Project Number: WY 002

RECYCLABLE ENZYMES IN THE BIODIESEL PRODUCTION FROM SOYBEAN OIL

A Major Qualifying Project Report
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of
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Approved

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Abstract

Biodiesel is an alternate and environmentally friendly fuel. Various catalysts, including enzymes, can accelerate the process of biodiesel production from animal fat and plant oils. Enzymatic transesterification for biodiesel is expensive due to the activity loss of enzyme after recycling. When chemically bonded to the surface of solution dispersible magnetic iron (II, III) oxide nanoparticles (Fe_3O_4), the enzymes can be recycled and reused. Lipozyme TL IM, a 1, 3-position specific lipase from *Thermomyces lanuginosus*, was bonded to Fe_3O_4 nanoparticles in this work. The enzyme-nanoparticle (ENP) complexes can be separated from the reaction products simply by using a conventional magnetic bar. Our preliminary results demonstrated that the recyclable enzyme-nanoparticle complexes were promising in enzymatic transesterification of soybean oil toward biodiesel with comparable activity to the free lipase. This research indicates an efficient way to separate the products and to reuse the enzymes, both leading to a significant decrease in the cost of biodiesel production.

Contents

Introduction

It has been increasingly important to find an alternative fuel source for diesel engines because of its biodegradability, renewability, and improved exhaust system. Many studies are targeting the possibility of using vegetable oils to convert into biodiesel. Biodiesel is a renewable fuel made from agricultural sources such as vegetable oils. Biodiesel can be produced by a process called transesterification, this method involve the usage of vegetable oils, industrial alcohol and a catalyst. The catalyst can be acid, base, or enzymatic (Steinbach, 2007). The transesterification of vegetable oils and animal fats have received considerable amount of attention for the past few years. Multiple routine have been studied for both chemical and enzymatic process. One of the researches today is focused on utilization of enzymes, usually lipase, for catalyzing the synthesis of vegetable oil or other agricultural lipid feedstock. Lipase-catalyzed transesterification is important in industrial application because such a system can avoid the problem of separation, chemical waste, toxicity, and flammability of organic solvents. Lipase-catalyzed transesterification of vegetable oil gives higher conversions than that of the conventional methods (Von de Fakultat). Lipozyme TL IM, a 1, 3-specific lipase from *Thermomyces lanuginosus*, is used as the enzyme to produce biodiesel.

One of the challenge with using enzymes as the catalyst is its expensive cost due the loss of activities after each cycle. In this project, our goal is to immobilize the enzyme so that the enzymes can undergo multiple reactions. Murthy and Wong, enzyme immobilization can be done through the combination of physical adsorption, microencapsulation, and physical entrapment. This route is based on the nanoparticles assembly synthesis of organic/inorganic hollow capsule structure. The enzyme is encapsulated by using surface coated nanoparticles, once encapsulated the enzymes can be recycle and reused.

One of the reasons why nanoparticles are widely used today is its magnetic property. We therefore took advantage of this property in our project to extract lipase out of the produced biodiesel. Lipases from *Thermomyces lanuginosus* were crosslinked by EDC with the carboxyl group on the surface of the iron (II, III) oxide nanoparticles. The crosslink is permanent therefore any reverse process will result in loss of lipase activity. The Enzyme-Nanoparticles (ENP) complex can be use to catalyze the biodiesel production from soybean oil. Once reaction is completed, the ENP can be extracted via a conventional magnetic bar and recycle and reuse in another cycle. Our preliminary results showed that the ENP is promising toward the recycle and reuse of enzyme in the production of biodiesel.

Background

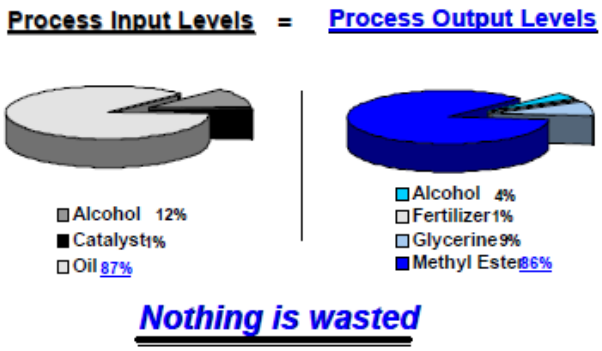
Biodiesel

Biodiesel is a domestic, renewable fuel for diesel engines. Biodiesel are developed from natural oils like soybean oil or animal fat. Biodiesel can be used in any concentration with petroleum based

diesel fuel in existing diesel engines with little or no modification (National Biodiesel Board). Biodiesel is produced by a chemical process which removes the glycerin from natural oils. It is comprised of mono-alkyl esters of long chain fatty acids derived from vegetable oils or animal fats. Biodiesel is typically produced by a reaction of vegetable oil or animal fat with an alcohol such as methanol or ethanol in the present of a catalyst; this will yield a variety of mono-alkyl esters (National Biodiesel Board).

