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Qualitative Analysis of Organic Catalysts for Biodiesel Production

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Qualitative Analysis of Organically Derived
Catalyst for Biodiesel Production

A Major Qualifying Project Report:
Submitted to the Faculty of
WORCESTER POLYTECHNIC INSTITUTE
In Partial Fulfillment of the Requirements for the
Degree of Bachelor of Science

Submitted to:
Professor William M. Clark, Advisor-CM, Worcester Polytechnic Institute

By:
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Abstract

The purpose of this Major Qualifying Project (MQP) was to create a successful methodology for a calcined eggshell biodiesel reaction and apply the same methodology to other forms of natural calcium carbonate, such as clam shells, seashells, and snail shells. This was achieved through troubleshooting steps that compared the effect of catalyst mass, mixing environment, calcining conditions, and methanol vs. ethanol on the biodiesel production process. The methodology that worked best for the egg shell reactions was then applied to other catalysts in a scaled up reaction.

The catalysts being considered were calcium oxide and calcined calcium carbonate, clam shells, seashells, and snail shells. All catalysts passed the majority of qualitative 3/27 tests proving that to some extent these catalysts allow a reaction to occur. It was hoped that these catalysts would be a more economical alternative to the common alkali metal catalysts, but on a one liter scale the calcining costs alone in the oven available prove to be more expensive than the catalyst. There were also concerns considering large scale production due to the difficulty associated with separating the biodiesel product and cleaning the equipment.

As it stands, using calcined calcium carbonate catalysts is a reasonable small scale biodiesel production method, but further testing is needed to determine how it quantitatively compares to more traditional methods and what additional costs would be associated with scaling up.

Executive Summary

Introduction

In recent years, the drive to become more environmentally conscious has led to the search for fuels with a lesser impact than traditional fossil fuels. One promising option is biodiesel, which is a diesel like fuel derived from biomass. Biodiesel is a relatively versatile fuel to produce, as it can be derived from both plant and animal lipids. Past projects at WPI have explored the production of biodiesel with different reagents and catalysts. This project qualitatively explored the effectiveness and viability of calcium carbonate based catalysts, such as eggshells, clamshells, seashells, snail shells, pure calcium carbonate, and pure calcium oxide.

In the 1930s, scientists determined the transesterification process that is used for most commercial biodiesel production and the product is largely compatible with conventional diesel engines. The feedstock for biodiesel can be any type of triglyceride, usually in the form of plant oils such as canola, corn, peanut, or vegetable oil. The transesterification process, as shown in Figure 1, is typically catalyzed using an alkali metal hydroxide such as KOH or NaOH to produce fatty acid methyl esters (FAME).

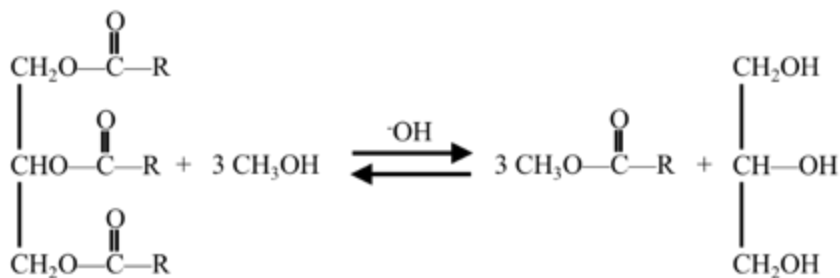


Figure 1: Biodiesel Transesterification Reaction

While alkali metal hydroxides are currently the dominant catalyst for biodiesel production, calcium oxide (CaO) catalysts are readily available from the thermal decomposition of CaCO₃, commonly found in eggshells, sea shells, and snail shells. To create these catalysts, the source of the CaCO₃ is heated to high temperatures in a process known as calcination, which decomposes the CaCO₃ into CO₂ and the desired CaO. Previous research has produced a process for eggshell preparation, which involves washing, blending, and calcining between 600-900°C for ranges of 2.5-12 hours.

Typically, methanol is used as a reagent to process the oils, but ethanol can be used as a safer alternative. Factors to consider when deciding between the use of MeOH and EtOH include price, safety, and energy potential. Although methanol is cheaper, it is more toxic and less energy dense than ethanol.

Objectives

The objects of this project were to:

- I. Develop a methodology for biodiesel reaction that uses calcined egg shell catalyst
- II. Replicate objective I with various natural forms of calcium carbonate, lab grade calcium carbonate, and calcium oxide

Methodology

The first testing steps involved calcining the catalysts. For this project, calcining involved subjecting washed, crushed calcium carbonate based catalysts to 900°C temps for 6 hours. This allowed the calcium carbonate catalysts to thermally decompose into calcium oxide.

The reactions took place in small (250 mL) and large (500ml) glass reactors maintained at 35°C and stirred continuously. The reactants included 30 mL methanol, 75.2 mL canola oil and 10 g of catalyst. This reaction was allowed to proceed for 24 hours at constant conditions. Reactions with each catalyst took place in each reactor 3 times for a total of 36 runs.

The 3/27 test was conducted to qualitatively determine the purity of the biodiesel produced in the above reaction. 3 mL of the “biodiesel” sample was added to 27 mL of methanol. If the mixture separated, that meant there was still a large amount of triglycerides remaining in the “biodiesel”, which implied the reaction had not proceeded far enough. If the mixture was uniform, it meant the “biodiesel” had converted enough to probably meet quality standards and dissolve in the methanol.

Results and Discussion

Prior to establishing the above methodology, a large period was spent determining the optimum procedures. During this time, experiments with both MeOH and EtOH were conducted using eggshells catalyst to determine whether a difference could be noted in their performance. It was found that EtOH did not consistently produce a biodiesel product and would therefore make test results less reliable, thus it was decided that testing would proceed with MeOH only. A well-defined ratio for the mixing of reactants was also developed.

Different environments for conducting the reactions were also tested. Reactions in an oscillating water bath, the larger self-contained reactors, and small beakers using stir bars were tested. The beakers and stir bars showed the best bench-scale results because they most effectively kept the reaction “well-

mixed”, but it was decided that using the larger, self-contained reactors would provide a more controlled reaction space while properly mixing reactants.

Calcining methods were tested first on eggshells between 400 and 900 C and 4-12 hours. The results showed that 900C for 6 hours best calcined the catalyst.

Each other calcium carbonate based catalyst was reacted and tested 6 times; 3 times in both the small and large sized reactor. The calcium oxide was unique as it did not require any calcination as it was already in the desired state. The calcium oxide performed well, passing in all 6 of the tests. The calcium carbonate was tested in the same way following calcination. It had the worst results of any of the catalysts, passing the 3/27 test 4 times and failing twice. It is worth noting, however, that the catalyst in failed tests was calcined over two weeks prior to their usage in the testing, alluding to a time criterion for catalyst usage. Clamshells were calcined generally whole and then crushed following the calcining process. They displayed 4 passes, 1 near pass, and 1 fail. The testing displayed a preference for the larger vessel, as both the near pass and the fail occurred in the smaller vessel. The seashell testing very closely mirrored the clamshell testing with 1 failure in the large reactor and 1 near pass in the small reactor. The other 4 tests resulted in passes. The calcining of the snail shells resulted in a color change from brown to white. For the testing, 5 of the 6 passed with the only failure occurring in the large reactor. Overall, these tests qualitatively indicate that when properly calcined any of the calcium carbonate based catalysts can work in the production of biodiesel.

Cost analyses on both a small and large scale were conducted in order to determine if there was economic potential in the utilization of these materials. For the small scale, the alkali metal hydroxides handily beat the calcium carbonate based catalysts due to cost of calcining and cost of purchase. This largely holds up within the large scale cost analysis as well. However, it is worth noting that the calcium carbonate based catalysts have the potential to be reused, leaving the possibility that they could be more cost effective over a greater period of time.

Conclusion

Unless there are specific incentives or the catalysts prove themselves to be extensively reusable, the catalysts are less cost efficient as their conventional competition. Additionally, the calcium carbonate based competition raises new issues with manufacturing, separation of product, and cleaning of production equipment.

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List of Abbreviations

By order of appearance:

DPF	Diesel Particulate Filter
FAME	Fatty Acid Methyl Esters
EtOH	Ethanol
MeOH	Methanol
CaCO ₃	Calcium Carbonate
KOH	Potassium Hydroxide
TBD	1,5,7-triazabicyclo[4,4,0]dec-5-ene
TMAH	Tetramethylammonium Hydroxide
CaO	Calcium Oxide
CO ₂	Carbon Dioxide
NaOH	Sodium Hydroxide
NaClO	Sodium Hypochlorite (Bleach)
H ₂ O ₂	Hydrogen Peroxide
AcOH	Acetic Acid
XRD	X-Ray Powder Diffraction

Introduction

As fossil fuels become increasingly scarce and their impact on the planet becomes evident, alternative energy sources become more appealing but necessary to ensure our future. Fossil fuels are responsible for 98% of annual carbon emissions, encouraging not only the pursuit of energy sources that are both more efficient and less environmentally impactful. Biodiesel is a promising energy source because it is nonflammable, biodegradable, and nontoxic (Balat, 2010). This alternative also has significantly fewer emissions when burned in comparison to petroleum based energy sources. Compared to regular diesel, biodiesel contains a higher level of oxygen and lower levels of nitrogen and sulfur, thus releasing fewer toxins into the air during combustion (Tica et al., 2010). Biodiesel is a viable energy source not only for developed countries but also for their less developed counterparts as their greater land availability, lower labor costs and favorable climates all lend themselves to biodiesel production (Balat, 2010).

Biodiesel fuels have been successfully incorporated into petro diesel blends used by vehicles in many countries, including Germany, France, and Brazil. These blends include 5%, 20%, and 100% biodiesel by volume, and are named B5, B20, and B100 corresponding to these compositions (Janjuan, Ellis, 2010). Blends up to B20 can be used in almost all diesel equipment with few to no engine modifications being necessary. Higher blends, such as B100, can be used in many pieces of diesel equipment after minor engine modifications. (Balat, 2010).

Raw materials for biodiesel production come from a variety of sources. Current research focuses mainly on ethanol production derived from major crops. The most widely studied crops used for this purpose are corn, sugar cane, and soybeans. These crops serve as the raw materials for ethanol that is eventually used as a gasoline additive. While their established infrastructure and proliferation of academic resources on the subject make these crops an excellent option, soil resource depletion, water requirements and availability, and the diversion of agricultural and food resources in the face of food shortages around the world discourage large scale biodiesel production by these means. Algae is another possible source of biodiesel that is being researched. Oil and alcohol can be produced by and extracted from algae to then be converted into biodiesel. However, the oils that are harvested are not always stable, resulting in a product that cannot be used as an energy source (Lin Chen, Wei Zhang, Xiaolin Chen, 2011). The complexity and relatively low yield of large scale bio-reactors to harvest algal products is a major inhibiting factor in producing biodiesel by this method. Biofuel production has yet to be perfected and many other options are being explored in order to find an alternative that can replace fossil fuels.

Biodiesel is produced via an acid or base catalyzed esterification reaction of vegetable oil and alcohol, which is most commonly methanol. This Major Qualifying Project (MQP) explored several different methods of making biodiesel from canola oil such as with ethanol (EtOH) in place of methanol (MeOH) and various catalysts. The goal of this project was to qualitatively compare the effectiveness and

viability of various catalysts while also taking into consideration the hazard associated with certain materials used in biodiesel production. Different catalysts were explored with the hopes of seeing if a nontraditional catalyst such as calcined egg shell could produce a high quality biofuel. The development of a safe, easily accessible biodiesel catalyst could make biofuels much more practical around the world and decrease dependency on fossil fuels.

Background

Biodiesel History and Relevance

People have been trying to utilize naturally formed fuels for engine power since the mid-19th century. These fuels were largely derived directly from the plant oils with little to no processing involved. This did not change until the 1930s when Belgian scientists discovered the transesterification process that allows for biodiesel as it is known today. (History of Biodiesel Fuels, 2016) Biodiesel enthusiasts have long found that biodiesel is similar enough to petroleum derived diesel to be used in most conventional diesel engines. Many European nations now mix biodiesel with regular diesel fuel as a way to reduce foreign oil dependence. Biodiesel is commonly available in 5%, 20% and 100% variants. Since 1993, nearly all new diesel vehicles have been made compatible with 100% biodiesel fuels, commonly known as B100. However, recent additions to certain vehicle lines of a diesel particulate filter (DPF) have made some vehicles incompatible with pure biodiesel requiring lower percentage mixtures.

In recent years there has been increased exploration of green, low cost biodiesel production methods. This area of study ranges from alternative catalysts to varied temperatures and pressures to the use of waste vegetable oil. One representative study used egg shell catalyst to produce biodiesel from waste cooking oil at ambient temperature and pressure without any esterification treatment of the oil (Piker et al, 2016). The study demonstrated a 97 weight percent yield of fatty acid methyl ester as well as the ability to reuse the egg shells for 5 cycles (Piker et al, 2016). This unorthodox process inspires further investigation of alternative biodiesel production methods.

Biodiesel Reaction

Biodiesel can be produced from a multitude of products including vegetable oil, waste cooking oil, and animal fats. The starting materials can be produced indefinitely, thus production of biodiesel is more favorable from a sustainability standpoint than petroleum diesels (University of Strathclyde, n.d.).

Biodiesel is the product of a triglyceride that has undergone transesterification. Although there are several methods of transesterification for biodiesel purposes, the most common is base catalyzed transesterification, which is the reaction method used throughout this study. The most common form of biodiesel production is illustrated in Figure 1, which summarizes the reaction of vegetable oil with methanol in the presence of NaOH or KOH to produce fatty acid methyl esters (FAME) and crude glycerol. Due to the difference in densities, the glycerol can be removed by phase separation if left undisturbed in a separating flask (University of Strathclyde, n.d.).

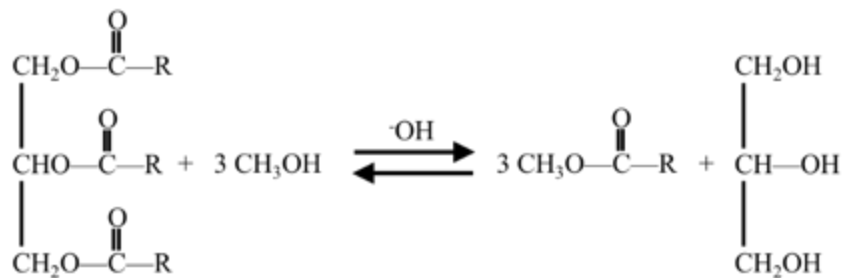


Figure 1: Biodiesel Transesterification Reaction

Although there were many potential variables to be studied in this process, this MQP considered the use of EtOH in place of MeOH and calcined sources of calcium carbonate (CaCO_3) in place of the alkali metal catalyst as a means to make the production of biodiesel safer and less expensive.

Relevant Major Qualifying Projects

Biodiesel studies and production are nothing new to WPI. A previous MQP developed the biodiesel reaction lab for the Unit Operations II class (see Appendix A). In this lab, students study the pseudo second order transesterification reaction and the effect of temperature on the reaction. MeOH and vegetable oil are used in a 6:1 weight percent ratio at 35°C using a KOH catalyst. Samples are removed from the reactor at timed intervals and tested for glycerol content using an enzyme assay to determine the extent of the reaction (see Appendix B). Due to the toxic nature of the MeOH in this reaction, the reactor is controlled by the laboratory computer (Clark, 2016).

