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# Biobutanol- A Fuel Additive with more Bang for the Buck

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# Research and Creative Experience for Undergraduates (RCEU) Program, Summer 2016


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Project Title: Biobutanol – a fuel additive with more bang for the buck


Project Summary:

Butanol is an interesting fuel additive that currently receives significant consideration. Compared to ethanol, a common fuel additive, butanol has twice the number of carbon atoms, therefore it provides more energy. Butanol mixes better with petroleum products such as gasoline and diesel than ethanol does. It has a lower vapor pressure and is more hydrophobic than ethanol, therefore less water will be introduced into any given fuel system that is substituted with an alcohol fuel additive.

Bacteria of the *Chlostridium* genus are of industrial importance because they perform a so called **ABE** fermentation process; that means, they produce **A**cetone, **B**utanol and **E**thanol when grown on sugars. *Chlostridium pasteurianum* is of particular interest, because this strain produces mostly butanol, and the strain is capable of fermenting glycerol. The strains ability to ferment glycerol, instead of needing sugar, is a tremendous advantage and makes the organism highly interesting to the (bio)fuel industry.

Biodiesel is a "green" fuel source; it is produced from vegetable oil and waste fat and oil sources such as used cooking oils and animal fats from the poultry industry. The biodiesel industry grew steadily over the past decade and a record 1.8 billion gallons were produced in 2013, which is a 65% increase over 2012. The enormous growth of the industry becomes even more evident when considering that in 2000 only two million gallons biodiesel were produced.[1] Yet, for every ton of biodiesel produced there are also 100 kg of glycerol produced, which is currently considered waste. In fact, the piled-up glycerol is such a severe problem that it received its own name, being called the "glycerol glut". The "glycerol glut" severely hampers the industry and has led to the closing of several biodiesel manufacturing sites.

Hence it is urgently necessary to address this "glycerol glut" problem by finding ways to convert this waste product into a new and value added product. *Chlostridium pasteurianum* could be a solution to the problem, as this organism is capable of converting waste crude (as received from the biodiesel industry) glycerol into the fuel additive butanol. Since this butanol is produced from "new" carbon, as opposed to the petroleum derived carbon, it carries the attribute "bio"butanol, indicating that it is obtained from a renewable and sustainable resource.

Our previous research has shown that *C. pasteurianum* can utilize crude glycerol anaerobically as a sole carbon source for butanol production.[2] Our previous studies, which focused on assessing growth behavior, toxicity and cellular inhibition due to impurities present in crude glycerol and homeoviscous cellular responses to increasing butanol titers were conducted in batch cultures. Our current research, funded through a NSF project, focuses on the development of a continuous culture approach using lipid vesicle mediated extraction to continuously collect butanol from a fermentation process.

Student Duties:

The student will work closely with a graduate student on the fermentation of *C. pasteurianum* on pure, simulated crude or crude glycerol to evaluate the cellular response of the microorganism to substrate and product toxicity using a continuous culture. The student will be responsible for conducting fermentation experiments. Learning objectives: (i) set up anaerobic fermentation conditions and conduct fermentation of *C. pasteuerianum*; (ii) record growth curves (iii) maintain the strain by continuous sub-culturing and stock preparation (iv) determine bioenergetics parameters associated with cell growth and cell maintenance.

When satisfactorily results have been achieved for the fermentation of *C. pasteuerianum* fermentations will be expanded to butanol challenged fermentation experiments, i.e. the bioenergetics and homeoviscous response will be studied as the fermentation broth is rendered with increasing butanol content. Learning objective: (i) extraction of cell membrane (ii) determine degree of unsaturation in lipids that constitute the cell membrane by $^{1}$H NMR

Mentor Supervision and Interaction:

I will meet with the student every morning for 15 to 20 minutes and discuss results of the previous day, and plan experiments for that day, thus he/she will have the benefit of daily supervision by me. Either the graduate student or I will teach the student the use of all the equipment and instrumentation that is needed to fulfill this project. I will be available continuously throughout the day if questions or problems arise. The RCEU student will participate in my bi-weekly group meetings and will be expected to provide research updates using power point presentations, as it is standard in my group. This will be an excellent opportunity for him/her to learn how to present research data and how to interpret and defend them in a group of fellow researchers. The student's progress on the project will be evaluated at these group meetings. He/she is also expected to summarize the entire research work in a formal setting by presenting the results to his/her peers and prepare a poster to be presented at the RCEU poster session in September 2016.

Expected workload: 10 weeks at 40 hrs/week

1 Taconi, K.A., Venkataramanan, K.P., Johnson, D.T. *Environm Prog Sustain* **2009**, 28, 1000**;** 2 Venkataramanan, K.P. Boatman, J.J., Kurniawan, Y., Taconi, K.A., Bothun, G.D., Scholz, C. Appl Microbiol Biotechnol 2012, 93, 1325