Biodiesel is the first alternative fuel that has successfully completed the EPA-required Tier I and Tier II health effects testing under the Clean Air Act. These independent tests showed the biodiesel's significant reduction of virtually all regulated emissions and showed that biodiesel does not pose a threat to human health (Nation Biodiesel Board). A U.S. Department of Energy study showed that the production and use of biodiesel resulted in a 78.5% reduction in carbon dioxide emissions, compared to petroleum diesel (National Biodiesel Board). The use of biodiesel showed a substantial decrease in unburned hydrocarbons and particulate matter. Biodiesel contains little or no sulfur or aromatics. Polycyclic aromatic hydrocarbons and nitrated polycyclic aromatic hydrocarbons, produced in petroleum diesel, have been identified as potential cancer causing compounds (Nation Biodiesel Board). With the prices of agricultural commodity approaching record low and petroleum prices approaching record highs, it is clear that more can be done to utilize domestic surpluses of vegetable oils while enhancing our energy security.

Biodiesel can be produced via three different routes from oils and fats. The first is base catalyzed transesterification of the oil with alcohol. The second is direct acid catalyzed transesterification of the oil with methanol. The third route is to convert the oil to fatty acids and then convert to alkyl esters with acid catalysis (National Biodiesel Board).



(National Biodiesel Board)

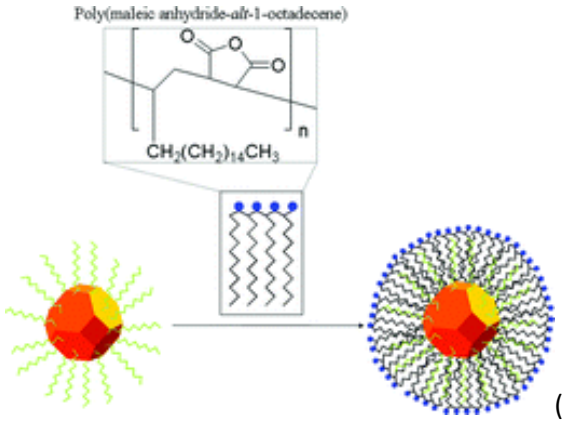
Figure 1: The levels of process input and process output are showed the bar graph. Both levels of input and output showed that nothing is wasted in the reaction.

Iron (II, III) Oxide Nanoparticles

Since the beginning of this century there has seen a rapid in interest for materials at nano-scale. Statistic from Lux Research, Inc. shows that there are more than $8 billion have been spend on research and development of nanotechnology. Nano-materials have attracted such a strong interest because of the physical, electronic, and magnetic properties resulting from their quantum size.

Iron oxide nanoparticles are of considerable interest for application in nanotechnology related fields such as magnetic storage, medicine, and catalysis. In biomedical application, iron oxide nanoparticles have appeared in MRI, hyperthermia, magnetically guided drug delivery, tissue repair, and molecular diagnostics. Ultimately, nanoparticles has the potential to offer new, inexpensive, and more efficient materials for a greater range of applications than achievable by bulk materials today.

There are many techniques for the synthesis of magnetic nanoparticles including: micro-emulsion, reduction of metal-salts, gas-phase reduction of metal complexes, thermolysis of metal-polymer complexes, and thermal decomposition of metal-carbonyl complexes. For most applications, a polymeric coating is needed to improve the stability, biocompatibility, and conjugation properties of the aqueous nanoparticles. For the iron (II, III) oxide nanoparticles used in this project, the nanoparticles were coated with Poly (maleic anhydride-alt-1-octadecene) or PMAO.



(Pellegrino, Corato, & Ricardo, 2008)

Figure 2: Nanoparticles coated with PMAO

The nanoparticles when coated with PMAO will result in long chain of polymers on the surface of the nanoparticles. At the end of the polymers are carboxyl groups that are capable of being activated by EDC.

Lipase from Thermomyces lanuginosus

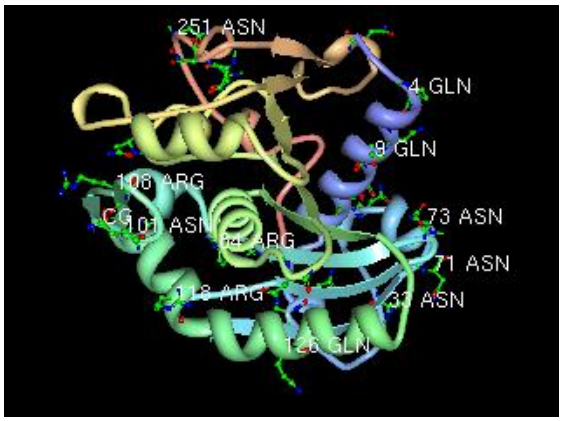


Figure 3: Lipase from Thermomyces lanoginosus

The picture is showing subunits of the lipase with some of the amino acids containing amine groups on the R group are labeled.

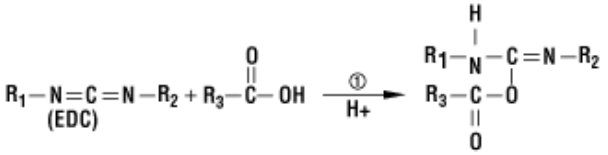
Lipases are esterases that hydrolyze long chain acyl-triglycerides to di- and monoglycerides (Patkar & Svenden, 2000). Lipase catalyzed biodiesel production from vegetable oils by transesterification reaction with methanol or ethanol. Most lipases are similar in structures; lipases are consisting of five or more parallel central β-sheet flanked by several α-helices and catalytic triad of serine, histidine and carboxylic acid (Turkan & Kalay, 2008). The active site structure for lipases can vary to determine the specificity of the substrate and the mechanism of the reaction (Patkar & Svenden, 2000). An important feature of most of lipases is that the lipases are able to hide their catalytic site from external environment by loops or helices which lie over the catalytic triad (Patkar & Svenden, 2000). The detailed mechanisms for the movement of the lipase's lid are still poorly understood.