Although the computer helps eliminate some of the dangers of working with MeOH, studies were conducted in 2016 to compare the methanolysis reaction to a similar ethanolysis reaction. In both the methanolysis and ethanolysis reactions, “glycerol overshoot” was observed. This meant that the glycerol readings from the enzyme assay were higher than stoichiometrically possible. However, the “S” shape curve for time vs. Glycerol concentration was still modeled. The study also found that the calculated activation energy for methanolysis, 59.9 kJ/mol , and ethanolysis, 46.9 kJ/mol , fell within the literature values of $26.8\text{--}61.5 \text{ kJ/mol}$ and $3.4\text{--}51 \text{ kJ/mol}$, respectively. The main difference between using MeOH and EtOH is observed when comparing the mass transfer capabilities of the two reactions: unlike MeOH, EtOH is not limited by mass transfer, allowing the reaction to be less inhibited. It was concluded in this study that ethanolysis is favorable at higher temperatures and has a higher heating value relative to petroleum diesel (Boule, Gallagher, Zonfrelli, 2016).

Biodiesel Catalysts

A number of catalysts exist to process vegetable oil into biodiesel. The one most often used commercially is KOH, although similar alkali metal hydroxides are used as well. These are useful due to their strong basicity, low cost, and relative abundance. They do have certain drawbacks however; at the end of the reaction, it is not economically advantageous to separate and recover the catalyst and it therefore must be neutralized out. Additionally, feedstocks with high concentrations of free fatty acids will readily consume the catalyst, leading to much higher catalyst requirements. Other catalysts include 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD), tetramethylammonium hydroxide (TMAH), as well as common egg shells that have been calcined to convert calcium carbonate to calcium oxide. Notable advantages of these catalysts include the ability to reduce unwanted side reactions (TBD), the ability to operate well in waste vegetable oil (TMAH), and the easy abundance of fresh material (egg shells). A major limitation with some of these catalysts is cost. While alkali metal catalysts are widely available and can be purchased cheaply for around \$0.01/gram other catalysts like TBD cost approximately \$12/gram, and TMAH costs over \$6/gram. This makes TBD and TMAH roughly 5,400 and 2,700 times, respectively, more expensive than their alkali metal competition.

Heterogeneous catalysts have been thoroughly studied in the context of biodiesel production. These catalysts are either acids or bases. Acids, while capable of esterifying rapeseed, or canola oil, require high temperatures and long reaction times for only relatively low rates of catalytic activity. In contrast, basic catalysts have shown high catalytic activities without the aforementioned limitations. Calcium oxide (CaO) is one such catalyst. It is well researched due to its advantages over KOH, namely its higher basicity, lower solubility, lower price, and greater safety (Lin Chen, Wei Zhang, Xiaolin Chen, 2011). Its catalytic activity is improved with calcination and pretreatment with MeOH. CaO is readily obtained by thermal decomposition of CaCO₃. This allows use of natural sources of CaCO₃ to decrease the material cost of the catalyst and therefore the cost of biodiesel production.

Calcining

The purpose of calcining is to heat and transform a chemical without the occurrence of fusion (Lenntech, 2017). In the case of CaO, calcining is used to oxidize the substance, specifically, as seen in Figure 2, converting CaCO₃ into CaO and carbon dioxide (CO₂) gas. It is important to note, however, that this reaction is reversible and exposure to CO₂ runs the risk of turning the CaO back into CaCO₃ (Shakharhiri). Calcium carbonate is naturally abundant and is a major component of egg shells, seashells, and snail shells (Industrial Minerals Solution-North America). The forward reaction allows forms of naturally occurring calcium carbonate that often go to waste to be repurposed into a catalyst. Previous

studies have shown that calcining eggshells effectively oxidizes CaCO_3 into CaO , which can then be used as a catalyst for the transesterification biodiesel reaction.

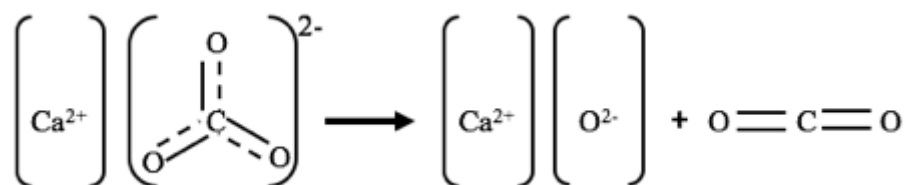


Figure 2: Oxidation Reaction of CaCO_3

There are several ways to prepare egg shells for calcination, but all start with washing and rinsing the egg shells in water (sometimes more than once) to remove any impurities and unwanted materials, such as leftover shell membrane. After washing the egg shells, they must be dried between $100\text{-}105^\circ\text{C}$ for approximately 24 hours. Once they are dried, the shells are broken into smaller pieces. This can be done by simply crushing them, or they can be ground in a blender. While this is the end of egg shell preparation for some methods, others are more involved. These more thorough methods involve more heating and rinsing of the shell pieces. The additional heating helps transform the CaCO_3 in the egg shells into CaO , the desired catalyst.

In a study performed by Viriya-empikul et al, the egg shells were heated at 800°C for 4 hours and then kept sealed until the biodiesel reaction was performed to avoid the egg shells reacting with the air (Viriya-empikul, Krasae, Puttasawat, et al). Niju et al performed several more steps to prepare their shells. After the initial heating, they calcined the egg shells at 900°C for 2.5 hours. Next, the egg shells were refluxed in 60°C water for 6 hours and dried at 120°C overnight. Finally, the product was calcined once more at 600°C for 3 hours to change the hydroxide form to oxide (Niju, Meera, Begum, Anantharaman). While more thorough preparation may yield a more effective catalyst, one run of calcination would be a more cost effective solution. One run of calcination would also be more time efficient, as it can be finished in less than a day after the initial drying.

Use of Methanol vs. Ethanol

MeOH is commonly used in biodiesel production, however, EtOH is a viable option as well. Despite being cheaper ($\$1$ per gallon MeOH versus $\$1.60$ per gallon EtOH), MeOH is less desirable due to hazardous properties such as its ability to cause blindness, vomiting, and if not used carefully, it can result in death. MeOH is irritating to the lungs and, when ingested, is metabolized to formaldehyde. When using MeOH , sodium hydroxide (NaOH) is often the catalyst of choice. However, the heat evolved in dissolving NaOH in MeOH can cause the MeOH to boil, causing dangerous working conditions. (Rapier, 2010).

Despite being more expensive, EtOH is easier to obtain as it is produced by the fermentation of corn and other foodstuffs. The plethora of vegetable oils also means there are minimal environmental waste disposal problems. The catalysts used with EtOH are safer and the reactions take place at room temperature, requiring no additional heating. Catalysts, such as alkali metal, are commercially available making them easy to purchase and find. EtOH has a higher energy density than MeOH. 1 gallon of EtOH (E85) contains about 73-83% of the energy that is in one gallon of gasoline, while 1 gallon of MeOH contains 49% of the energy in the equivalent amount of gasoline. The energy content of E100 is 76,330 BTU/gal, and the energy content found in methanol is 57,250 BTU/gal. Another advantage to using EtOH is that its associated industrial practices are often similar or identical to those of conventionally fueled operations. Therefore, special lubricants are only sometimes necessary, but for MeOH special lubricants are always required. (Alternative Fuel Data Center, 2014).

Methodology

Objectives

The objects of this project were to:

- III. Develop a methodology for biodiesel reaction that uses calcined egg shell catalyst
- IV. Replicate objective I with various natural forms of calcium carbonate, lab grade calcium carbonate, and calcium oxide

The first objective for the project was to develop a repeatable method for testing CaCO_3 based catalysts. Egg shells were the most studied catalyst found and were thus chosen as the base case. Several conditions were studied and evaluated for this test including temperature, alcohol, method of agitation, and vessel. The evaluations can be found in the Troubleshooting section below.

The second objective entailed utilizing the set conditions found in the first objective to test alternative catalysts to see if they performed similarly. This meant replicating the conditions from the egg shell trials and testing quality under similar conditions. This allowed for an objective assessment of the performance of these catalysts relative to the more studied egg shells.

Introduction to Methodology

Although there was eventually a uniform reaction procedure, several other methods for reaction environment, calcining procedures, and analysis were attempted. Stated below are the final methodologies for calcining, reaction, and analysis. Additionally, the troubleshooting section describes the path taken to achieve the final methods.

Calcining

As previously discussed, calcining is the process in which CaCO_3 converts into CaO . Catalysts such as CaCO_3 (figure 3), egg shells (figure 4), seashells (figure 5), snail shells (figure 6), and clam shells (figure 7). A mortar and pestle was required for crushing the materials. A furnace (figure 8) capable of reaching 900°C and holding for at least 6 hours was necessary for the calcining process to occur.



Figure 3: Calcium Carbonate Before and After Calcining



Figure 4: Egg Shells Before and After Calcining



Figure 5: Seashells Before and After Calcining



Figure 6: Snail Shells Before and After Calcining



Figure 7: Clam Shells Before and After Calcining



Figure 8: Furnace in Goddard Hall

Procedure

1. Wash catalyst material to remove any unwanted material
2. Dry catalyst for 10-15 minutes in 100°C oven
3. Crush catalyst material in mortar and pestle
4. Place crushed catalyst in crucible. Use enough material to ensure approximately 60 g after calcining
5. Place crucible in 900°C oven
6. Remove from oven after 6 hours and allow to cool in fume hood
7. Cover once cooled to prevent reaction of catalyst with air

Reaction

The reaction to produce biodiesel was done in a heated reactor with an impeller (figure 9) to maintain the reaction at 35°C and proper mixing. The reaction was done with catalysts including calcium oxide and calcined catalysts such as CaCO₃, egg shells, seashells, snail shells, and clam shells. The catalysts were run in the reactor with MeOH and virgin canola oil.

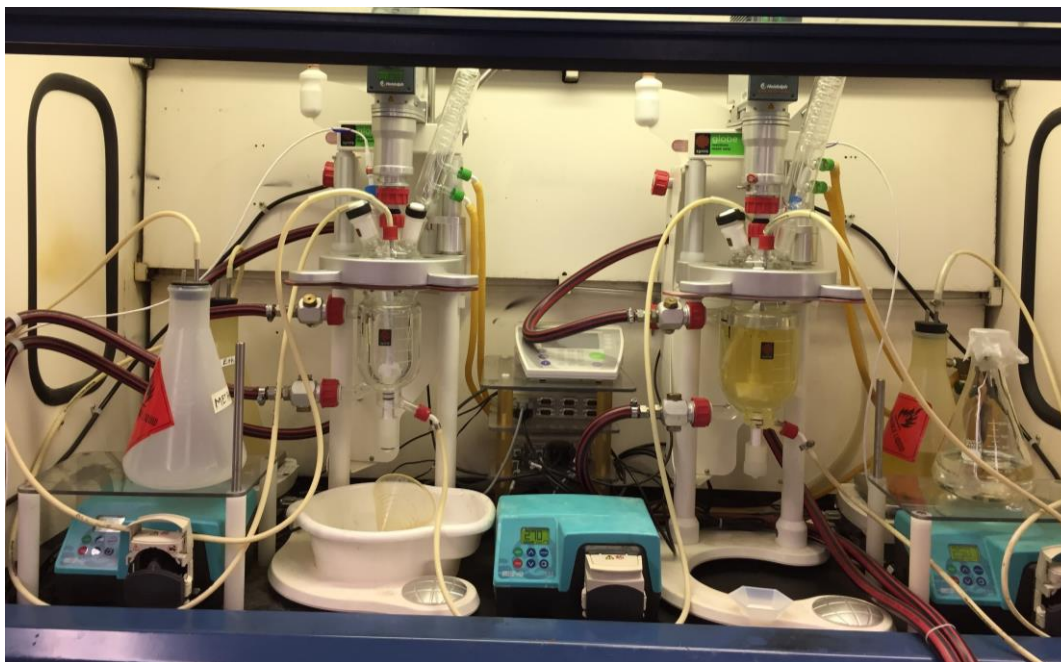


Figure 9: Biodiesel Reactor in Unit Operations Lab

Procedure

1. Ensure all equipment is dry. Place crucibles and graduated cylinders in drying oven at 100°C if necessary
2. Set 250 mL reactor temperature to 35°C
3. Using graduated cylinder measure 30 mL of MeOH and place in reactor
4. Measure 10 g of catalyst and add to reactor
5. Set impeller speed to 670 rpm
6. Once catalyst is mostly dissolved in the methanol add 75.2 mL of canola Oil to the reactor
7. Wait 24 hours for reaction to run to completion
8. Remove product from reactor and place in separatory funnel
9. Clean reactor chamber with MeOH (Use brush if necessary)
10. Repeat steps 1-7 twice
11. Repeat steps 1-8 in 500 mL reactor

3/27 Analysis

The reactions were qualitatively analyzed using a method referred to as 3/27 Analysis. This requires a 9:1 ratio of MeOH to reaction product. If there are two visible layers, that means the reaction still has unconverted tri-glycerides and the reaction was considered unsuccessful.

Procedure

1. Reactor products are placed in flask and covered for approximately 24 hours to allow phase separation via gravity
2. 27 mL of methanol is pipetted into centrifuge tube
3. 3 mL of the separated biodiesel product is removed from the top layer of flask and placed in centrifuge tube with methanol
4. Centrifuge tube is capped and shaken lightly to allow complete mixture
5. After 10 minutes the contents are observed to see whether two layers have formed

Troubleshooting Steps

The initial step before designing a procedure was to replicate the biodiesel unit operations lab experiment. This reaction took place in the same 500 mL heated reactor mentioned in previous sections. It involved mixing MeOH, canola oil, and KOH catalyst at 35°C for roughly 2 hours. The final reaction procedure and glycerol assay procedure can be seen in Appendix C. The reaction was also attempted with EtOH in place of MeOH, where the same amount of EtOH and MeOH were used in their respective reactions.

Bench-scale reactions were also done to find a proper reaction mechanism. The first tests compared an oscillating water bath seen in figure 10 to magnetic stir bars. The expected advantage of the oscillating water bath was the ability to run the reaction at 35°C and not room temperature. Like the unit operations reaction, the bench-scale reactions were also done to compare MeOH and EtOH. Additionally, reactions were run with 0.5 g, 1.0 g, and 1.5 g calcined egg shell catalyst.

There were also comparisons of the calcining procedure to find a temperature and duration necessary to convert CaCO_3 present into CaO. To achieve this the CaCO_3 was heated at high temperatures for certain periods. The first attempt to calcine egg shells was at 400°C for 6 hours. Further, the temperature was increased to 900°C for both 6 and 12 hours. Additionally, conversion was attempted using eggshells in bleach (NaClO) and eggshells in hydrogen peroxide (H_2O_2).

A summary of these reactions can be found in Appendix E.



Figure 10: Oscillating Water Bath

Results and Discussion

Troubleshooting Steps

Initial Unit Operations Reaction with Potassium Hydroxide Catalyst and Glycerol Assay Analysis

The reaction was also done with EtOH in place of MeOH. However, the EtOH samples did not result in an obvious glycerol layer when put in the separatory funnel so visual qualitative analysis was not able to be completed. In addition, the assay proved to be particularly troublesome as it required very precise liquid measurements to determine how the concentration of glycerol changed over time. As this project progressed further from determining the kinetics of given reactions and more towards qualitative analysis, it was decided that the visual separation would suffice while working on the bench-scale egg shell reactions and the assay would not be used. Due to the evolution of this project throughout the course, the initial KOH reactions did not play a role in the final results; the results to these specific reactions can be referenced in Appendix D.

