# Increasing Acetogenic Bacterial Density for Syngas Conversion Using A Porous Membrane

## Background & Significance

In the sustainability field, one of the most abundant resources is lignocellulosic biomass, a plant-based biowaste, prompting a considerable body of research on how to utilize it.[1] One method is by using gasification to convert it to synthetic gas, or syngas. This consists of multiple gases, of which carbon monoxide and hydrogen are the focus for this research. Syngas can be used for electricity, but the value is low for that or conversion to ethanol. Thus, it is desirable to convert it to something else more valuable. One such product are single cell proteins (SCPs). SCPs can be used as an protein substitute in animal feed or supplements due to a crude protein content of up to 80%.[2] Calculated for worldwide production, this could amount to three times the amount of protein currently in annual soybean meal production.[2] This is a two-step process, the first being syngas fermentation, an anaerobic conversion of syngas to acetic acid and ethanol. Those products are then put in an aerobic environment with different bacteria that will convert them to SCPs. Since the inputs of this process are biowaste or gas common in carbon emissions, improvement of this process could also help to create a more sustainably circular economy.

The focus of this study is on the improvement of syngas fermentation, specifically for the product of acetic acid using acetogenic bacteria such as *Clostridium aceticum* or *Clostridium ljungdahlii*. Both have been proven to be able to convert carbon monoxide to acetic acid.[3,4] Currently, a problem in syngas fermentation is the low solubility of gas as a substrate. This decreases the ability of utilization. The amount of reacted gas substrate is directly proportional to the gas-liquid interfacial area per unit volume.[5] A method called immobilization, used during the growth of the bacteria, increases this. The material used for this provides the bacteria more surface area to grow on, creating support and a more evenly spread biofilm. From this an

increase of bacterial density can occur, as well as increasing working surface area for syngas to access the bacteria for conversion. For this project we will use a porous material such as biochar for immobilization. Created from pyrolysis of biomass, biochar is an easily accessible and porous material. This gives more spaces and surface area for bacteria to be immobilized.

**Related Research**

Overall, there are studies which use biochar for improvement of syngas fermentation, such as one done at Jiangnan University in Wuxi, China, but like others, the focus was on improvement for ethanol production.[6] In such studies acetic acid production is either not mentioned due to use as an intermediate, or it is briefly mentioned. Most used poultry litter biochar, and all used either *Clostridium ragsdalei* or *Clostridium carboxidivorans* as their bacteria. In the past, Dr. Ruan's Center for Biorefining has done research in bioenergy and bioproducts as well as food science engineering. In the bioproducts sector, they have long been doing work with gasification, microorganism manipulation, and waste utilization. Syngas fermentation is an extension of these areas.

**Hypothesis & Aims**

With biochar deposited in the growth medium for acetogenic bacteria, the aim is to increase bacterial density and cause less random or clumped growth of the bacteria. This would improve acetic acid production during syngas fermentation. The biochar is predicted to do just that due to its porous surface.

**Methods and Analysis**

For 12-16 weeks, I will spend 8-10 hours per week in Dr. Ruan's lab. My time will consist of initially preparing mediums and biochar for each iteration of fermentation. The first one will be to test the porous material's ability to immobilize the acetogenic bacteria. If for any

reason that is proven to fail, the experiment will be modified. During the growth of the acetogenic bacteria and syngas fermentation, I will be visiting to monitor the reactors' progress. For control purposes, the syngas used in the experiments will be simulated from commercial gases, so its creation will not be needed. Before any syngas fermentation, the bacteria will first need to be grown. After mixing an enrichment medium which includes fructose as a food source, this and the biochar will be sterilized in an autoclave. These will then be placed together with the bacteria inside a reactor. The bacteria will then be left to grow in this medium for 4-5 days. Simultaneously, two controls will also be separate reactors, one with porous biochar equivalent to that in the experimental medium, and one with an equivalent volume of medium and bacteria without the biochar. After this, the biochar for each reactor will be weighed for weight change. The air in each reactor will then be displaced with syngas to start syngas fermentation, which will happen over the course of another 4-5 days. After syngas fermentation has commenced, the amount of acetic acid in the experimental and control reactors will be measured using high-performance liquid chromatography (HPLC).

**Expected Outcomes**

Using the biochar during bacterial enrichment stage, I will be able to quantify the effect of biochar on bacteria growth rate. This can then lead to quantification of the effect on the gas utilization efficiency of acetogenic bacteria during the fermentation stage. The improvement of acetate productivity may indicate the biochar can enhance the liquid-gas transfer efficiency of the syngas fermentation by building up biofilm to have more specific surface area for access to the syngas for conversion.

**References**


1. Ge, X.; Chang, C.; etc. Conversion of Lignocellulosic Biomass Into Platform Chemicals for Biobased Polyurethane Application. In *Advances in Bioenergy*; Elsevier, 2018; Vol 3; pp 161-213 https://doi.org/10.1016/bs.aibe.2018.03.002 (accessed October 12, 2020).
2. Matassa, S.; Papirio, S.; Pikaar; etc. Upcycling of biowaste carbon and nutrients in line with consumer confidence: the "full gas" route to single cell protein. *Green Chem* [Online] **2020**, *15*, 4912-4929. https://doi-org.ezp2.lib.umn.edu/10.1039/D0GC01382J (accessed September 28, 2020).
3. Sim, J. H.; Kamaruddin, A. H.; Long, W. S.; etc. *Clostridium aceticum*—A potential organism in catalyzing carbon monoxide to acetic acid: Application of response surface methodology. *Enzyme and Microbial Technology* **[**Online**] 2006**, *5*, pp 1234-1243. https://doi.org/10.1016/j.enzmictec.2006.09.017 (accessed September 28, 2020).
4. Younesi, H.; Najafpour, G.; Mohamed, A. R.; etc. Ethanol and acetate production from synthesis gas via fermentation processes using anaerobic bacterium, *Clostridium ljungdahlii*. *Biochemical Engineering Journal* [Online] **2005**, *2*, pp 110-119. https://doi.org/10.1016/j.bej.2005.08.015 (accessed September 28, 2020).

5. Klasson, K. T.; Ackerson, M.D.; Clausen, E.C.; etc. Bioreactor design for synthesis gas fermentation. *Fuel* [Online] **1991**, *5*, pp 605-614. https://doi-org.ezp1.lib.umn.edu/10.1016/0016-2361(91)90174-9 (accessed October 12, 2020).

6. Sun, X. Title of Thesis. Ph.D. Dissertation, Oklahoma State University, Stillwater, Oklahoma, 2018. https://search-proquest-com.ezp1.lib.umn.edu/docview/2287508049/fulltextPDF/B3AF51B77BFB4212PQ/1?accountid=14586 (accessed October 12, 2020).