TO: Texas Hazardous Waste Research Center

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SUBJECT: Annual Progress Report

PROJECT NUMBER: 513LUB0022H

PROJECT TITLE: Heterogeneous Catalyst Development for the Conversion of Phospholipid-Containing Feedstocks to Renewable Transportation Fuels

PROJECT PERIOD: 1 Sept 2013 – 31 Aug 2015

DATE: 15 Sept 2014

Project Description

To eliminate concerns with using food type oils for fuel, researchers have identified several potential feedstocks that are noncompetitive with the food industry. These oils are microbial derived oils (MDOs) and contain not only acylglycerides, but also vast amounts of free fatty acids and phospholipids, which are not amenable to biodiesel processing. The plant-derived oils (PDOs) currently used in biodiesel manufacturing contain ≤2% of phospholipids, which can be cheaply removed *via* a degumming pretreatment before converting the triglyceride oils to biodiesel. Phospholipids are the major component of cellular membranes; their function is to regulate nutrients into and wastes out of the cell. The oleaginous yeast R. Glutinous contains 17 wt% phospholipids and microalgae up to 40 wt%. Studies by Revellame and co-workers, which Dr. Benson is a co-author, suggest that wastewater sludges offer a viable feedstock solution for the displacement of petroleum-derived fuels. In fact, 25 – 65% of operating expenses of wastewater treatment facilities are associated with excess sludge removal. Therefore, conversion of microbially activated sludges into salable fuels and chemicals offers profit potentials for these facilities.

Furthermore, the U.S. Department of Energy (DOE) has invested significant resources over the past decade to develop the biorefinery concept. Rather than removing and discarding phospholipids in a pretreatment step, using phospholipids (and their acylglyceride counterparts) as a biofuel would be most beneficial. In addition, this research would allow for the recycling of phosphorus, which can then be used for biochemical processes, as flame-retardants, fertilizers, and pesticides. The process developed in Dr. Benson's laboratory (Fig. 1) requires no wash water, and thus, is an environmentally friendlier, sustainable process.

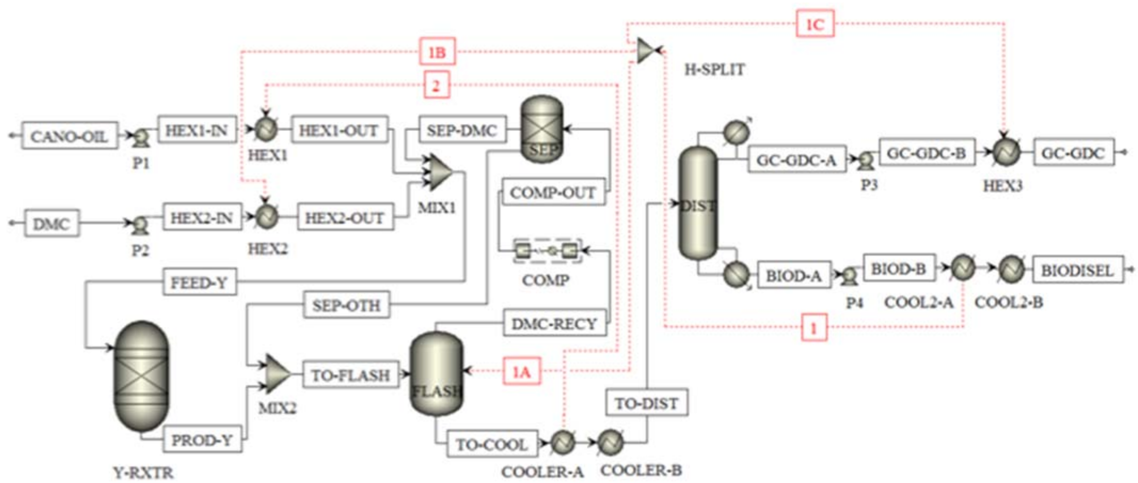


Fig. 1. Process flow diagram using Aspen Plus simulation for a glycerol-free biofuel production facility. The red dotted lines indicate heat integration techniques that reduce overall energy input. This process is 15% more energy efficient than current biodiesel production facilities. . http://dx.doi.org/10.1016/j.fuproc.2013.03.030