At this stage of the project, it was determined that this methodology worked well for pre-established experiments, such as the unit operations reaction, but running at such high volumes and using the glycerol assay while trying to build a methodology for the egg-shell biodiesel production reaction would be wasteful and time consuming with unreliable quantitative results. The chosen amount of catalyst was derived from the series of tests on the bench top comparing 0.5 g, 1.0 g, and 1.5 g of calcined egg shell. Tests showed that 1.0 g allowed the reaction to yield the best results without excess of catalyst.

Bench-Scale Calcined Eggshell Catalyst Reaction with Qualitative Analysis

Oscillating Bath vs. Stir Plate with Magnetic Stir Bars

When comparing the magnetic stir bar and oscillating water bath, the reaction only proceeded when using the stir bar. The stir bar had the advantage because the bar was inside the beaker during the reaction and allowed the catalyst to disperse throughout the mixture. The oscillating bath moved the beaker around, but the mixture stayed separated in its initial layers. In other words, the catalyst remained on the bottom of the beaker instead of mixing with the alcohol and oil. The egg shell catalyst tended to form its own layer, so one of the keys to running the reaction was ensuring the catalyst would interact with the reactants.

Use of Methanol vs. Ethanol

The MeOH runs resulted in two different layers excluding the layer of catalyst at the bottom of the container while the EtOH runs resulted in no visible varying layers. Due to the lack of qualitative results, the experiments that followed were not done using EtOH. Additionally, EtOH had difficulty with CaCO₃ based catalysts.

Mass of Calcined Eggshell Catalyst

Each reaction with varying amounts of catalyst yielded similar qualitative results, producing comparable amounts of biodiesel. It was decided that 1.0 g catalyst would be the standard used throughout following experiments as it was equally effective to using 1.5 g with easier separation of product at the end of the experiment and found in literature.

Calcining process

The first attempt to calcine egg shells was at 400°C for 6 hours and yielded a product that was an ashy grey color indicating it did not fully burn off many organics present. Trials with the product showed it was not useful as a catalyst. Further, the temperature was increased to 900°C for both 6 and 12 hours. No detectable difference was found between the 6 and 12 hour times so the 6-hour time period at 900°C was adopted as the standard calcining process. Additionally, the attempts done using eggshells in bleach (NaClO) and eggshells in hydrogen peroxide (H₂O₂) did not show signs of desired product formation.

Reasoning for Modifications to Unit Operations Procedure

The catalyst material is washed in order to remove any organic material such as dirt or inner membrane (in the case of egg shells) and placed in an oven to remove any excess water remaining from washing. The catalyst material was crushed to improve surface area interactions and mixing during the reaction. Catalysts were calcined in an oven located in the fume hood then cooled in the fume hood to avoid any strong odors from the heating of the catalyst.

The heated reactor was necessary to maintain a temperature of 35°C as it is more favorable for the reaction kinetics. Although the initial troubleshooting eggshell reactions were done at 25°C (room temperature) without regulation, it was observed during the Unit Operations lab that increased temperature allows the transesterification reaction to run to completion. The impeller allows for the reaction to keep conditions close to “well-mixed”. Even with the increased temperature, the reaction time was set to approximately 24 hours, as seen in literature, to ensure that the reaction will run to completion. Testing in the 250 mL reactor and the 500 mL reactor was done to see whether or not the decreased surface area would affect the reaction extent.

Alkaline catalysts, such as KOH, are the most commonly used in this transesterification reactions; however, it is known that CaO works similarly as a catalyst for this reaction. Thus, this series of reactions examined CaO catalysts derived from various forms of naturally occurring CaCO₃ including egg shells, seashells, snail shells, and clam shells as well as CaCO₃ through calcining to examine if they will work as well as KOH.

Following the biodiesel reaction, the reactor potentially contained the following: solid catalyst, methanol, glycerin, converted biodiesel, and unconverted and partially converted glycerides. In allowing the reactor products to sit, the mixture separated into two phases with the catalyst and glycerin forming one phase and the MeOH, biodiesel, and glycerides forming the top phase.

For the 3/27 Test, a 3 mL sample is taken from this top layer. The test relies on the solubility of biodiesel, MeOH and monoglycerides in MeOH. The large amount is so no material gets saturated in the MeOH. The key is that while the above soluble components dissolve, unconverted di- and triglycerides form a separate phase at the bottom. The test is designed so that a clear (one phase) test should indicate that the biodiesel would typically pass quality control.

Egg Shells

The egg shell tests were completed on a smaller scale than the Unit Operations Reactor. These tests were run in 20 mL beakers using varying forms of alcohol, calcination procedures, and using an oscillating water bath or a stirring plate with a stir bar. The success or failure of the reaction was largely dependent on the mixing process employed. The first round of trials was conducted in an oscillating water bath where the temperature was set to 35°C. Although the amount of catalyst used was varied, the outcomes were consistent. Whether 0.5 g, 1.0 g, or 1.5 g of egg shell catalyst was used, the end product did not separate into 2 different layers (excluding the layer of catalyst) thus qualitatively concluding a reaction did not occur in any of the 6 beakers after 19 hours. The failure of this test run of the egg shell catalyst can be attributed to the water bath not stirring the catalyst as much as necessary for the reaction to occur. After these tests did not achieve the desired results, the exact same reaction was attempted but using a stir plate and placing a stir bar into the beakers. Although these reactions were run at room temperature, after 22-24 hours 6 of the 6 reactions using methanol resulted in 2 visible layers (again excluding the layer of catalyst). The catalysts for these successful reactions were all calcined at different times within a 9-day range and for varying durations but days and this did not have any effect on the completion of a reaction thus from these results it was concluded that although the catalyst for the water bath tests sat for 7 days this was not a factor for the incompletion of the water bath reactions.

Stir-bar tests were also conducted using ethanol opposed to methanol and varying the calcination process of the catalysts used in methanol. The ethanol tests had the same procedures as the tests using

methanol but had different outcomes. All 4 solutions using ethanol did not separate into two distinct layers. As previously mentioned, the amount of time the catalyst sat was not taken into consideration as a factor in the completion of the reaction, thus concluding that ethanol in this case was not ideal for making biodiesel. Moving forward from these results, ethanol was not used with any other catalysts due to its lack of desired biodiesel production. Other inconclusive tests were completed using different calcination processes. In these trials, the eggs were placed in bleach or peroxide and allowed to soak for a day. These eggs were then removed and rinsed, they were not however, calcined using heat. It was hoped the chemical treatments would calcine the egg shells. After allowing the 4 reactions (2 bleach, 2 peroxide) to run for 27 hours, all 4 solutions that resulted, although having two visibly different layers, did not contain biodiesel. The two layers clearly presented the separation of the methanol from the canola oil. It was concluded from these tests that the bleach and peroxide calcination process did not properly convert the egg shells into CaO resulting in a product that did not contain biodiesel. A summary of these reactions and pictures can be seen in Appendix E and Appendix F, respectively.

X-Ray Powder Diffraction

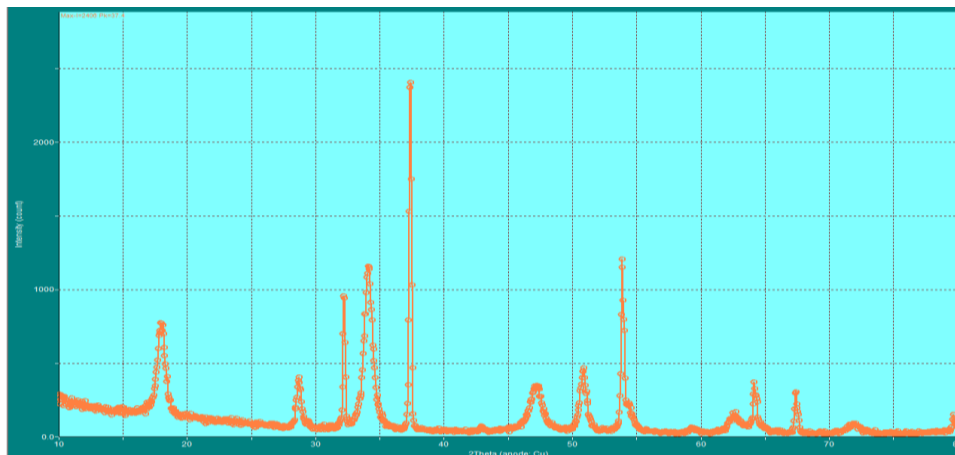


Figure 11: Egg Shell XRD

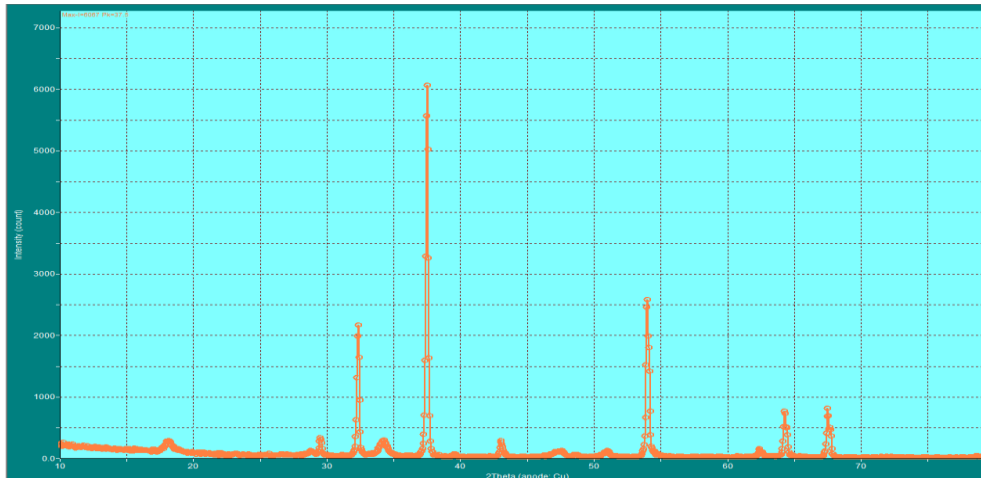


Figure 12: Calcium Oxide XRD

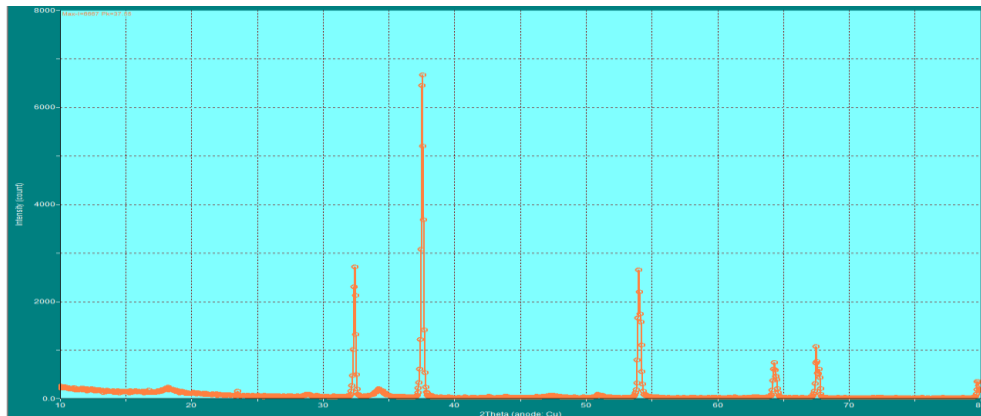


Figure 13: Clamshell XRD

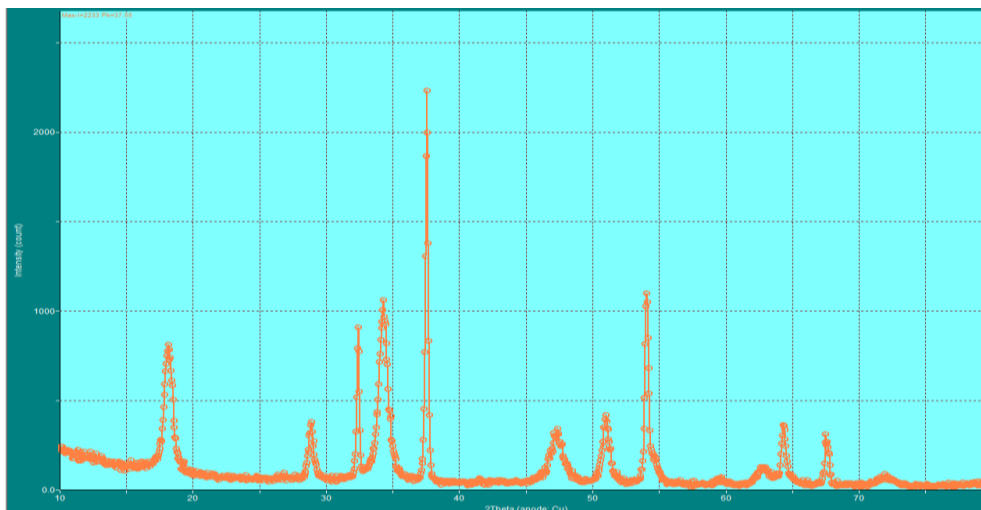


Figure 14: Calcium Carbonate XRD

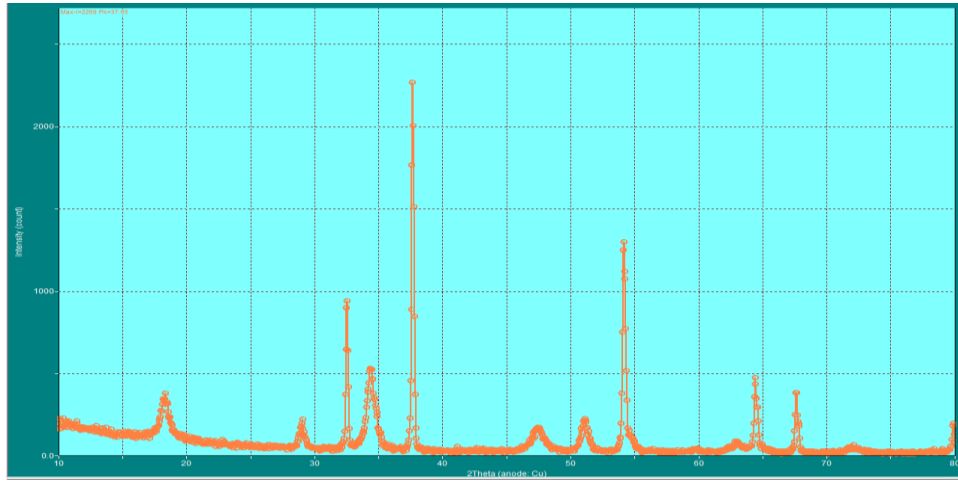


Figure 15: Seashell XRD

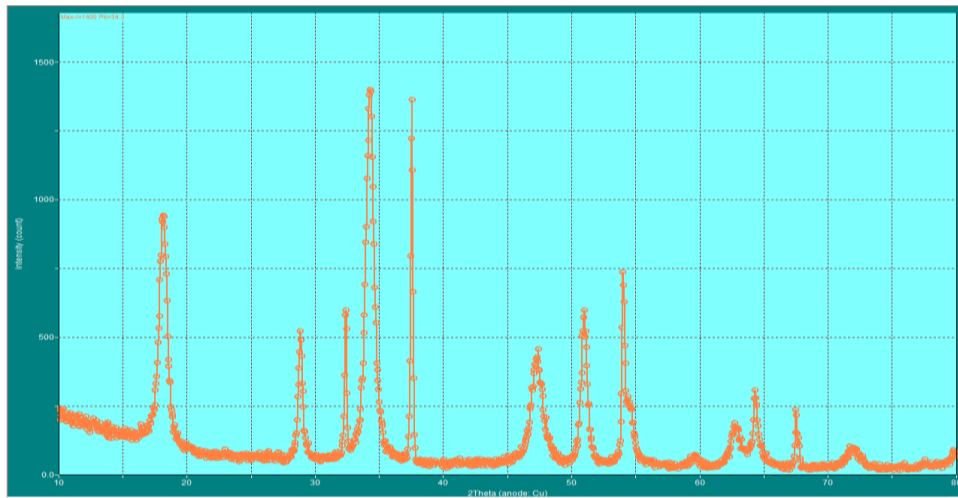


Figure 16: Snail Shell XRD

Figures 11 through 16 were obtained through the technique of x-ray powder diffraction (XRD). This technique is used to identify crystalline material or provide information on unit cell dimensions. D-spacing can be determined using the Bragg's Equation and each material has specific d-spacing. The space between the diffracting planes of the atoms is what determines or leads to the peaks in the graphs. From these graphs you can see the similar peaks, for example none of the peaks split. The peaks are of comparable heights and widths. Any differences could be attributed to differences in the arrangement of their lattices or variations of crystallite size.