Even though there are distinct differences among the lipase family, there are some general statements that can be made about the structure/function relationships within the lipase family: (a) most lipases experience profound conformational changed during interfacial activation; the lid that blocks the active site move from closed to an open conformation. This results in the formation of large hydrophobic protein. (b) Some of the lipases may be present in solution that is in dynamic equilibrium between the closed and open conformation. (c) Some organic solvents can help facilitate the opening of the lid (Patkar & Svenden, 2000).

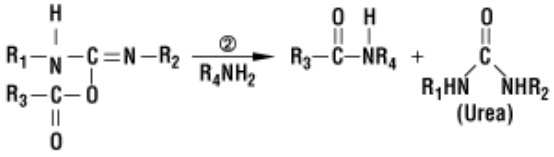
1-Ethyl-3(3-dimethylaminopropyl) carbodiimide HCl (EDC)

In this project, EDC was used to crosslink the iron (II, III) oxide nanoparticles with the lipases. EDC are a group of organic compounds which have the resonance formulation N=C=N (Chemicalland21). Carbodiimides are produced from the dehydration of urea or thiourea. Carbodiimides are readily active with various forms of amine and hydroxyl functional groups. Carbodiimides are used as dehydration agents and as activating agent of carboxylic acid to form esters or amides (Chemicalland21). EDC reacts

with carboxylic acid group and activates the carboxyl group to form an active O-acylisourea intermediate, allowing it to be coupled to the amino group in the reaction mixture (Chemicalland21). An EDC by-product is released as urea derivative after displacement by the nucleophile.



EDC reacts with carboxylic acid group and activates the carboxyl group, allowing it to be coupled to the amino group (R_4NH_2) in the reaction mixture.



EDC is released as a soluble urea derivative after displacement by the nucleophile, R_4NH_2.

(Thermo Scientific, 2009)

Figure 4: EDC Reaction

This diagram shows a schematic version of how EDC interacts with the carboxyl group to couple with the amino group.

Materials and Methods:

Establishing the GC condition

Perkin Elmer Clarus 500 Gas Chromatography was used to test the result of the experiment. The column used was a Supelco® SPB-17 which is a 50% Phenyl – 50% methylpolysiloxane with a dimension of 15m x 0.54mm x 1µm. Several FAMEs compounds were used to test for the GC condition. Those FAMEs included methyl linoleate, methyl linolenate, methyl stearate, methyl palmitate, and methyl oleate. The methyl esters were obtained from Sigma and they are >99% GC pure. Each methyl esters were heavily diluted in methanol to about 5% and 1µL of the solution was injected to the GC. The GC condition was micro-adjusted to obtain desire peaks for corresponding methyl esters.

The appropriate method established for methyl esters for Supelco® SPB-17 is as follow: the oven temperature start at 100°C and increase to 260°C at a rate of 40°C/min and hold at 260°C for one minute. The detector temperature is 260°C and injector temperature is 250°C. Helium is the carrier gas and has a flow rate of 5.00mL/min which is approximately 43.8cm/sec. The split ratio is at standard flow which is 20mL/min. The total run time is six minutes.

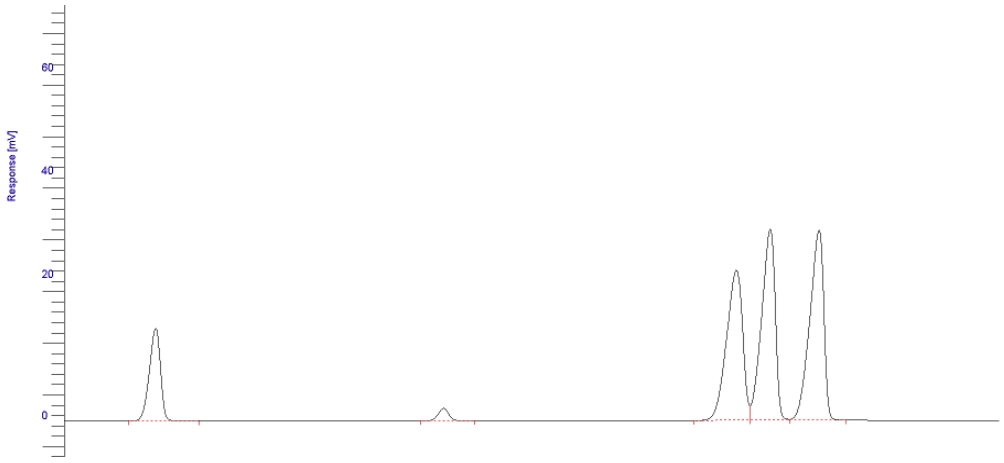


Figure 5: GC Analysis of Known Methyl Esters.