This project is a bio-inspired concept for developing a heterogeneous catalyst that will selectively cleave the sn-2 acyl oxygen and both the sn-3 acyl and alkyl oxygens (Fig. 2), similar to phospholipases (PL) PL-A2, PL-C, and PL-D, respectively. Cleavage at positions 1 and 2 will yield FAME and glycerophosphocholine (GPC), but cleavage at position 3 will yield a phosphate ester. The main constraint when converting phospholipids to biofuel is that the fate of phosphorus-containing compounds is not known. Therefore, the intellectual merit of this work would be the development of a bifunctional catalyst, mimicking lipase enzymes, and determining the fate of the phosphorus component. Elimination of unwanted byproducts, elimination of wastewaters, and recyclability of phosphorus will be at the forefront throughout all phases of this work.

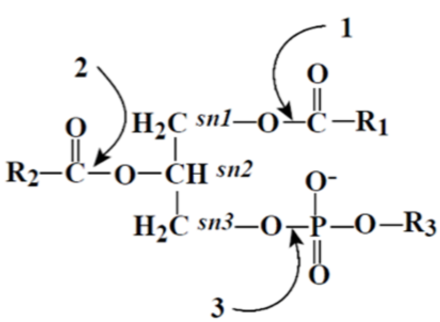


Fig. 2. Illustration of a phospholipid molecule where R_1 and R_2 are generally saturated and unsaturated hydrocarbon chains (14 – 22 C's in length) and R_3 can be $C_2H_4NH_3$ (ethanolamine), $C_6H_{12}O_6$ (inositol), or $C_3H_7NO_3$ (serine).

Objectives

The overall goal of this project was to develop a catalytic process for the conversion of crude lipid oils to green fuels. Previous work by the Benson Research Group has shown that refined row-crop oils (i.e. canola oil, soy bean oil, etc.) can be converted to a biofuel that similar to biodiesel but without unwanted, unsalable byproducts and without generated process wastewater. However, approximately 85% of the cost of biodiesel manufacturing lies within the refining steps that remove free fatty acids, phospholipids, and oderants from crude oils before being converted to biodiesel. Since the lipid feedstocks envisioned by Dr. Benson would be not only from row-crop oils but also from microbial-based feedstocks and used greases, a research effort is needed to determine the extent of conversion and any deleterious affects that these feedstocks would have on the catalysts developed in Dr. Benson's group. Therefore, the objectives of this project were to use crude oils, containing high free fatty acid concentrations, and phospholipids to test for overall conversions, kinetics, and catalyst robustness.

Methodology

A series of LDH catalysts have been synthesized containing a wide range of ligand/TBD complexes and exposed cationic LDH surfaces to create a bifunctional catalyst. Mechanistically, phospholipase (PL) enzymes use metal cations to polarize the sn-2 carbonyl oxygen or the phosphonyl oxygen while coordinating with a water molecule to hydrolyze the sn-2 acyl bond. PL-C phospholipase contains a triad of amino acids (Asp33, Arg69, and His82) to interact with the oxygen atoms of the phosphate group at the non-bridging and bridging (i.e. leaving group) positions. This interdependency leads to a major quantitative change in the transition state structure with an effective stabilization of the negative charge on the leaving group. To develop heterogeneous catalysts that mimic PLs, proper calcination time and temperatures of layered double hydroxides (LDH) produce a surfaces that are abundant in Mg^{2+} and Al^{3+} sites (Fig. 3).