Table 1: Presence of Compounds other than Calcium Oxide retrieved from Jade 9 Software

	CaO	CaCO ₃	Ca(OH) ₂	C ₂ Ca	C ₂ H ₂ O ₄ ·2H ₂ O
Egg Shell	X	X	X		
Calcium Oxide	X		X		
Calcium Carbonate	X		X		X
Seashells	X	X	X		
Clam Shells	X				X
Snail Shells	X		X	X	

From the Jade 9 software, results showed the chemical composition and content percentage of the different samples. This is a search and match software. Observed were nearly identical peaks that were formed when all the given materials matched with CaO. The different samples matched with other materials. Eggshells and seashells matched with CaCO₃. While CaCO₃ and clamshells matched with hydrogen oxalate hydrate (C₂H₂O₄·2H₂O). Eggshells, seashells, CaCO₃, snail shells, and CaO all matched with Ca(OH)₂. Lastly, snail shells were the only to match with calcium carbide (C₂Ca). Most of the different calcined materials had different percentages due to having slightly different make-ups, however, egg and seashells were identical in make-up as well as percentage. A summary of these results can be seen in Table 1. A reason for the presence of compounds other than CaO as would be expected could be due to the time the powders were allowed to sit after calcination. Interaction with the air is a possibility for the impure CaO.

Calcium Oxide

The CaO tests were unique in that the powder required no preparation; this meant that there was no waiting period due to calcination. All six trials passed. The point of calcining the other catalysts was to produce CaO, so it stands to reason that lab grade CaO would perform well. The calcination reaction is reversible, so exposure to the air will convert CaO back to CaCO₃. Due to the tight seal on the container and the nonexistent wait time, there was virtually no risk of the CaO reacting with the air. Another advantage of not calcining the CaO was mass loss. The various forms of CaCO₃ all lost some mass through

the calcining process, so additional catalyst would be required to achieve the desired amounts. This issue is not present with pure CaO, so all purchased catalyst could be used for reactions.

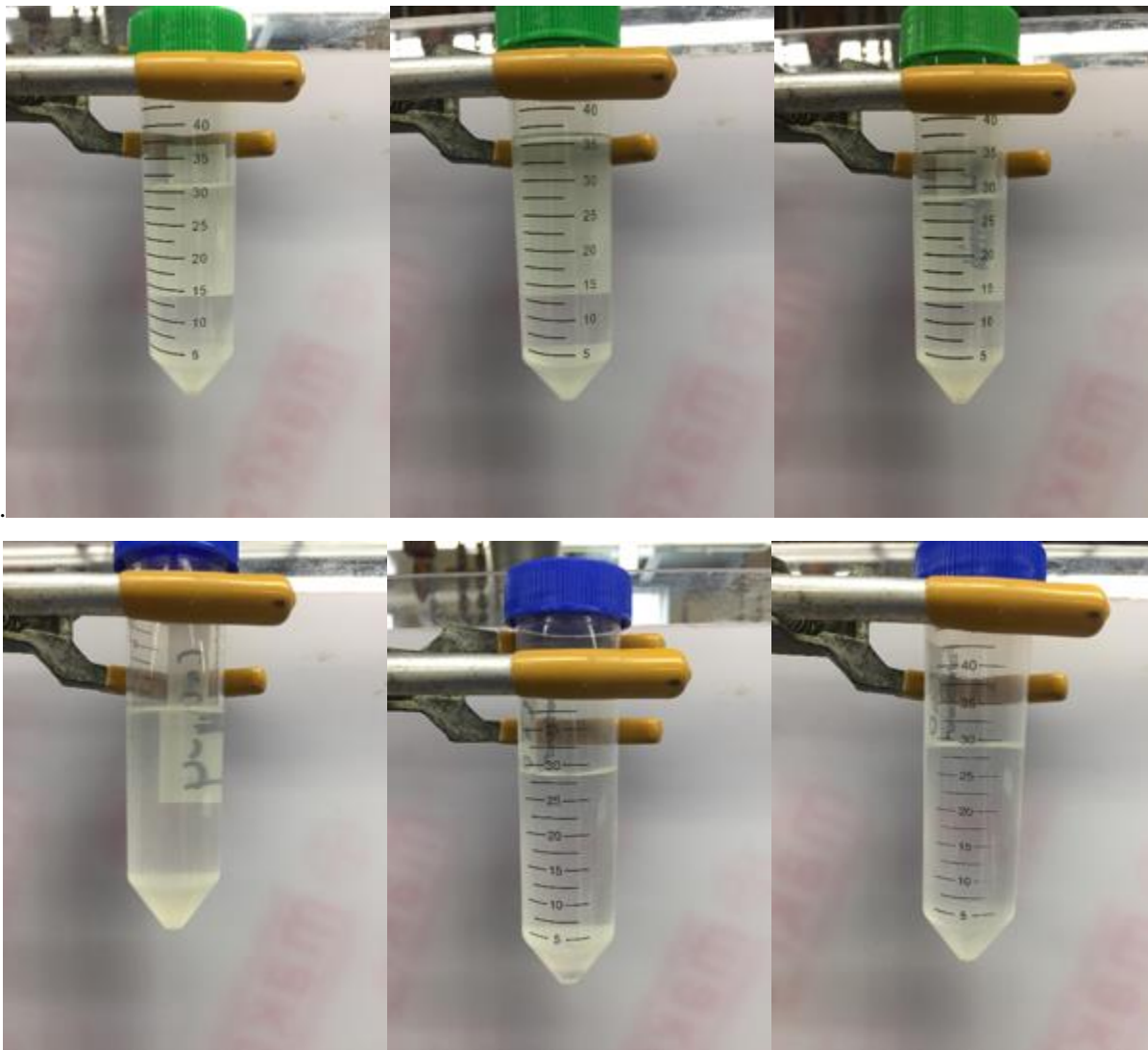


Figure 17: Calcium Oxide 3/27 Tests (Top Row Small Reactor, Bottom Row Large Reactor)

Calcium Carbonate

The calcined CaCO₃ tests made a few considerations apparent. At face value, this test arguably had the worst results with two failures and only four passes. There are, however, possible explanations for this. (1) The failures all occurred when the reaction took place in a large reactor. This could imply that the reaction was not well-mixed or the heat was not distributed evenly enough for a complete reaction to take place. However, this trend did not occur for other catalysts. (2) The reactions failed due to length since the catalyst had been calcined. The catalyst used failed reactions were calcined 16 and 17 days opposed to 1,

7, 8, and 9 days for the "passed" reactions. This is significantly longer than another catalyst sat. This can be attributed to two things: the catalyst degrades over time or the catalyst was exposed to water and CO_2 and converted back to CaCO_3 . It is believed that the age of catalyst had more to do with these failures than reactor size, and future tests should be done to determine what can be attributed to the aging process.

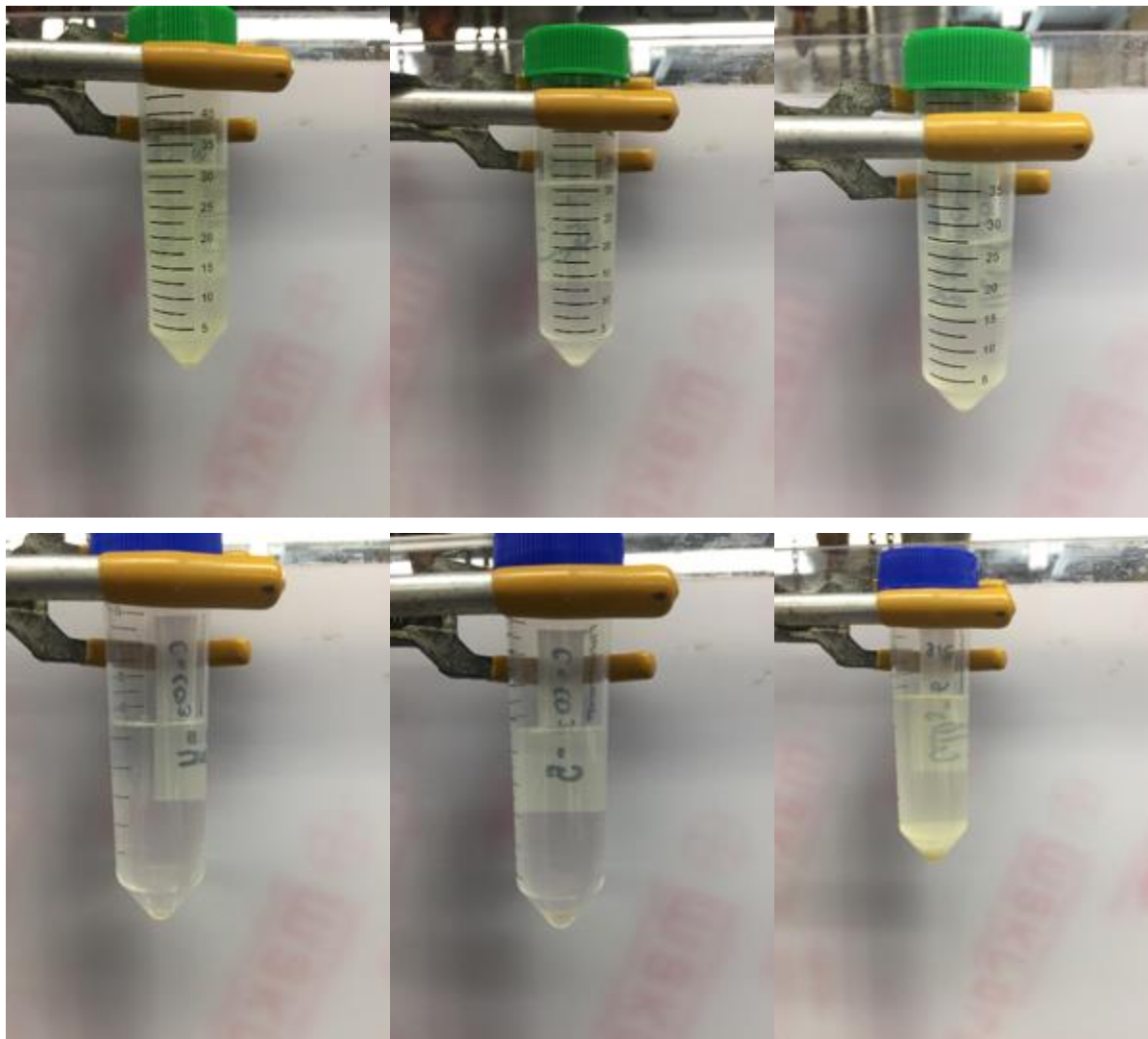


Figure 18: Calcium Carbonate 3/27 Tests (Top Row Small Reactor, Bottom Row Large Reactor)

Clam Shells

For the clam shells, the calcining process yielded a product that was largely still in the shape of the original clam shell chunks. They had turned white and begun to flake apart. They were soft however, and easy to grind in the mortar and pestle. The results of the testing yielded one fail and one near pass among six tests. The fail and near pass both occurred during the testing in the smaller vessel with the larger vessel exhibiting all passes.

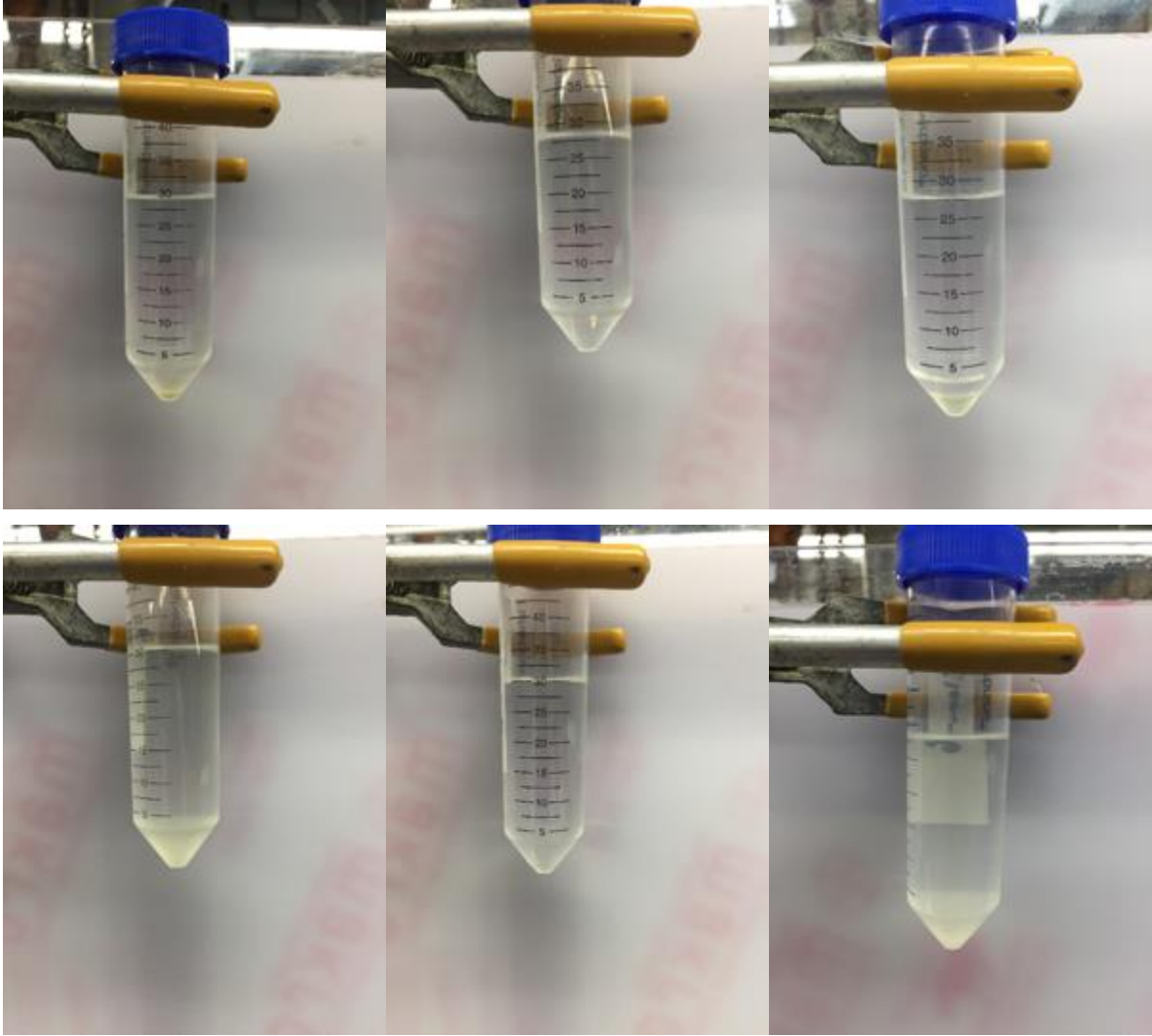


Figure 19: Clam Shell 3/27 Tests (Top Row Small Reactor, Bottom Row Large Reactor)

Seashells

The seashells were broken into approximately quarter-sized pieces before being calcined. After calcining, the shells mostly kept their shape with some flaking apart. The shells required much more effort in reducing them to powder using a mortar and pestle than was required for the egg shells. In order to completely break down the calcined shells, only a small amount was placed in the mortar and pestle at a time. While powdering likely did not have an impact on the calcining process it was done prior to calcining. The results of the 6 biodiesel tests yielded only one failure in the large reactor and one near pass in the small reactor. The near pass, which corresponded to the first run in the small reactor, yielded a biodiesel product that was much more yellow than any of the other seashell products. The failure corresponded to the first run in the large reactor, although the remaining two trials in the large reactor were both successful. Trials 2, 3, and 5, corresponding to the second and third trials in the small reactor and the second trial in the large reactor, each displayed a cloudy, bubbly-textured film above the settled catalyst in the collected product.