In order to obtain desire GC condition to run the experiments, several known concentration of methyl esters were used as the preliminary step. The peaks shown are methyl palmitate, methyl myristate, methyl stearate, methyl linoleate, and methyl oleate, respectively.

Iron (III) Oxide Nanoparticles and Lipase Crosslink

The entire reaction takes place at room temperature. Lipozyme TM lipase from *Thermomyces Lanuginosus* was obtained from Sigma Aldrich. The lipase has an activity of 10000U/g. The iron (III) oxide nanoparticle has a diameter of 25nm. The nanoparticles were synthesized by a WPI graduate student in the lab. The iron (II, III) oxide nanoparticles have a density of 5.18g/cm^3. EDC was bought from Sigma.

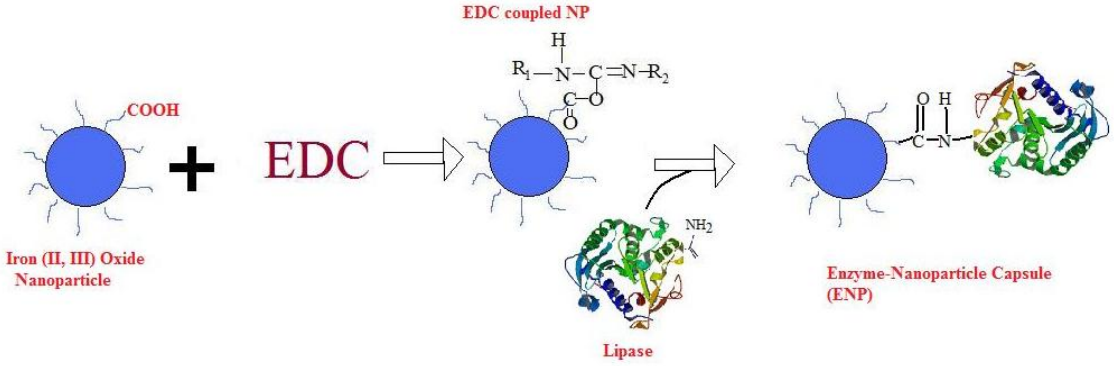


Figure 6: Crosslinking Iron (II, III) Oxide Nanoparticles with Lipases

The picture shows a schematic theory of how the nanoparticles can be coupled with the enzymes. EDC was used as the intermediate step to stabilize the activated carboxyl group.

The reaction was carried out in a 50mL-flask capped with a septum. The flask was equipped with magnetic stirrer. The reaction mixture contains 0.3ml of lipase (Novozyme Corp, L0777, solution ≥100,000U/g) pre-diluted with 1ml of DI-water, 0.3mL of Fe_3O_4 nanoparticles (5.18g/cm^3) diluted in 5mL of DI-water. The nanoparticles solution was subsequently added into the enzyme solution over a period of three minutes. This will allow the nanoparticles to be separated in the solution. EDC was added last, 0.02g of EDC was measured out and dissolve in water to a concentration of 1mg/ml before adding to the reaction mixture. The mixture was left to react for two hours. To avoid bubbles in the solution, the cap was frequently removed to let air out of the flask.

After two hours of reacting, the ENP was removed from solution by placing it on a conventional magnetic bar. The solution was left overnight for complete extraction of nanoparticles and ENP. Because most of the nanoparticles get extracted out of solutions, there are black dots on the side of the flask which indicates NP/ENP assembly. The solution was carefully removed from the flask without disrupting the NP and ENP. Once all of the solution is removed, the mixture of NP and ENP was used in the reaction for production of biodiesel.

Conversion of Soybean Oil to Biodiesel

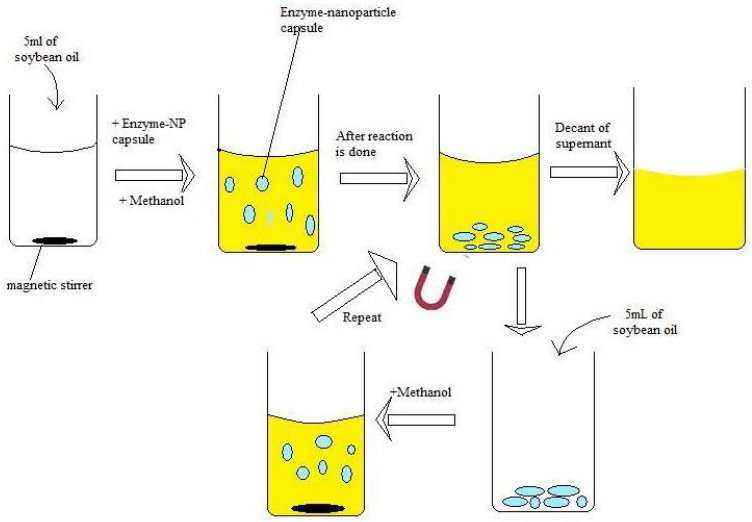


Figure 7: Process for Biodiesel Production Using ENP

The process for the catalytic production of biodiesel from soybean oil by enzyme-nanoparticles complex is shown above. The solution contains only soybean oil, methanol, and ENP. There were no additional solvent included in this reaction. Once the reaction is done, ENP is extracted by a conventional magnetic bar, supernatant is removed and the ENP undergoes another reaction to produce biodiesel.