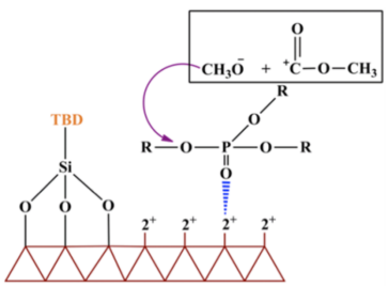


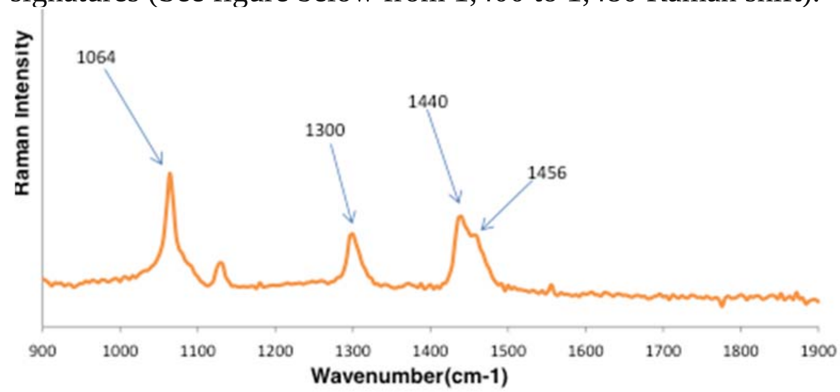
Fig. 3. Illustration of the proposed phospholipid adsorption through interaction of surface cations, which will stabilize the phosphonyl. Reaction of DMC into its ionic components occurs via the TBD, thus having dual functionality.

Another aspect of this project is the evaluation of kinetic parameters (i.e. rate constants, reaction orders, activation energies, etc.) for lipid oils containing triglycerides, FFAs, and phospholipids. Of particular interest is the determination of co-adsorption, competitive adsorption, and cross interaction adsorptive properties for the conversion of a simulated feedstock on the modified LDH catalysts. Kinetics analysis is the best method for determining true mechanisms throughout the reaction sequence. For these experiments, *in situ* measurements, which require small sample sizes (<5 mL), will yield complimentary Raman and GC/MS analysis. However, a Raman spectroscopy method must be developed for analyzing these types of reactions, containing reactants, products, as well as catalysts.

Accomplishments/Problems

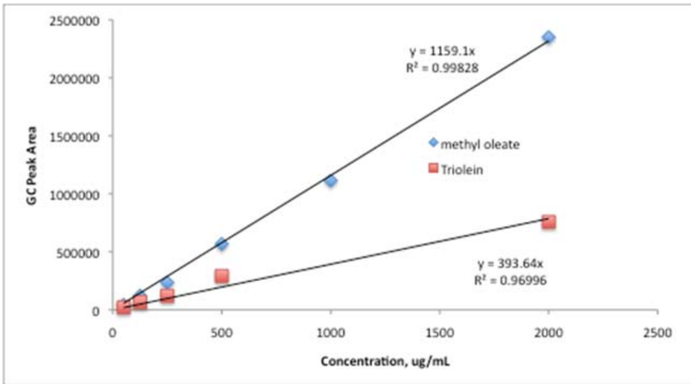
Dr. Benson has had two graduate students, both Masters level, and one undergraduate student working on this project. Mr. Keyvan Mollaeian and Mr. Josh Borton are the two graduate students, while Ms. Bleinie Dickerson is the undergrate student and has worked closely with Mr. Mollaeian. Mr. Mollaeian has been tasked with developing the *in situ* Raman analysis for this project. Mr. Borton's focus has been on the kinetic evaluation as well as evaluating crude lipids oils, containing 4.5, 14.5, and 45 wt% FFAs, obtained from Renewable Biofuels (Port Neches, TX).