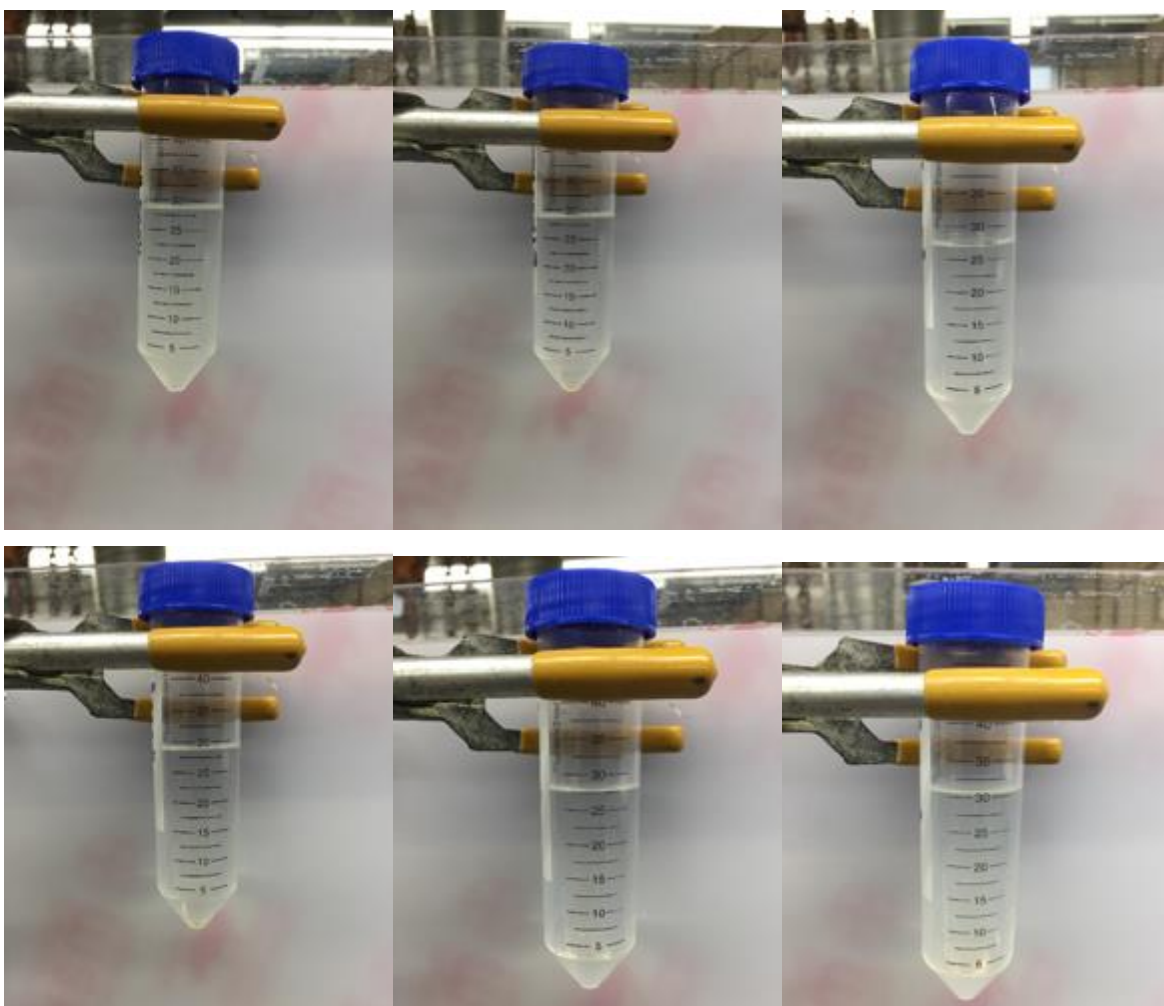


Figure 20: Seashell 3/27 Tests (Top Row Small Reactor, Bottom Row Large Reactor)

Snail Shells

The snail shells color and hardness changed through calcination; originally a brown, hard shell, the shells turned white, flaked, and were easily crushed. The reactions using the snail shells were run in a large and small reactor simultaneously. From this, it would be presumed that the biodiesel resulting from these two runs, using the same MeOH, canola oil, and shells which sat exposed to air, should be identical. From these runs, 5 of the 6 passed. The failure was in the large reactor on the second trial day, with only 2 days between calcination and the start of the reaction. Due to the catalysts being the same and the reaction passing in the small reactor, the time the catalyst sat was not taken into consideration. Instead, possible explanations for failure could be the reactor not being completely, properly cleaned before use. These reactions led to accumulation of old catalyst and possibly liquids within the reactor and if not all were flushed out during cleaning, it is possible these hindered the reaction. The liquid resulting from this failed reaction was different from those of the reactions that passed. It was cloudier, white liquid and bubbles seemed to be present while the other reactions all concluded with a clear yellow liquid.

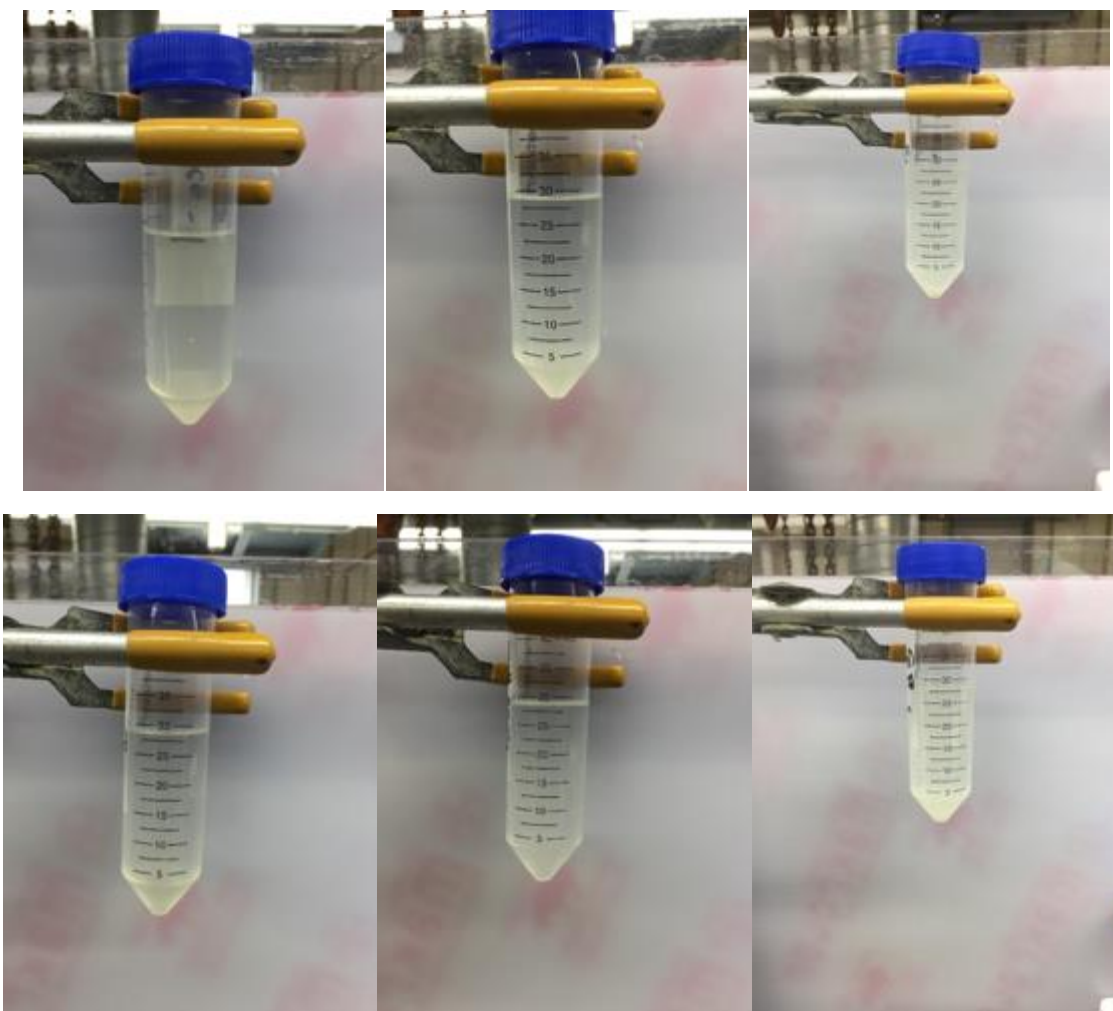


Figure 21: Snail Shell 3/27 Tests (Top Row Small Reactor, Bottom Row Large Reactor)

Small Scale Cost Analysis

It is not only important that these catalysts work, but that they are also less expensive than what is currently being used. Due to the scale of this project, the cost to produce only 1 liter of biodiesel was analyzed. Considered in this analysis were the costs of raw catalyst and calcining, but not universal costs such as jacket heating, impeller electricity, or MeOH and canola oil.

Based on the 1800W output of the oven and the assumption that roughly 400g of uncalcined catalyst can fit in the oven for any given batch, it would cost roughly \$3.89 to calcine 1kg of catalyst for 6 hours. However, it was observed that each catalyst experienced a loss of mass during the calcining process which resulted in different costs of electricity per hour. One element not added in this analysis is reusability which is an advantage the organically derived catalysts might have. As the products were difficult to separate and no techniques exist to do so easily it was not made a part of this analysis but could potentially make those catalysts more economic. Table 1 was developed using the prices paid for catalysts, electrical costs, and the equations available in Appendix F. It was determined that KOH is still by far the cheapest catalyst. Even for the catalysts that do not need to be purchased, like the eggshells, clam shells, and seashells, the electrical costs alone are still higher per liter of biodiesel than the cost of KOH. Notably, all the calcined catalyst loses between 40 and 50% of the mass through the process meaning even more catalyst would need to be purchased than anticipated. However, the fact that most retentions are in the same ballpark means that if purchasing costs are actually similar the prices will be more equivalent. Based on the numbers seen in Table 1, eggshells, clam shells, and seashells are the most economical option that is not KOH, even when compared to the lab grade chemicals.

Table 2: Small-Scale Estimated Cost

Catalyst	Catalyst Cost [\$/kg]	Catalyst lost Calcining [%]	Catalyst per Liter MeOH [kg/L]	Catalyst Cost per Liter Biodiesel [\$/L]	Electrical Cost per Liter Biodiesel [\$/L]	Total Cost [\$/L]
KOH	6.59	-	0.0175	0.01	-	0.01
CaO	39.64	-	0.333	1.61	-	1.61
CaCO ₃	13.23	41	0.333	0.92	0.27	1.18
Eggshells	0.00	46	0.333	0.00	0.29	0.29
Clam shells	0.00	46	0.333	0.00	0.29	0.29
Seashells	0.00	45	0.333	0.00	0.29	0.29
Snail Shells	123.02	47	0.333	9.50	0.30	9.80

Importantly, this is a very rough estimate for pricing. There are more things that need to be taken into consideration when working in larger scales that could potentially lower or raise the price per liter. The benefit to working on this sort of scale is that it is reasonable to collect the naturally available catalysts. Finding a kilogram of eggshells or seashells is relatively reasonable for one or a few people to do because it can easily be a byproduct of personal consumption. However, as scale increases, this only gets more difficult and raises potential sourcing costs.

Large Scale Cost Analysis

The large scale cost analysis was done to determine the magnitude of difficulty in using alternative catalysts vs conventional catalysts (KOH, NaOH) for a mid to large size producer. For this analysis, a producer produces >100 liters of biodiesel a week. There are three main components where extra cost can be applied: sourcing, cleaning and preparation, and separation. Sourcing costs vary heavily on the size of the producer, with larger producers able to work with commercial partners to secure supplies at little to no cost as some catalysts are seen as waste products. Cleaning and preparation of the incoming catalyst material is likely to be the most expensive step in both capital investment and operation cost. While cleaning can be done to the extent as desired by the producer and is not likely to dramatically increase costs the calcining process will require an oven capable of 900°C. This is likely the largest cost to any alternative system. Separation systems would involve holding tanks which would add some capital cost but minimal maintenance. They potentially are already involved in the conventional process and might not add any

additional cost. One cost saving measure is likely to be the reusability of the organically derived catalysts which do not need to be discarded after use unlike alkali metal catalysts. For firms looking to investigate alternative biodiesel manufacture it is recommended location selection be considered in detail as proximity to suppliers (food processing centers) and cheap energy could greatly reduce costs.

Issues with Cleaning and Manufacturing

One of the largest issues found with the testing was the difficulty of cleaning the glassware and ensuring proper mixing of the reactor. The CaO based catalysts would clump and settle out quickly, making emptying the reactor vessels, separating the products, and cleaning the glassware quite difficult.

The reactor products were found to be impossible to forcibly separate. Centrifuging the products yielded a gel within the product which consumed most of the desirable biodiesel. This made using separatory funnels the best way to divide the products, but yielded new problems as the catalyst, once settled to the bottom of the separatory flask, would solidify and become lodged in the bottom.

Once the testing was complete, the greatest issue found was the difficulty of cleaning out glassware and other vessels. When the catalyst was mixed with either the biodiesel or the unconverted oil, it formed a paste that was resistant to most solvents. Against soap, this paste would merely smear and spread. The best solution found for dealing with this was a mild (5-10% by volume) acetic acid (AcOH) bath. This generally dissolved the catalyst and allowed for easier cleaning of the glassware. Additional solutions were to cook off the reaction product in an oven and then soak in the bath for better cleaning.