Control production of biodiesel

Reaction takes place at room temperature. The reaction was carried out in a 50ml volumetric flask equipped with magnetic stirrer. The reaction mixture contains 4.595g of soybean oil and 0.5ml of lipase. 0.03mL, 0.03mL, and 0.006mL (of methanol was added at t=0, t=2hr, and t=4hr respectively. The reaction was vigorously stirred and left to react overnight. Once reaction is finished, sample of 0.1ml was withdrawn from solution and mixed with 1ml of methanol.0.1ml of methyl tridecanoate also added to the mixture. Methyl tridecanoate was used as the internal standard. The sample was analyzed via gas chromatography.

Production of biodiesel using Enzyme-Nanoparticle Capsules

Procedures for the production of biodiesel are the same as the above control experiment with the exception that instead of lipase enzymes, ENP was used instead. Once the reaction was done, samples were taken for analysis using gas chromatography. The solution was then placed next to a magnetic bar for extraction of the ENP. The solution was left overnight for complete extraction of ENP. The supernatant was removed from the flask and ENP was used to catalyze another cycle of biodiesel production. This process was repeated to react in another cycle of biodiesel production.

Because the solution was very viscous due to the high concentration of soybean oil and FAMEs, the ENPs had a hard time migrating toward the magnet. There was a great barrier for ENPs migration

than the ENPs in water therefore; the solution must be diluted to a lower concentration of oil and FAMEs. After every react, before extracting the ENPs, the solutions were diluted with 5-10mL of water (to fill the bottle) so that the ENPs can be easier to mobile when using a magnet to extract them

Analysis of Biodiesel Using Gas Chromatography

After every reaction of biodiesel production, 0.1mL of biodiesel was diluted with 1mL of methanol. Methanol was used as the solvent. 0.1ml of internal standard was added to the solution. Methyl tridecanoate was used as the internal standard. The condition for the GC was established above.

Calculation for Unknown Samples

In order to determine how much biodiesels were produced in each reaction, an internal standard was used to analyze the quantity of the sample. An internal standard is known amount of compound, different from the analyte that is added to the unknown sample (The University of Adelaide Department of Chemistry).The internal standards are useful for analysis samples where the instrument response varies slightly from run to run. The relative response for the standard is usually standard over a wide range of condition. The internal standard allows you to determine the amount of unknown analyte in the sample, as long as the concentration of the internal standard is known.

The amount of methyl esters can be determined from the results of the GC. Unknown weight of methyl esters can be determined via the following equation. From previous GC runs, we have already determined the constant (k) for each sample of known amount of methyl ester (data shown in table 1 below). From that, we can substitute in k and find W_1, the weight of unknown methyl esters.

$$\frac{W_1}{W_0} = k\frac{A_1}{A_0}$$

W_1= the weight of unknown methyl esters
W_0 = the weight of internal standard
A_1 = the area of unknown methyl esters resulted from GC run
A_0 = the area of internal standard resulted from GC run
k = the internal standard constant which can be determined from the GC run with known sample of methyl esters

Internal Standards					
	A_o (area)	**A1 (Area)**	**Mass (W_o)**	**Mass (W_1)**	**k**
M. Myristate	153092.9	153188.2	0.0868	0.0866	0.997075
M. Palmitate	169296.7	31224.56	0.0868	0.0162	1.011923
M. Stearate	169879.1	25069	0.0868	0.013	1.014908
M. Linoleate	173692.9	181658	0.0868	0.0886	0.975981
M. Oleate	167586.4	170929.8	0.0868	0.0874	0.987215

Table 1: Constant (k) Calculation

Based on the equation above, known concentration of methyl esters (obtained from Sigma) and internal standard, methyl tridecanoate, were used to calculate the constant (k). W_1 is the weight of the methyl esters and W_0 is the weight of methyl tridecanoate.

Results and Discussion

The experiments were not designed to optimize the production of biodiesel. Instead, the objective of the experiment is to determine whether or not the Enzyme-Nanoparticles complex can catalyze the production of biodiesel from soybean oil. Therefore the results show a low production of biodiesel.

