

Synthesis of Biodiesel from Hydrolyzed Rice By-Products Fermented with Engineered *B. subtilis*

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Current methods for production of biodiesel are not cost competitive when prices of crude oil are low. This study proposes a biodiesel production method that produces surfactant as a coproduct, making it profitable even when oil wholesale prices are low. In this study, Arkansas-grown rice hulls were hydrolyzed and fermented with genetically-engineered *B. subtilis* bacteria to produce FA-Glu surfactant. This fermentation was analyzed using a standard assay to confirm the production and consumption of glucose. The FA-Glu was then treated with 6 M HCL and heated in an oven at 100° C until the surfactant separated into its oil and amino acid components. After this separation, a hexane extraction was performed to make the oil easier to collect. Next, the oil layer was extracted using a pasteur pipette, the hexane was evaporated, and the mass of the oil was measured and found to be 0.110 g. The amino acid layer of the separation was then analyzed through a glutamate assay to determine if the predicted reaction occurred by testing for the presence of glutamate, the byproduct of the FA-Glu separation. The assay showed a significant amount of glutamate was present in the samples showing the predicted separation did take place. According to NREL publications, if this oil were processed with a hydro-treatment to remove the oxygen atoms from the fatty acid, making it chemically indistinguishable from petro-chemical-derived diesel fuel.