All of these issues pose significant challenges for mass production. The tendency to form deposits would make continuous agitation of solution a necessity. Separation issues would necessitate large separation vessels, which would accumulate material as the catalyst builds up. Lastly, cleaning out the separation and reaction vessels, would require potentially caustic chemicals, ease of access to internals, and regular maintenance time. The solution to these issues was not extensively researched however and could potentially be abated by other means.

Conclusion and Recommendations

As seen in the Results and Discussion section, all of the calcined CaCO_3 qualitatively produced biodiesel. The majority of the 'failures' can be accounted to other factors. Unfortunately, the 3/27 test is only a qualitative analysis. Although it proved calcined catalyst worked, there is no guarantee that it works to the same extent as the KOH reactions. Further testing needs to be done to compare how much biodiesel each catalyst is producing and exactly how much calcined CaCO_3 catalyst is necessary. Due to the results of this project, it is recommended that a quantitative analysis be done; this could be done in a handful of ways: 1) using the previously mentioned glycerol assay analysis to determine how much glycerol is produced to calculate the extent of the reaction or 2) evaporating the alcohol off the product and measuring the volume of unconverted alcohol to calculate extent. Both accomplish the same goal, but the first option is better if the change in concentration over time is important. Additionally, either gas chromatography or high pressure liquid chromatography could be better methods if quantifying all of the reaction components is important, rather than just the biodiesel product.

Although it was hypothesized that using calcined catalyst would be a cheaper alternative than the KOH catalyst, the cost of calcining alone is more than the cost of catalyst. Although recycling the catalysts, such as eggshells, seashells, and snail shells, would be a more environmentally conscious option, they could potentially be difficult to collect in bulk. Another major concern is that the equipment is extremely difficult to clean and the separation of biodiesel from catalyst and glycerol is very difficult. This does not lend itself to being mass produced. As it stands, using calcined CaCO_3 works well in small scale, but using KOH is judged to be more practical for large scale.

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Appendix

A. Unit Operations II Lab Document

Biodiesel Reaction Safety and Kinetics

Renewable fuels such as ethanol and biodiesel are becoming increasingly popular alternatives to petroleum based fuels. In this laboratory exercise, you will study temperature and mass transfer effects on the base-catalyzed transesterification reaction used to produce biodiesel from vegetable oil. In addition to studying the fundamental chemical engineering principles required to optimize the reaction, you will study the safety requirements for this process and gain experience with following a standard operating procedure and maintaining an electronic batch record.

The overall reaction for production of biodiesel from vegetable oil using methanol is shown in Figure 1. When ethanol is used instead of methanol, three ethanol molecules react with the triglyceride to form 3 molecules of ethyl esters.

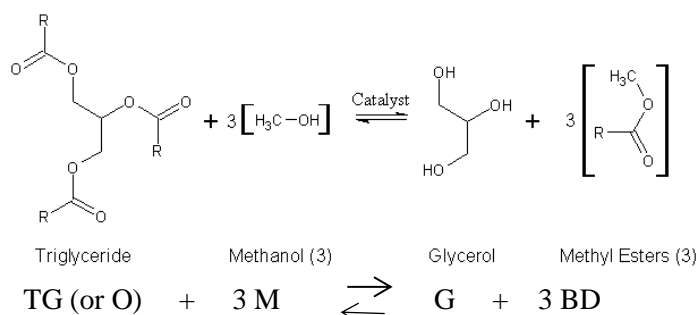


Figure 1. Overall biodiesel production reaction [1].

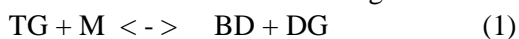
Vegetable oil is primarily composed of triglycerides with long chain aliphatic R groups of the form $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7$, but different types of oils have different amounts of saturated and unsaturated fats of various types in the R groups [2]. The methyl (or ethyl) esters, also called fatty acid methyl (or ethyl) esters (FAMEs or FAEEs), are the biodiesel products we seek. The reaction can be either base-catalyzed or acid-catalyzed. Vegetable oil can be burned directly but is not a good engine fuel due to its high viscosity. Transesterification converts the high viscosity oil into 3 biodiesel molecules with viscosity and other properties similar to those of petroleum diesel fuel [3].

The apparent simplicity of the process along with the readily available supply of waste vegetable oil from deep fry food preparation has given rise to a large number of small scale and “home brew” processors who usually use potassium hydroxide as catalyst. As you will learn in this laboratory experiment, this base-catalyzed biodiesel process is neither particularly simple nor particularly safe. Methanol and ethanol are toxic and highly flammable. KOH is caustic and when mixed with methanol (or ethanol) forms an extremely dangerous potassium methoxide (or ethoxide) solution. Your first objective for the pre-lab exercise will be to obtain and read the MSDS information for KOH, methanol, ethanol, potassium methoxide, and potassium ethoxide. You will need to write a brief discussion of the safety precautions required for handling and processing these materials and the safeguards we have included in the lab. Your second objective for the pre-lab will be to locate (on the web or elsewhere) a report of an accident involving a fire with this process. You will need to write a brief summary of the accident and a discussion of what could have been done to prevent it.

We have converted a 5 ft wide fume hood into a mini biodiesel pilot plant. Our computer controlled reactor system consists of a 250 ml jacketed glass catalyst preparation reactor and a 500 ml jacketed glass process reactor connected to feed and product vessels and each other via peristaltic pumps [4]. Sparkless and brushless overhead electronic stirrers are used to control and monitor the stirrer rpms and torque in each reactor. A constant temperature is maintained with a separate temperature bath circulating water through the jacket of each reactor. A third circulating temperature bath is used to circulate chilled water through condensers attached to each reactor vessel to minimize evaporation losses at elevated process temperatures. Using a computer control panel outside of the hood to operate the process gives it the feel of a larger scale industrial process and minimizes some of the dangers from the hazardous and flammable materials involved.

For a typical experiment, methanol (or ethanol) and vegetable oil are introduced into the catalyst prep reactor and process reactor, respectively, by computer controlled gravimetric dosing from feed vessels on electronic balances working together with the peristaltic pumps. Once both reactors reach the target temperature, solid KOH catalyst is manually added and dissolved in the catalyst prep reactor. The process reaction is initiated by pumping the contents of the catalyst prep reactor into the process reactor. Samples are withdrawn from the process reactor at regular intervals and analyzed for glycerol content via an enzymatic assay to follow the reaction progress [5]. At the end of the experiment all samples can be analyzed at once using a 96 well plate reader at a wavelength of 570 nm. The reactor system is washed with methanol (or ethanol) and allowed to air dry between experimental runs. The ReactorMaster software [4] that controls the process also collects data on each piece of equipment, allows for pauses to insert comments, and effectively keeps an electronic batch record of everything that happens in each experimental run. You will be required to input comments into the batch record indicating that you have either performed or witnessed various aspects of the standard operating procedure as is often required in the bioprocess industry.

As noted above, the biodiesel production process is more complex than it may seem from looking at Figure 1. It has been observed that the production process depends on the type of oil used, the water and free fatty acid content of the oil, the type and amount of catalyst, the alcohol to oil ratio, and operating conditions such as temperature, pressure, and mixing rate [6, 7]. The methanolysis reaction is believed to involve multiple steps as indicated in Figure 2 where it can be seen that triglyceride (TG) is first attacked by the methoxide ion CH_3O^- (present in the basic KOH/methanol solution) to produce one biodiesel (BD) and a di-glyceride (DG). The DG is next converted to a second BD and a mono-glyceride (MG). Finally, in a third reaction step, the MG is converted to a third BD and glycerol (G). Each of these reactions can be considered reversible, giving rise to a forward and a reverse rate constant for each of the three reactions. Side reactions that produce soap (via saponification) instead of biodiesel often occur, especially if water is present in the mixture, but we will neglect side reactions in our analysis. To complicate matters further, methanol and oil are essentially insoluble in one another requiring good stirring to bring them into contact. The reaction mixture begins as two phases, then goes to one phase, and finally back to two phases because the glycerol and biodiesel are also essentially insoluble in one another. This has caused many researchers to believe that the reaction cannot be properly modeled without understanding the phase behavior and droplet size changes that occur during the course of the reaction [8, 9]. Others have shown that in cases with sufficient stirring, experimental data can be modeled using only the reversible reactions shown in Figure 2.



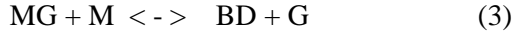


Figure 2. Multiple step biodiesel reaction mechanism.

For example, Vicente et al [10] studied base-catalyzed transesterification of sunflower oil with 6:1 mole ratio of methanol to oil and stirring rates that they believed were sufficient to avoid mass transfer limitations. They varied the KOH catalyst concentration and the process temperature and measured the concentration of TG, DG, MG, BD, M, and G with time during the reaction. Assuming that the forward rate constants for reactions 1, 2, and 3 are given by k_1 , k_3 , and k_5 , while the reverse rate constants for the three reactions are given by k_2 , k_4 , and k_6 , they found the values of the rate constants that fit their data according to the mathematical model shown in Figure 3.

$$\begin{aligned} TG(0) &= TG_0 & M(0) &= M_0 & DG(0) &= 0 & MG(0) &= 0 & BD(0) &= 0 & G(0) &= G_0 \\ TG'(t) &= -k_1 \cdot TG(t) \cdot M(t) + k_2 \cdot BD(t) \cdot DG(t) \\ DG'(t) &= k_1 \cdot TG(t) \cdot M(t) - k_2 \cdot BD(t) \cdot DG(t) - k_3 \cdot DG(t) \cdot M(t) + k_4 \cdot BD(t) \cdot MG(t) \\ MG'(t) &= k_3 \cdot DG(t) \cdot M(t) - k_4 \cdot BD(t) \cdot MG(t) - k_5 \cdot MG(t) \cdot M(t) \\ M'(t) &= -k_1 \cdot TG(t) \cdot M(t) + k_2 \cdot BD(t) \cdot DG(t) - k_3 \cdot DG(t) \cdot M(t) + k_4 \cdot BD(t) \cdot MG(t) - k_5 \cdot MG(t) \cdot M(t) \\ BD'(t) &= k_1 \cdot TG(t) \cdot M(t) - k_2 \cdot BD(t) \cdot DG(t) + k_3 \cdot DG(t) \cdot M(t) - k_4 \cdot BD(t) \cdot MG(t) + k_5 \cdot MG(t) \cdot M(t) \\ G'(t) &= k_5 \cdot MG(t) \cdot M(t) \end{aligned}$$

Figure 3. Mathematical model equations for multistep biodiesel reaction shown in Figure 2.

Note that in the mathematical model, the symbol $TG'(t)$ represents the time derivative of the triglyceride concentration, $dTG(t)/dt$. At 35 °C and 0.5 weight percent KOH (on a percent weight of oil basis) they found the following rate constants with units of L/(mol min): $k_1 = 0.20$, $k_2 = 0.98$, $k_3 = 1.67$, $k_4 = 2.18$, $k_5 = 0.27$, and $k_6 = 0.01$ (sufficiently small that it has been omitted in the model). A Mathcad file that solves this model is available on the course website for your use.

In your experiments you will use canola oil rather than sunflower oil and will only be able to follow overall reaction conversion by following the glycerol concentration with time. It will, therefore, be difficult, if not impossible; to fit all six rate constants to your data. Some researchers have suggested that it is advantageous to consider the reaction in three stages: a brief initial mixing/mass transfer limited stage, an irreversible chemical reaction controlled stage, and a reversible equilibrium reaction controlled stage near the end [11]. These authors have further suggested that the pseudo 2nd order reaction:

$$O'(t) = dO(t)/dt = -k O(t)^2 \quad (4)$$

provides an approximate model for the overall reaction shown in Figure 1, at least in the middle stage.

You will want to test this hypothesis using the Mathcad model of the reaction at 35 °C. That is, use the multistep model results for concentration with time to test if a 2nd order model could fit those results and determine the pseudo 2nd order rate constant, k . You should consult a chemical reaction engineering text for information on how to fit reaction data to a 2nd order model. Note that in the 2nd order model for oil (O) of Equation 4 there is no accounting for DG or MG, and the oil concentration, O, is used instead of TG. To compare results of this simple model to those of the multistep Mathcad model that solves the

equations in Figure 3, you will need to follow total oil, $O = TG + DG + MG$, as indicated in the multistep Mathcad model. To test the hypothesis, once you find the rate constant k , you will want to solve Equation 4 to generate a plot of $O(t)$ vs time and compare that to the $(TG + DG + MG)$ vs time results from the multistep mathematical model.

Some studies [12] using ethanol instead of methanol with sunflower oil have also found that the reaction can be modeled with an irreversible 2nd order reaction followed by a reversible 2nd order reaction as the process nears completion. Interestingly, these studies didn't observe any initial mass transfer limited lag phase when using ethanol.

Others studies [13] using ethanol with castor oil also found no mass transfer limited lag phase, but found that the reaction followed 1st order kinetics.

For your lab exercise, you will want to use ethanol with canola oil and determine if the results can be described with either a first order or second order reaction. You will also want to study the temperature dependence and evaluate an activation energy for canola oil conversion to BD with 0.5 weight % KOH and 6:1 ethanol to oil ratio. You will also want to discuss possible mass transfer limitations of the reaction. For the pre-lab you should explain how you will study the T dependence, evaluate the activation energy, and how you could study the mass transfer limitations if there was sufficient time in the lab. Densities and molecular weights for reactants and products are given in Table 1.

Table 1. Densities and molecular weights of canola biodiesel reactants and products [14].

component	density (g/ml)	molecular weight (g/mol)
canola oil	0.92	887.3
methanol/ethanol	0.792/0.789	32.0/46.1
glycerol	1.26	92.1
biodiesel	0.88	297.1

Summary of deliverables for your pre-lab report:

- 1) Locate, copy, and read the MSDS for methanol, ethanol, KOH, potassium methoxide, and potassium ethoxide.
- 2) Write a brief discussion of the safety precautions needed in handling and processing these materials and include a brief discussion of the safeguards we have included to conduct this reaction safely.
- 3) Locate (on the web or elsewhere) a report of an accident involving a fire in biodiesel production. Write a brief summary of the accident and a discussion of what could have been done to prevent it.
- 4) Use the data generated in the Mathcad file that solves the multistep biodiesel reaction mathematical model to test the validity of using a pseudo 2nd order rate equation for sunflower oil at 35 °C, 0.5 weight % KOH, and 6:1 methanol to oil ratio. Report the pseudo 2nd order rate constant and compare the predicted O vs time curve from Equation 4 to the $TG+DG+MG$ vs time curve from the multistep model.
- 5) Explain how you can test whether the results from your ethanolysis experiments follow 1st order or 2nd order kinetics.

- 6) Explain how you will study the T dependence and evaluate the activation energy for ethanolysis of canola oil in the lab.
- 7) Explain how you could investigate mass transfer limitations of the transesterification reaction in the lab. (You will probably not have time to do actually do the investigation, but you should discuss what could be done to study this).

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B. Glycerol Assay Product Sheet

BioAssay Systems Glycerol

EGLY003.pdf

EnzyChrom™ Glycerol Assay Kit (Cat# EGLY-200) Quantitative Colorimetric/Fluorimetric Glycerol Determination

DESCRIPTION

GLYCEROL [GLYCERIN or GLYCERINE, C₃H₈(OH)₃] is widely used in foods, beverages and pharmaceutical formulations. It is also a main by-product of biodiesel production. Simple, direct and automation-ready procedures for measuring glycerol concentrations find wide applications. BioAssay Systems' glycerol assay uses a single Working Reagent that combines glycerol kinase, glycerol phosphate oxidase and color reactions in one step. The color intensity of the reaction product at 570nm or fluorescence intensity at λ_{em}/ex = 585/530nm is directly proportional to glycerol concentration in the sample.