Biodiesel Production by Enzyme-Nanoparticles Complex

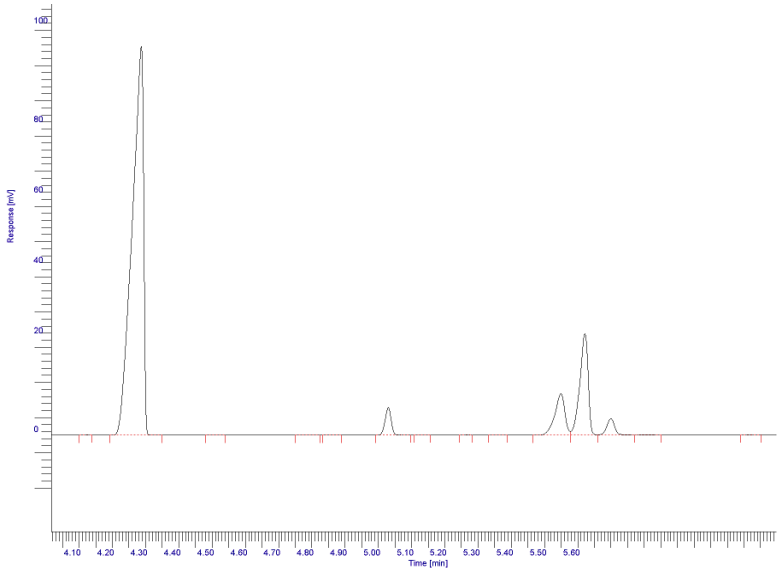


Figure 8: GC Analysis of Biodiesel Production with Free Lipases

Free lipases were used to produce biodiesel in this cycle. This serves as the control to check for the activities of the lipases. The reaction contains 5ml of soybean oil and 2ml of methanol and 0.5ml of lipases. The first peak corresponds to the internal standard and the next four peaks are the biodiesel produced by free lipases. The first peak from the left is the internal standard. The next four peaks are methyl palmitate, methyl stearate, methyl linoleate, and methyl oleate, respectively.

Control Reaction					
	A_o'	**A1'**	**k**	**W_0**	**W_1**
M. Palmitate	264451.5	9919.41	1.011923	0.0868	0.003295
M. Stearate	264451.5	24546.1	1.014908	0.0868	0.008177
M. Linoleate	264451.5	50230.73	0.975981	0.0868	0.016091
M. Oleate	264451.5	7884.76	0.987215	0.0868	0.002555

Table2: Amount of Methyl Esters Produced

Table 2 shows the amount of biodiesel (W_1) produced from free lipases. The amount of methyl esters (W_1) were calculated with respect to the internal standard (W_0). The equation is given in previous section.

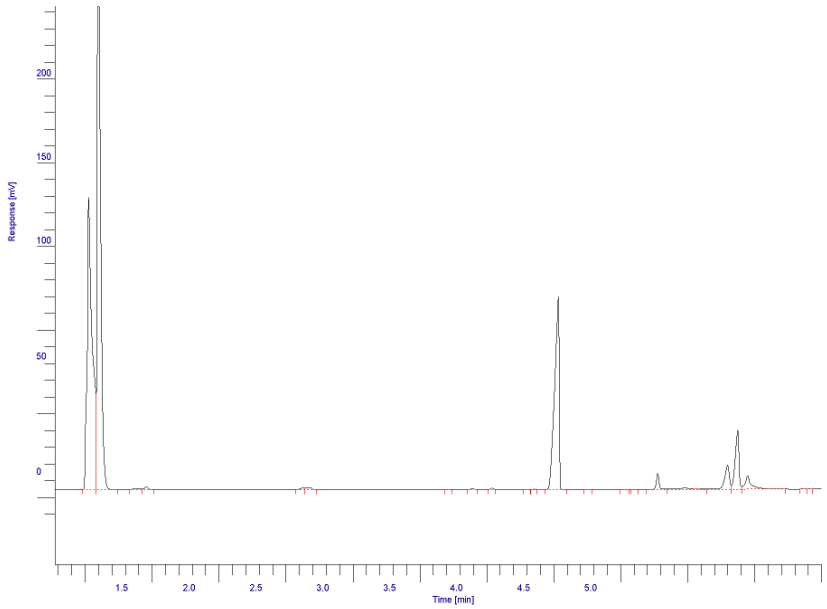


Figure 9: GC Graph of Biodiesel Production by ENP

Lipases were covalently attached to iron (II, III) oxide nanoparticles by EDC coupling agent resulting in an enzyme-nanoparticle complex. The ENP complexes were used to convert soybean oil to biodiesel. The result is shown above. The peak at time 4.75 correspond to the internal standard and the four peaks following the internal standard are methyl esters, component of biodiesel.

Enzyme-Nanoparticles	**Reaction 1**				
	A_o'	**A1'**	**k**	**W_0**	**W_1**
M. Palmitate	270769.77	12931.79	1.011923	0.0868	0.004195
M. Stearate	270769.77	34066.21	1.014908	0.0868	0.011083
M. Linoleate	270769.77	67871.2	0.975981	0.0868	0.021235
M. Oleate	270769.77	25986.03	0.987215	0.0868	0.008224

Table 3: Amount of Methyl Esters Produced from ENP

This table shows the amount of methyl esters that were produced by using ENP catalyst. The amount of methyl esters (W_1) produced is calculated from figure 9 with respect to the internal standard.

