**Title:** Optimization of Solvent-Free Enzymatic Esterification of Free Fatty Acids using Taguchi Design of Experiments

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**Project Goals:** Esterification of free fatty acids (oleic acid as model) with glycerol using immobilized lipase for deacidification of oil for improved biodiesel production.

**Abstract Text:** The preferred process of biodiesel production converts triglycerides into their ester derivatives (biodiesel). Free fatty acids affect the efficiency and yield of the process. In this work, immobilized lipase was used for esterification of free fatty acid into glycerides to decrease acidity of oil, making the oil more suitable for biodiesel production. Several process parameters and their levels were optimized using a Taguchi design of experiments to achieve maximum conversion. The reusability of the enzyme was evaluated to allow for reduction in cost of the enzyme in the process.

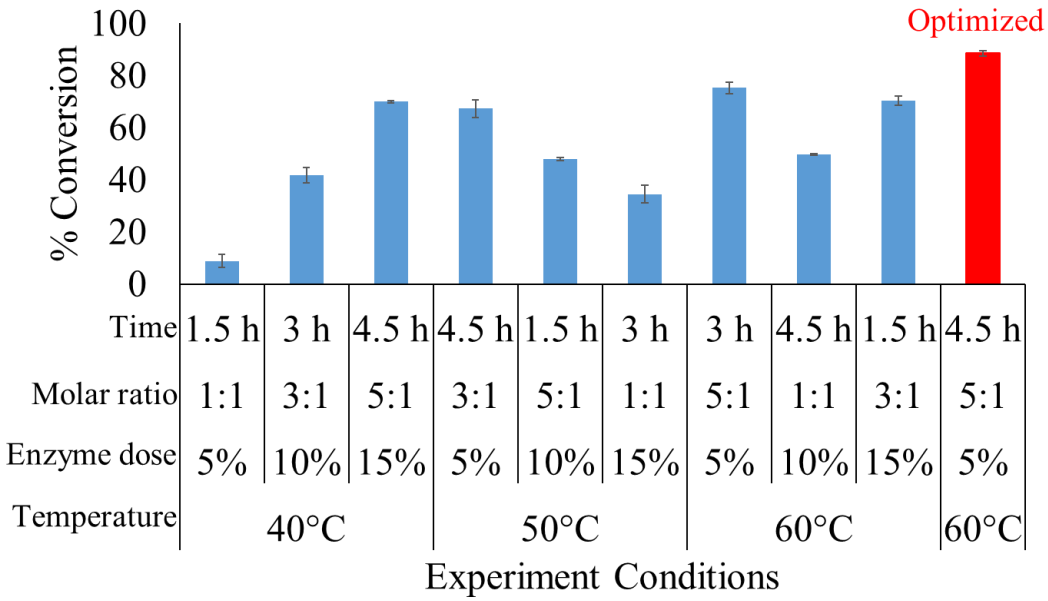
Biodiesel production using alkali catalyzed transesterification process is the preferred commercial process because it gives a high yield and requires lower capital and operating costs [1]. However, the process efficiency decreases in the presence of free fatty acids [2]. We have observed excess free fatty acids in oil recovered from engineered lipid producing crops developed by the Center for Advanced Bioenergy and Bioproducts Innovation (Urbana, IL). Hence, pre-processing of lipids for deacidification would be required for this new source of lipids. The conventional deacidification process requires high capital investment, generates waste, and causes loss of triglycerides and other phytoconstituents [3]. Therefore, a greener process of oil deacidification is desirable, which minimizes constituent loss and improves biodiesel yield.

In this work, immobilized lipase (*Candida antarctica* lipase b) was used for the esterification of oleic acid with glycerol. Oleic acid was chosen as it is the dominant fatty acid of lipids in vegetative tissue. Several parameters including temperature (40, 50 and 60°C), enzyme dose (5, 10, 15 wt%), molar ratio of oleic acid to glycerol (1:1, 1:3, 1:5), and reaction time (1.5, 3 and 4.5 h) were evaluated. The effect of process parameters and their levels were investigated using Taguchi design of experiments to maximize oleic acid conversion from a feasible number of experimental runs. Water produced *in-situ* during esterification reaction can decrease enzyme activity and thereby the product yield. Therefore, improvement of conversion by adding a molecular sieve for *in-situ* removal of water was tested. The reusability of the enzyme was evaluated under optimal conditions for maximum conversion.

The best experimental conditions (60°C, 5 wt%, 5:1 and 3 h) allowed 75.23 ± 2.19% conversion of oleic acid to esters. Using molecular sieves (type 3 Å) to remove produced water increased the

conversion to 86.73 ± 1.09% (15% increase). However, the Taguchi design predicted optimal parameters were 60°C, 5 wt%, 5:1 and 4.5 h. By applying these conditions, conversion of oleic acid was increased to 88.5 ± 1.11% and, furthermore, this was achieved without the added expense of a molecular sieves. The immobilized enzyme could be reused up to seven times with only a 10% decrease in conversion.

Thus, the developed enzymatic esterification process provides a greener alternative for oil deacidification. In addition, it will allow the valorization of glycerol which is produced abundantly in the biodiesel process.

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