KEY FEATURES

Sensitive and accurate. Use as little as 10 μL samples. Linear detection range in 96-well plate: 10 to 1000 μM (92 μg/dL to 9.2 mg/dL) glycerol for colorimetric assays and 2 to 50 μM for fluorimetric assays.

Simple and convenient. The procedure involves addition of a single working reagent and incubation for 20 min at room temperature, compatible for HTS assays.

Improved reagent stability. The optimized formulation has greatly enhanced the reagent and signal stability.

APPLICATIONS:

Direct Assays: glycerol in biological samples (e.g. serum and plasma).

Drug Discovery/Pharmacology: effects of drugs on glycerol metabolism.

Food and Beverages: glycerol in food, beverages, pharmaceutical formulations etc.

KIT CONTENTS

Assay Buffer: 24 mL **Enzyme Mix:** 500 μL **ATP:** 250 μL

Dye Reagent: 220 μL **Standard:** 100 μL 100 mM Glycerol

Storage conditions. The kit is shipped on dry ice. Store Assay Buffer at 4°C and other reagents at -20°C. Shelf life of 6 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

COLORIMETRIC PROCEDURE

Note: SH-group containing reagents (e.g. mercaptoethanol, DTT) may interfere with this assay and should be avoided in sample preparation.

1. Equilibrate all components to room temperature. Keep thawed Enzyme Mix in a refrigerator or on ice. Dilute standard in distilled water as follows (diluted standards can be used for future assays when stored refrigerated).

No	STD + H ₂ O	Vol (μL)	Glycerol (mM)
1	10 μL + 990 μL	1000	1.0
2	6 μL + 994 μL	1000	0.6
3	3 μL + 997 μL	1000	0.3
4	0 μL + 1000 μL	1000	0

Transfer 10 μL standards and 10 μL samples into separate wells of a clear 96-well plate.

2. For each reaction well, mix 100 μL Assay Buffer, 2 μL Enzyme Mix, 1 μL ATP and 1 μL Dye Reagent in a clean tube. This Working Reagent should be used on the same day of preparation. Transfer 100 μL Working Reagent into each reaction well. Tap plate to mix.
3. Incubate 20 min at room temperature. Read optical density at 570nm (550-585nm).

Note: if the Sample OD is higher than the Standard OD at 1.0 mM, dilute sample in water and repeat the assay. Multiply result by the dilution factor.

CALCULATION

Subtract blank OD (water, #4) from the standard OD values and plot the OD against standard concentrations. Determine the slope using linear regression fitting. The glycerol concentration of Sample is calculated as

$$[\text{Glycerol}] = \frac{\text{OD}_{\text{SAMPLE}} - \text{OD}_{\text{H}_2\text{O}}}{\text{Slope}} \quad (\text{mM})$$

OD_{SAMPLE} and OD_{H₂O} are optical density values of the sample and water. **Conversions:** 1mM glycerol equals 9.2 mg/dL, 92 ppm.

FLUORIMETRIC PROCEDURE

For fluorimetric assays, the linear detection range is 2 to 50 μM glycerol. Mix 10 μL 100 mM Standard with 990 μL H₂O (final 1 mM).

No	1 mM STD + H ₂ O	Vol (μL)	Glycerol (mM)
1	50 μL + 950 μL	1000	0.050
2	30 μL + 970 μL	1000	0.030
3	15 μL + 985 μL	1000	0.015
4	0 μL + 1000 μL	1000	0

Dilute standards as above. Transfer 10 μL standards and 10 μL samples into separate wells of a black 96-well plate.

Add 100 μL Working Reagent (see *Colorimetric Procedure*). Tap plate to mix.

Incubate 20 min at room temperature and read fluorescence at λ_{em} = 530nm and λ_{ex} = 585nm.

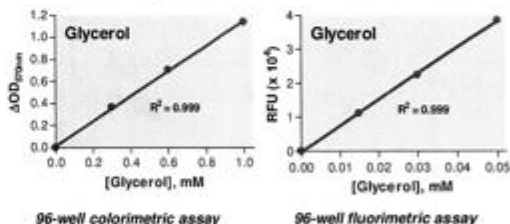
The glycerol concentration of Sample is calculated as

$$[\text{Glycerol}] = \frac{F_{\text{SAMPLE}} - F_{\text{H}_2\text{O}}}{\text{Slope}} \quad (\text{mM})$$

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipeting devices, centrifuge tubes, Clear flat-bottom 96-well plates, black 96-well plates (e.g. Corning Costar) and plate reader.

Glycerol Standard Curves



LITERATURE

1. Duncan RE, et al. (2007). Regulation of lipolysis in adipocytes. *Annu Rev Nutr.* 27:79-101.
2. Moller F, Roomi MW. (1974). An enzymatic, spectrophotometric glycerol assay with increased basic sensitivity. *Anal Biochem.* 59(1):248-58.
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C. Summarized Unit Operations Reaction and Glycerol Assay Procedures

Reaction

1. 100 mL Methanol put in catalyst prep reactor and 400 mL Vegetable oil put in process reactor using “computer controlled gravimetric dosing from feed vessels on electronic balances working with peristaltic pumps.”
2. Reactors are heated up to 35°C
3. 1.75 g of KOH is manually added and dissolved to catalyst reactor
4. Contents from catalyst reactor are pumped to process reactor
5. Samples are drawn at intervals of 0.5, 1, 2, 5, 10, 15, 20, 25, 30, 40, 50, 60, 90, and 120 minutes and placed in plastic centrifuge tubes.
6. Remove Biodiesel glycerol mixture into separatory funnel.
7. Wait for two distinct layers to be present to qualitatively signify reaction occurrence and drain off glycerol portion from the bottom.

Glycerol Assay (As Specified from Product Sheet)

1. Before reaction, remove enzyme kit from freezer.
2. Dilute standards as specified in the table below

No	STD + H2O	Total Volume (μL)	Glycerol (mM)
1	10 μL+ 990 μL	1000	1.0
2	6 μL+ 994 μL	1000	0.6
3	3 μL+ 997 μL	1000	0.3
4	0 μL+ 1000 μL	1000	0.0

3. Transfer standards to well plate
4. For each sample taken from reactor, mix 100 μL Assay Buffer, 2 μL Enzyme Mix, 1 μL ATP and 1 μL Dye Reagent in a clean tube
5. Transfer 100 μL Working Reagent into each reaction well
6. Incubate samples for 20 minutes at room temperature
7. Place in machine and read at optical density of 570 nm (550-585nm)
8. Subtract blank No. 4 from the standard OD values
9. Plot OD against standard concentrations
10. Measure slope using linear regression fitting
11. Calculate using:

$$[\text{Glycerol}] = \frac{\text{OD}_{\text{Sample}} - \text{OD}_{\text{H}_2\text{O}}}{\text{Slope}}$$

D. Unit Operations repeated reaction with KOH and Glycerol Assay Results

Table 3: MeOH Linear Regression

		Optical Density	Correlating Std 4 Optical Density	Adjusted Optical Density	[Glycerol] (mM)	Slope	R2
Run 1	Std1	1.4928	0.1146	1.3782	1	1.0563	0.44734
	Std2	1.0206	0.1139	0.9067	0.6		
	Std3	1.5902	0.1127	1.4775	0.3		
	Std4	0.1127	0.1127	0	0		
Run 2	Std1	1.4454	0.0813	1.3641	1	1.5006	0.85643
	Std2	1.4142	0.0806	1.3336	0.6		
	Std3	0.4309	0.0808	0.3501	0.3		
	Std4	0.0808	0.0808	0	0		
Run 3	Std1	1.5535	0.0826	1.4709	1	1.2633	0.58094
	Std2	1.4269	0.0817	1.3452	0.6		
	Std3	1.5031	0.082	1.4211	0.3		
	Std4	0.082	0.082	0	0		
Run 4	Std1	1.7232	0.1895	1.5337	1	1.3678	0.70426
	Std2	1.508	0.1873	1.3207	0.6		
	Std3	1.4523	0.1871	1.2652	0.3		
	Std4	0.1871	0.1871	0	0		

Table 2 shows the optical density values for the glycerol standards. The most important column in this table is the slope. In this case, slope comes from the line of best fit created by the four standard optical densities. It is used to convert optical densities into concentrations by dividing the optical density at each point by the slope. This allows the user to observe the results in terms of concentration vs. time, rather than the less meaningful optical density.

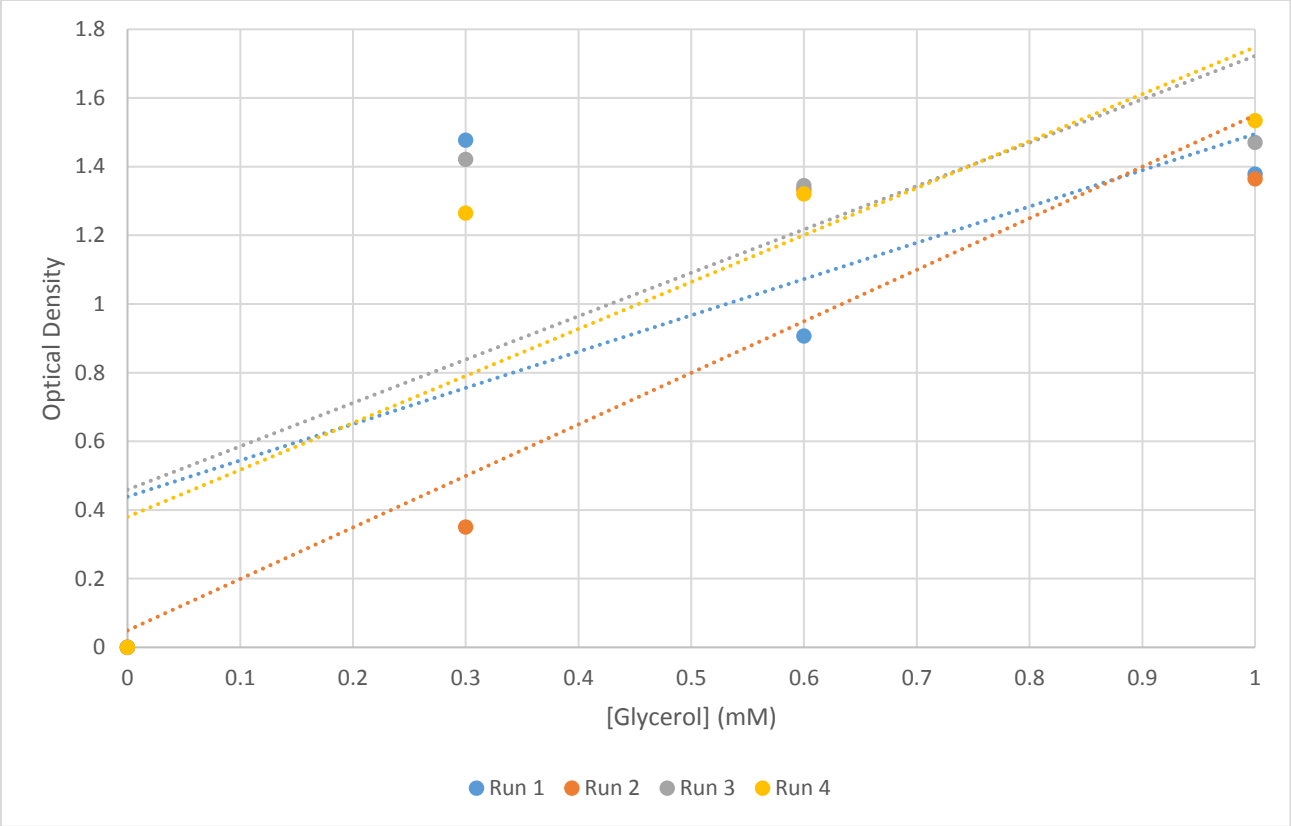


Figure 22: MeOH Linear Regression

Table 4: MeOH Run 1

Time (min)	Optical Density	Correlating Std 4 Optical Density	Adjusted Optical Density	[Glycerol] (mM)
0.5	0.1297	0.1146	0.0151	0.0143
1	0.1459	0.1146	0.0313	0.0296
2	0.1691	0.1146	0.0545	0.0516
5	0.1583	0.1146	0.0437	0.0414
10	0.1256	0.1139	0.0117	0.0111
15	0.1993	0.1139	0.0854	0.0808
20	0.1772	0.1139	0.0633	0.0599
25	0.1525	0.1139	0.0386	0.0365
30	0.1807	0.1139	0.0668	0.0632
40	0.1373	0.1139	0.0234	0.0222
50	0.1382	0.1127	0.0255	0.0241
60	0.1345	0.1127	0.0218	0.0206
90	0.0996	0.1127	-0.0131	-0.0124
120	0.1101	0.1127	-0.0026	-0.0025
Slope	1.0563			

Table 5: MeOH Run 2

Time (min)	Optical Density	Correlating Std 4 Optical Density	Adjusted Optical Density	[Glycerol] (mM)
0.5	0.0909	0.0813	0.0096	0.0064
1	0.0986	0.0813	0.0173	0.0115
2	0.1335	0.0813	0.0522	0.0348
5	0.4923	0.0813	0.411	0.2739
10	1.0548	0.0813	0.9735	0.6487
15	0.8485	0.0806	0.7679	0.5117
20	0.9171	0.0806	0.8365	0.5574
25	1.1118	0.0806	1.0312	0.6872
30	0.8231	0.0806	0.7425	0.4948
40	1.0132	0.0806	0.9326	0.6215
50	0.9962	0.0808	0.9154	0.6100
60	0.9349	0.0808	0.8541	0.5692
90	0.0375	0.0808	-0.0433	-0.0289
120		0.0808	-0.0808	-0.0538
Slope	1.5006			

Table 6: MeOH Run 3

Time (min)	Optical Density	Correlating Std 4 Optical Density	Adjusted Optical Density	[Glycerol] (mM)
0.5	0.077	0.0826	-0.0056	-0.004
1	0.1015	0.0826	0.0189	0.015
2	0.1228	0.0826	0.0402	0.032
5	0.5469	0.0826	0.4643	0.368
10	0.8755	0.0826	0.7929	0.628
15	1.262	0.0817	1.1803	0.934
20	0.7699	0.0817	0.6882	0.545
25	0.9633	0.0817	0.8816	0.698
30	0.6452	0.0817	0.5635	0.446
40	0.7313	0.0817	0.6496	0.514
50	0.9363	0.082	0.8543	0.676
60	1.0325	0.082	0.9505	0.752
90	0.9471	0.082	0.8651	0.685
120	0.9679	0.082	0.8859	0.701
Slope	1.2633			

Table 7: MeOH Run 4

Time (min)	Optical Density	Correlating Std 4 Optical Density	Adjusted Optical Density	[Glycerol] (mM)
0.5	0.0876	0.1895	-0.1019	-0.0745
1	0.0903	0.1895	-0.0992	-0.0725
2	0.1725	0.1895	-0.017	-0.0124
5	0.6807	0.1895	0.4912	0.3591
10	1.0903	0.1895	0.9008	0.6586
15	1.4777	0.1873	1.2904	0.9434
20	0.9439	0.1873	0.7566	0.5532
5	1.4551	0.1873	1.2678	0.9269
30	1.5106	0.1873	1.3233	0.9675
40	1.4015	0.1873	1.2142	0.8877
50	1.0526	0.1871	0.8655	0.6328
60	1.4922	0.1871	1.3051	0.9542
90				
120				
Slope	1.3678			