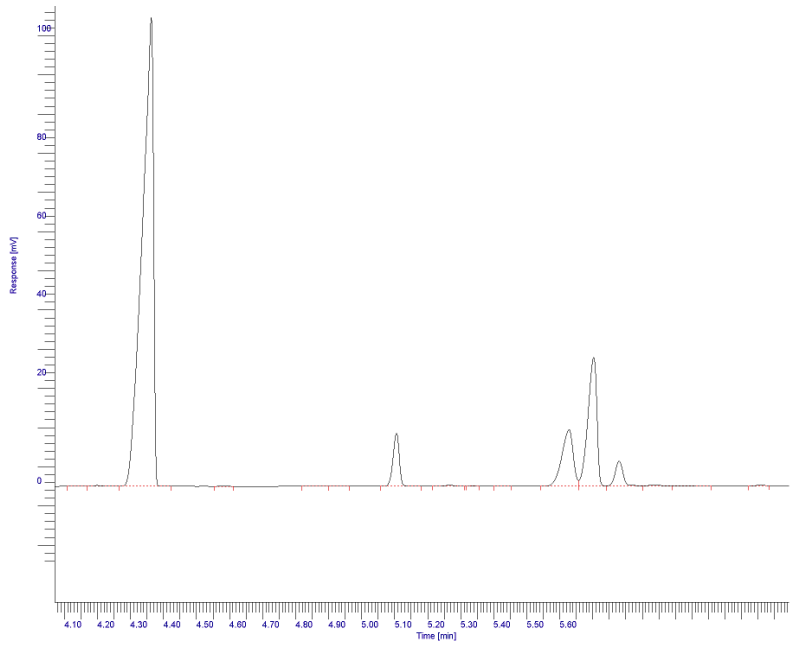


Figure 10: Round 2 of Biodiesel Production Using ENP from Previous Reaction

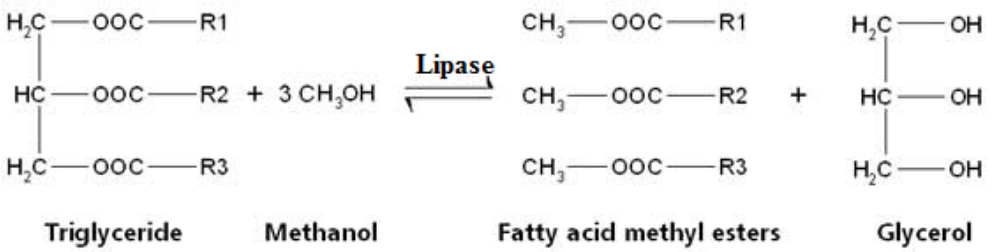
The recycled ENPs from previous reaction were reused to produce the biodiesel in this reaction. The ENPs were magnetically extracted from the solution in reaction 1 and immediately reused to catalyze this reaction. The first peak is the internal standard and the next three peaks are methyl palmitate, methyl stearate, methyl linoleate, and methyl oleate respectively.

Enzyme-Nanoparticle	**Reaction 2**				
	A_o'	**A1'**	**k**	**W_0**	**W_1**
M. Palmitate	286840.95	17791.69	1.011923	0.0868	0.005448
M. Stearate	286840.95	34375.59	1.014908	0.0868	0.010557
M. Linoleate	286840.95	62203.18	0.975981	0.0868	0.018371
M. Oleate	286840.95	10662.09	0.987215	0.0868	0.003185

Table 4: Amount of Methyl Esters Produced by Reusing ENP

The amount of methyl esters (W_1) produced by the recycled ENP were calculated and shown above. There are no significant differences in the production of biodiesel in this experiment compare with the biodiesel production by free lipase.

The control production of biodiesel with free lipase showed that the lipases are in fact functional and are able to catalyze the production of biodiesel from soybean oil. The control experiment also serves a gradient for the production of biodiesel using enzyme-nanoparticles complexes. The system used in this experiment is feasible for biodiesel production. The ratio of methyl esters produced from the experiments was similar to the ratio of methyl esters expected to produce from soybean oil. The transesterification reaction of soybean oil produced five different fatty acid methyl esters in the solutions. The five methyl esters are methyl palmitate, methyl stearate, methyl linoleate, methyl linolenate (Figure 11), and methyl oleate. In this experiment, methyl linolenate did not show up in the GC analysis. This could be due to the fact that the both methyl linolenate and methyl linoleate have nineteen Carbons and two Oxygens. The only difference between the two is that methyl linolenate have one more double bond than methyl linoleate. Therefore it is probable that the GC cannot distinguish the two compounds apart and show up as one peak. When known samples of methyl linoleate and methyl linolenate were ran on separate GC, both shows up at the same retention time.



(Steinbach, 2007)

Figure 11: Process of Transesterification of Soybean Oil to FAMEs

The results show that the ENP complexes successfully catalyze the production of biodiesel from soybean oil for two cycles. Table 3 shows the result of using ENPs in the first cycle to produce biodiesel. In the first cycle, there were approximately 20% more methyl esters (W_1) calculated compares to the free lipase reaction (Table 2). In the second cycle, the ENPs produced about the same amount of methyl esters as in the first cycle. There were no signs of enzyme activities loss in either cycle. The reasons for the increase of biodiesel using ENP complexes are unknown. The rate of reaction for both free lipases and ENP complexes did not change. From this result, we concluded that the lipases were successfully coupled to the iron (II, III) nanoparticles without disrupting the activities of the lipases.

