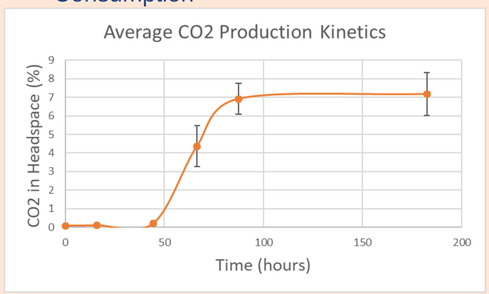

Figure 5: Percentage of CO_2 in serum bottle headspace during cultivation of anaerobic digestor samples.

Change in Methanol Concentration after Two-week Culture		
Sample	Methanol (%)	Methanol Change (%)
Initial Concentration	0.739	-
Hemp Sample 1	0.558	-0.181
Wood Sample 1	0.565	-0.174
Polypropylene Sample 1	0.514	-0.225

Table 1: HPLC analysis of the decrease in methanol concentration after two weeks of testing with anaerobic digester samples.

Results Continued

Part 2: Attachment with Anaerobic Digester Sample

- Materials included: hemp rope, polypropylene, and wood

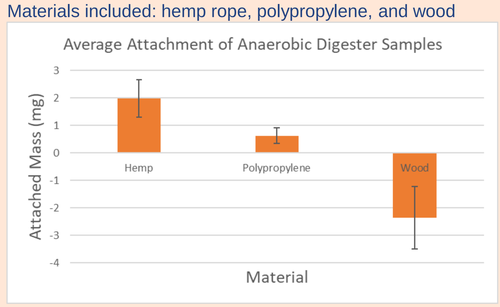

Figure 6: Average attachment of anaerobic digester bacteria to hemp rope, polypropylene, and wood samples.

Part 3: Attachment with *M. fibrata* Sample

- Materials included: hemp rope, polypropylene, nylon, and wood

Figure 7: Average attachment of *Methylosarcina fibrata* to hemp rope, polypropylene, nylon, and wood samples.

Figure 8: Average attachment of control samples with no inoculation.

Part 4: Bacterial Isolation from Anaerobic Digester Sample

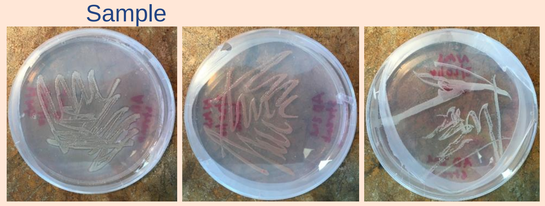

Figure 8: Three plate cultures streaked from the anaerobic digester sample. Plates were streaked until cultures appeared to be monocultures based on sight.

Discussion

- Analysis of CO_2 production kinetics in the anaerobic digester samples showed maximum CO_2 production from about 44-88 hours of cultivation. Further analysis of the methanol consumption kinetics would help determine if this is the maximum growth period for the methanotrophs.
- The attachment tests using the anaerobic digester sample resulted in statistically significant data showing hemp as the leading attachment material, averaging 1.98mg of attached mass. The hemp control sample also showed an average reduction in mass of -0.65mg; therefore, the attached mass to the hemp samples may actually be larger.
- Isolation of methanotrophic strains from the anaerobic digester sample appears to have been successful, but more testing on the samples is needed to confirm their taxonomic classification.

Conclusions

- Based on current results, hemp rope is the most effective material for the attachment of methanotrophic bacteria from the anaerobic digester sample with a two-week cultivation period based on average attachment of 1.98mg.
- Further testing on attachment to wood is necessary due to the loss of mass in the autoclave and media during testing.
- Future work on this project may include a better method of measuring attachment to wood and a wider variety of materials to test what material characteristics are best suited for attachment.

Acknowledgements

This project was supported by the University of Minnesota Undergraduate Research Program.

References

1. Christopher Hyner. A Leading Cause of Everything: One Industry That Is Destroying Our Planet and Our Ability to Thrive on It. Stanford Environmental Law Journal. [Online] 2015, n.p.
2. Philip K. Thornton; Mario Herrero. Potential for Reduced Methane and Carbon Dioxide Emissions from Livestock and Pasture Management in the Tropics. Proceedings of the National Academy of Sciences of the United States of America. [Online] 2010, 107(46), 19667-72.
3. Howard Dalton. The Leeuwanhoek Lecture 2000-The Natural and Unnatural History of Methane-oxidizing Bacteria. Philosophical Transactions of the Royal Society B: Biological Sciences. [Online] 2005, 360(1458), 1207-22.
4. Eric R. Sundstrom; Craig S. Criddle. Optimization of Methanotrophic Bacterial Growth and Production of Poly(3-Hydroxybutyrate) in a High-Throughput Microbioreactor System. Applied and Environmental Microbiology. [Online] 2015, 81(14), 4767-73.
5. Ahmad Abdolahi; Esah Hamzah; Zaharah Ibrahim; Shahrir Hashim. Application of Environmentally-Friendly Coatings Toward Inhibiting the Microbially Influenced Corrosion (MIC) of Steel: A Review. Polymer Reviews. [Online] 2014, 54(4), 707.