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From Rags to Riches, From Waste to Valuables: Towards a Sustainable Fuel Additive

Carmen Scholz
University of Alabama in Huntsville

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Faculty Mentor: Dr. Carmen Scholz
Professor
Department of Chemistry
301 Sparkman Dr. MSB 333

☎256-824-6188
256-824-6349
cscholz@chemistry.uah.edu

Project Title: From Rags to Riches, from Waste to Valuables: Towards a Sustainable Fuel Additive

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.

Funded by an NSF grant, our lab currently studies the production of biobutanol by *Chlostridium pasteurianum*. The butanol produced here is derived from "new" carbon, as opposed to the petroleum derived carbon, therefore it is called "bio"butanol, indicating that it is obtained from a renewable and sustainable resource. *C. pasteurianum* produces butanol by the fermentation of glycerol instead of sugar. The ability to use this unique Carbon-source makes this organism highly relevant to the biofuel industry, as glycerol is a waste-product of the biodiesel industry. Biodiesel is a "green" fuel source, produced from vegetable oil and waste fats and oils, such as used cooking oils and animal fats from the poultry industry. Annual biodiesel production has been steady for the past several years at around 1.4 billion gallons annually. However, 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. Converting this glycerol glut into an effective, i.e. value-added fuel additive (butanol) can be an elegant solution to the glycerol-waste problem.

This NSF-funded project started in Fall 2015 and our lab has made strides towards setting the parameters for the glycerol fermentation and the analysis of the fermentation products[1,2]. Current research focuses on establishing bioenergetics parameters and developing a time-efficient, NMR-based method for the determination of the butanol concentrations in the fermentation broth and during the butanol extraction process, replacing the current HPLC method, which is time-consuming and proved to be too sensitive to lipid vesicles used in the extraction. We are working towards a continuous culture system that is scalable and will steadily produce butanol, which then

will be continuously extracted using the lipid vesicle based extraction system that we designed.

Student Duties:

The student will work closely with the post-doctoral associate on (i) the fermentation of *C. pasteurianum* on pure, simulated crude or crude glycerol to determine the bioenergetics parameters for each carbon source and on (ii) the analytics of the fermentation products. The student will be responsible for conducting fermentation experiments. Learning objectives: (i) set up anaerobic fermentation conditions and conduct fermentation of *C. pasteuerianum*; (ii) monitor growth and collect growth data (iii) maintain the strain by continuous sub-culturing and stock preparation (iv) calculate bioenergetics parameters.

The student will also work on the analytics of the fermentation products, using quantitative NMR and comparing/verifying the results by HPLC. Learning objectives: (i) become proficient in the use 1H NMR and HPLC instrumentation, (ii) analyze 1H NMR and HPLC data, and determine concentrations. Mastering these two techniques is of significant benefit to the student as those are key chemical analytical techniques. They are used in various chemical industries for a variety of analytical goals. Hands-on proficiency in these key-techniques and the ability to read the spectra and analyze the data is a sizeable advantage for every graduating student, independent whether he/she is seeking employment in industry or continues his/her studies in a graduate program.

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. The post-doctoral associate 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 2017.

Expected workload: 10 weeks at 40 hrs/week

[1] Venkataramanan, K.P., Kurniawan, Y., Boatman, J.J., Haynes, C.H., Taconi, K.A., Martin, L., Bothun, G.D., Scholz, C. *J. Biotechnol*. **2014**, 179 C 8-14

[2] Bothun, G.D., Boltz, L., Kurniawan, Y., Scholz, C. *Colloids and Surfaces B: Biointerfaces*, **2016**, 139, 62-67