The Reaction System

In the first cycle, there are possibilities that there are some free lipases in the solution. Even though a magnet was used to extract the ENP complexes, all of the supernatant could not be completely disposed and the ENPs were not washed with any other fluids after they were extracted. Therefore free lipases could still be in solution. In the second cycle, though there could be free lipases but the chances and percentage are very small. We can also rule out the option that the enzymes can be detached from the nanoparticles because EDC covalently couple the lipases to the nanoparticles and the reverse react would most likely result in the lost of enzyme activities (Thermo Scientific, 2009). The ENPs are a mixture

of both inactive form and active form. EDC are not position-specific, lipase contains a high number of arginines, glutamine, and asparagines (Figure 3) that have amine group on their side chain therefore the iron (II, III) oxides nanoparticles can be attached anywhere to those amino acids. As a result, the concentration of active lipases in the reaction could be different from the free lipases. The magnetic strength of the iron (II, III) oxide nanoparticles is unknown. The ideal iron (II, III) oxide nanoparticles would carry multiple enzymes on its surfaces because there are multiple carboxylic groups on the nanoparticle's surface. This is more efficient because fewer nanoparticles would be needed in the system. The exact amount of enzymes attached to the surface of the nanoparticles is unknown. There could be one enzyme or there could more than one enzyme attached to the nanoparticle.

After the second cycles, the ENP complexes were no longer extractable by the conventional magnet. This could be due to the viscosity of the soybean oil which prevents the ENPs to be moved to toward the magnet bar. The oils could surround the ENPs so that the magnetic property of the ENPs cannot be detected by the magnet bar. One way to make the ENPs magnetically extractable is to use a solvent in the reaction. This way, you would have a higher percent yield for biodiesel production and there will be less soybean oil to interact with the ENPs. There will be less restriction with the ENPs if the amount of soybean oil is minimized at the end results. T-butanol was most often used as a solvent, the idea ratio of t-butanol to oil is 0.58 (Zong & Wang, 2008)

The overall advantages of this system are that the enzymes are able to catalyze multiple reactions without losing their activities and the enzymes and the enzymes are capable of withstanding at room temperature longer than normal enzymes. The reaction took place over a period of 4 days and over the four days, the enzyme were kept at room temperature whereas the free lipases are kept at 4˚C. The ENP complexes are very promising in the production of biodiesel from soybean oil. If the ENP complexes were made to react in 10 to 20 cycles and up to 95% of biodiesel produced, this could be potential a strong tool for industries to make biodiesel. By recycling the enzymes and reusing it in multiple reactions, industries could save millions of dollars on enzymes alone.

Future Experiments

This project have potential to be a great tool for industries to use toward biodiesel productions but there are multiple flaws that are needed to be corrected.

First of all, the data from the project are from one experiment. We were running out of time at the end therefore could not repeat the process for more results. This data are not sufficient enough to draw any major conclusion therefore further experiments are needed before drawing any solid conclusion for the Enzymes-Nanoparticles complexes.

Second, after the second reaction, the enzymes-nanoparticles could not be extracted from solutions. There could be many reasons to why this is happening, but either way, if this step cannot be overcome, the experiment cannot be sustained. Once we are able to do this, it is possible to try to maximize the number of cycles that the ENP complexes can catalyze before losing their activities.

Third, in this project, we were only interested in the activities of the lipases when crosslinked to the nanoparticles therefore the productions of biodiesel were not maximized. As a matter of fact, the productions of biodiesel are very efficient. You can get up to 95% of biodiesel produced under the right conditions. An additional solvent, such as t-butanol or ethanol, could be use in this reaction.

Works Cited

Chemicalland21. (n.d.). *Chemicalland21*. Retrieved April 20, 2009, from Chemicalland21: http://chemicalland21.com/lifescience/phar/N-(3-DIMETHYLAMINOPROPYL)-N'-ETHYLCARBODIIMIDE%20HYDROCHLORIDE.htm

National Biodiesel Board. (n.d.). *Biodiesel.* Retrieved April 20, 2009, from The Official Site of the National Biodiesel Board: http://www.biodiesel.org/

Patkar, S., & Svenden, A. (2000). Structural Origins of the Interfacial Activation in Thermomyces (Humicola) lanuginosa Lipase. *Biochemistry* , 15071-15082.

Pellegrino, T., Corato, D., & Ricardo. (2008). Water solubilization of hydrophobic nanocrystals by means of poly(maleic anhydride-alt-1-octadecene). *Journals of Materials Chemistry* , 1991-1996.

Steinbach, A. (2007, November). *A Comprehensive Analysis of Biodiesel.* Retrieved April 22, 2009, from Biodiesel Magazine: http://www.biodieselmagazine.com/article.jsp?article_id=1917&q=&page=1

The University of Adelaide Department of Chemistry . (n.d.). *Internal Standards*. Retrieved April 21, 2009, from Stage 2 Chemistry Social Relevance Projects: http://www.chemistry.adelaide.edu.au/external/soc-rel/content/int-std.htm

Thermo Scientific. (2009). *Protein Discovery Workflows: Introduction to Crosslinking*. Retrieved April 20, 2009, from A Pierce Protein Research Product : http://www.piercenet.com/Proteomics/browse.cfm?fldID=CE4D6C5C-5946-4814-9904-C46E01232683

Turkan, A., & Kalay, S. (2008). Study of Mechanism of Lipase-Catalyzed Methanolysis of Sunflower Oil in tert-Butanol and Heptane. *Turkish Journal of Biochemistry* , 45-49.

Zong, M., & Wang, Y. (2008). Improvement of biodiesel production by lipozyme TL IM-catalyzed methanolysis using response surface methodology and acyl migration enhancer. *Bioresource Technology* , 7232-7237.