Raman spectroscopy is commonly used for characterization of the materials such as chemical bonds and symmetry of molecules. This technique is a complement to FT-IR and elemental analysis. When using in situ Raman spectroscopy to monitor the reaction, time-resolved information about molecules structure, intermediates, mechanism, and rate of reaction can be obtained Using a Perkin Elmer Raman Flex 400 spectrometer (532 nm laser with a fiber optic probe), he has been able to elucidate, specifically, which Raman signatures are carbonyl's are product vs reactants. This is the most important, while also the most time consuming, aspect of the analytical method development. Nearly all of the reactants used in this study contain carbonyls, so differentiating between various species is difficult. However, we have been able to develop deconvolution algorithms to discriminate among the various components using a Gaussian fitting protocol. A Gaussian peak fitting program was written for parameter fitting of unconstrained nonlinear optimization using peak height, center of peak, and the full width at half max (FWHM) to calculate the peak area for peaks hidden (or obscured) by spectroscopic signatures (See figure below from 1,400 to 1,480 Raman shift).



Mr. Mollaeian and Ms. Dickerson have worked to synthesize additional LDH-TBD catalysts with varying degrees of crystallinity and cationic sites for adsorption and conversion of phospholipid compounds. By careful control of calcination time (1 – 3 hrs) and temperature (250 – 450°C), crystallinity can be adjusted 45 – 62%, as measured by a Bruker D4 XRD located at Lamar University. It is believed that the d-spacing between the LDH layers can be manipulated through adjustment of the calcination conditions. This work is still ongoing.

Mr. Borton has been performed a parametric study using crude lipid oils obtained from Renewable Biofuels, Inc. to determine reaction conversions depending on the quality of the oil. Using the TBD catalyst, he performed experiments using both methanol and dimethyl carbonate as methylating agents. Methanol was used as a base comparison since it is the industry standard for biodiesel production. DMC (or methanol):oil (6 – 24) and FFA content (4.5 – 45 wt%) were considered. Samples were analyzed using GC-FID in collaboration with colleagues at University of Louisiana – Lafayette (see figure below showing GC standards calibrations). Conversions as high as 90% were achieved even with 45 wt % FFA.



Future Work

Preliminary work has been performed towards the development of the kinetic parameters for the heterogeneous LDH-TBD catalyst (see figure below). However, it was found that LDH-TBD has limited shelf life, unless stored in desiccator to prevent oxidation of the TBD by moisture and air. LDH-TBD takes approximately one week to synthesize from Mg and Al salt solutions and can be stored for ~2 months in dessicator. Currently, we are working to produce larger batches of LDH-TBD catalyst (up to 500 g) before completing the kinetic evaluations. Future work will entail the kinetic evaluations and complete the *in situ* Raman analysis method development.

List of Publications and Presentations

25 Sept 2013 – "Catalytic and Process Development for Glycerol-free Biofuel from Lipids" Presented to Renewable Biofuels, Inc. (invited talk)

3 Nov 2013 – "Kinetic Evaluation and Reactor Modeling for Transesterification of Lipids With Dimethyl Carbonate Using the Homogeneous Catalyst Triazabicyclodecene" Presented at annual AIChE conference in San Franscisco, CA

7 Nov 2013 – "Development of a Heterogeneous Guanidine Base Catalyst for the Conversion of Lipids to a Sustainable Biofuel" Presented at annual AIChE conference in San Franscisco, CA.

6 May 2014 – "Overview: What's Around the Corner for the Glycerol Market?" Presented at the annual AOCS Conference in San Antonio, TX

Keyvan Mollaeian (MS Student) – Thesis Title "Layered Double Hydroxide Catalyst for the Conversion of Crude Vegetable Oils to a Sustainable Biofuel" (Expected Graduation Dec 2014)

Josh Borton (MS Student) – Thesis Title "Parametric Evaluation for the Conversion of Crude Lipid Oils Containing High Free Fatty Acid Content to a Sustainable Biofuel" (Expected Graduation Dec 2014)

Upon completion of theses by graduate students at least three peer reviewed manuscript will be submitted for publication: Parametric study, *In Situ* Raman methodology, and kinetics.