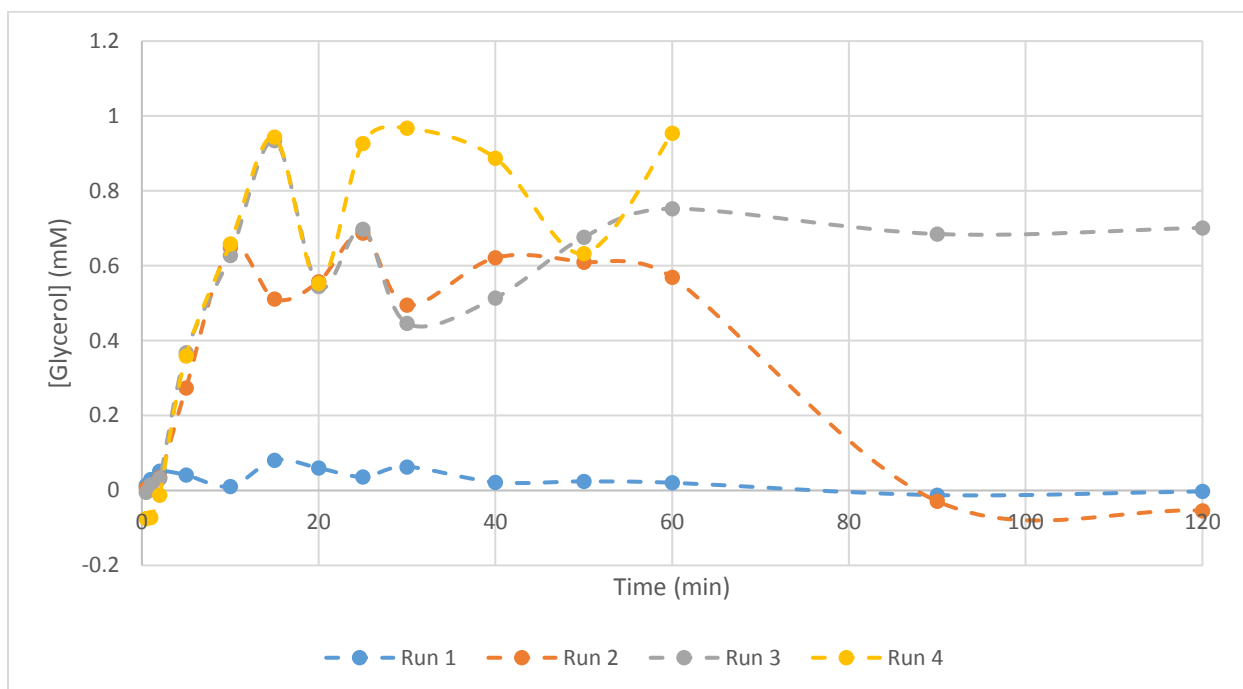


Figure 23: MeOH - [Glycerol] vs. Time

Table 8: EtOH Linear Regression

			Correlating Std 4	Adjusted	[Glycerol] (mM)	Slope	R2
Run 1	Std1	0.0729	0.0488	0.0241	1	-0.014	0.00442
	Std2	0.1627	0.0492	0.1135	0.6		
	Std3	0.2463	0.0491	0.1972	0.3		
	Std4	0.0491	0.0491	0	0		
Run 2	Std1	2.395	0.0662	2.3288	1	2.3774	0.99204
	Std2	1.6391	0.0656	1.5735	0.6		
	Std3	0.7377	0.0653	0.6724	0.3		
	Std4	0.0653	0.0653	0	0		

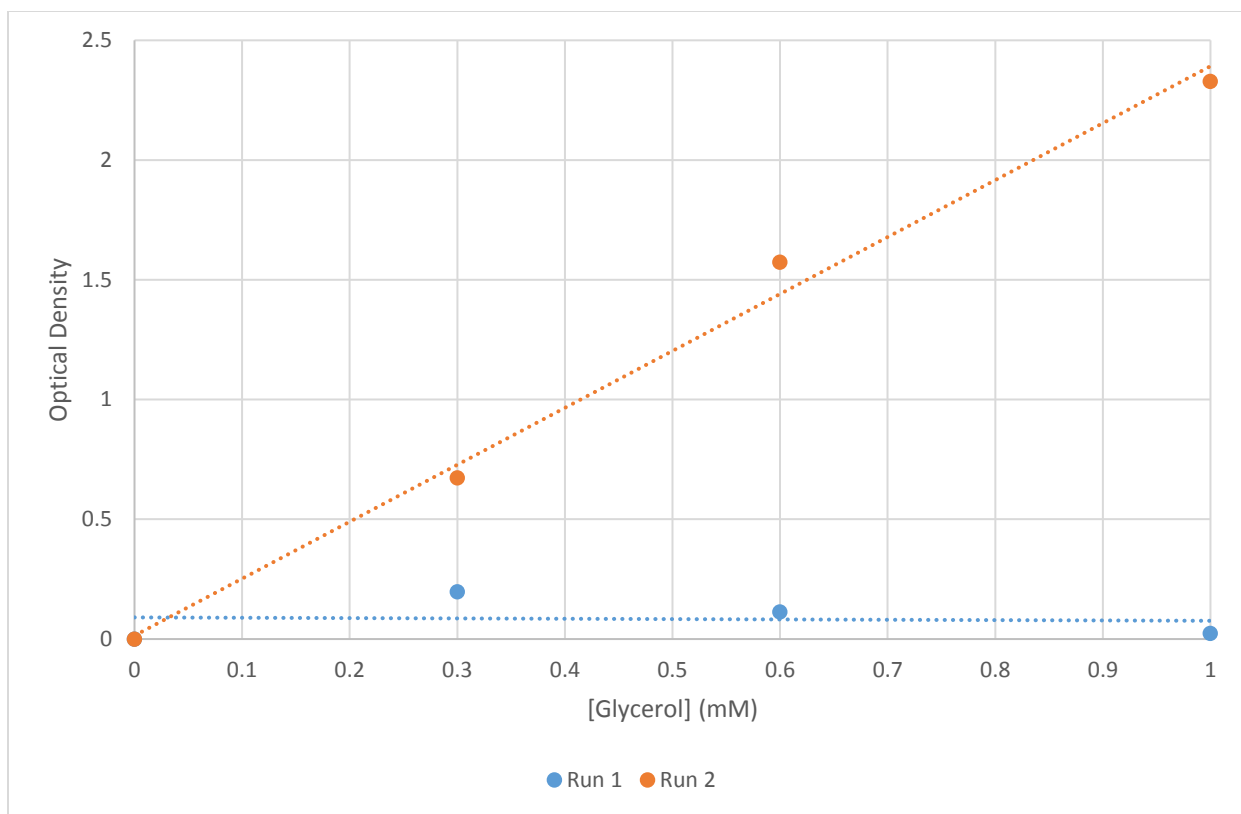


Figure 24: EtOH Linear Regression

Table 9: EtOH Run 1

Time (min)	Optical Density	Correlating Std 4 Optical Density	Adjusted Optical Density	[Glycerol] (mM)
0.5	0.1067	0.0488	0.0579	-4.135714
1	0.1224	0.0488	0.0736	-5.257143
2	0.2088	0.0488	0.16	-11.42857
5	0.2198	0.0488	0.171	-12.21429
10	0.3336	0.0488	0.2848	-20.34286
15	0.417	0.0492	0.3678	-26.27143
20	0.4962	0.0492	0.447	-31.92857
25	0.4639	0.0492	0.4147	-29.62143
30	0.5284	0.0492	0.4792	-34.22857
40	0.4588	0.0492	0.4096	-29.25714
50	0.6153	0.0491	0.5662	-40.44286
60	0.7513	0.0491	0.7022	-50.15714
Slope	-0.014			

Table 10: EtOH Run 2

Time (min)	Optical Density	Correlating Std 4 Optical Density	Adjusted Optical Density	[Glycerol] (mM)
0.5	0.127	0.0662	0.0608	0.0255742
1	0.1462	0.0662	0.08	0.0336502
2	0.1896	0.0662	0.1234	0.0519054
5	0.3841	0.0662	0.3179	0.1337175
10	0.4676	0.0662	0.4014	0.1688399
15	0.554	0.0656	0.4884	0.2054345
20	0.6517	0.0656	0.5861	0.2465298
25	0.6269	0.0656	0.5613	0.2360983
30	0.6064	0.0656	0.5408	0.2274754
40	0.6489	0.0656	0.5833	0.2453521
50	0.6414	0.0653	0.5761	0.2423235
60	0.6152	0.0653	0.5499	0.2313031
Slope	2.3774			

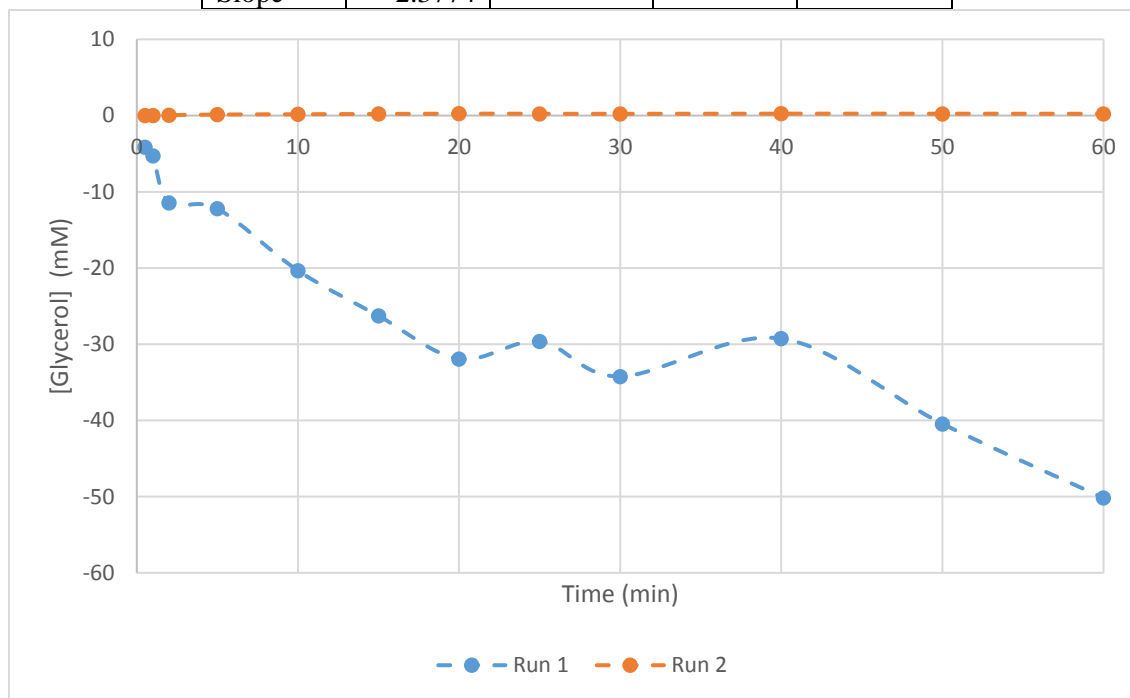


Figure 25: EtOH - [Glycerol] vs. Time

Figure 18 shows the change in glycerol concentration vs. time, with each data point representing a sample. The general trend of the curve is as expected, with glycerol concentration decreasing as time elapses, but

there are a few discrepancies where the concentration increases at later times as well as the fact that the concentration is negative which is impossible.

E. Troubleshooting Reactions

Table 11: Bench-scale Reactions

Date of Catalyst Production	Date of Reaction [Start]	Catalyst Type	Membrane Present?	Catalyst Size	Catalyst Process Temp [C]	Catalyst Process Time [hr]	Catalyst Process Temp 2 [C]	Catalyst Process Temp 2 [hr]	Mass Catalyst [g]	Reactant	Volume Alcohol [mL]	Volume Alcohol [mL]	Reaction Process	Reaction Temp [C]	Reaction Time [hr]	Reacted?
28-Nov	29-Nov	Egg Shell	No	Crushed	400	6	900	6	0.5	Methanol	3.00	7.52	Stirred	22	22	Yes
28-Nov	29-Nov	Egg Shell	No	Crushed	400	6	900	6	1.0	Methanol	3.00	7.52	Stirred	22	22	Yes
28-Nov	29-Nov	Egg Shell	No	Crushed	400	6	900	6	1.5	Methanol	3.00	7.52	Stirred	22	22	Yes
28-Nov	2-Dec	Egg Shell	No	Crushed	400	6	900	6	1.0	Ethanol	3.00	7.52	Stirred	22	23	No
21-Nov	28-Nov	Egg Shell	No	Crushed	900	3	-	-	0.5	Methanol	3.00	7.52	Water bath	35	19	No
21-Nov	28-Nov	Egg Shell	No	Crushed	900	3	-	-	1.0	Methanol	3.00	7.52	Water bath	35	19	No
21-Nov	28-Nov	Egg Shell	No	Crushed	900	3	-	-	1.5	Methanol	3.00	7.52	Water bath	35	19	No
21-Nov	30-Nov	Egg Shell	No	Crushed	900	3	-	-	1.0	Methanol	3.00	7.52	Stirred	22	24	Yes
21-Nov	2-Dec	Egg Shell	No	Crushed	900	3	-	-	1.0	Ethanol	3.00	7.52	Stirred	22	23	No
28-Nov	30-Nov	Egg Shell	No	Crushed	900	3	-	-	4	Methanol	12	30.08	Stirred/Heated	35	-	Yes
28-Nov	30-Nov	Egg Shell	No	Crushed	900	6	-	-	1.0	Methanol	3.00	7.52	Stirred	22	24	Yes
28-Nov	2-Dec	Egg Shell	No	Crushed	900	6	-	-	1.0	Ethanol	3.00	7.52	Stirred	22	23	No
28-Nov	30-Nov	Egg Shell	No	Whole	900	6	-	-	1.0	Methanol	3.00	7.52	Stirred	22	24	Yes
28-Nov	2-Dec	Egg Shell	No	Whole	900	6	-	-	1.0	Ethanol	3.00	7.52	Stirred	22	23	No
21-Nov	28-Nov	Egg Shell	No	Crushed	400	6	-	-	0.5	Methanol	3.00	7.52	Water Bath	35	19	Yes
21-Nov	28-Nov	Egg Shell	No	Crushed	400	6	-	-	1.0	Methanol	3.00	7.52	Water Bath	35	19	Yes
21-Nov	28-Nov	Egg Shell	No	Crushed	400	6	-	-	1.5	Methanol	3.00	7.52	Water Bath	35	19	Yes
N/A	2-Dec	KOH	-	-	-	-	-	-	0.043	Methanol	2.00	8.00	Stirred	22	23	Yes
N/A	2-Dec	KOH	-	-	-	-	-	-	0.043	Ethanol	2.00	8.00	Stirred	22	23	No
	3-Dec	Egg Shell		Crushed	Peroxide	24	-	-	1	Methanol	3	7.52	Stirred	22	27	No
	3-Dec	Egg Shell		Crushed	Peroxide	24	-	-	1	Ethanol	3	7.52	Stirred	22	27	No
	3-Dec	Egg Shell		Crushed	Bleach	24	-	-	1	Methanol	3	7.52	Stirred	22	27	No
	3-Dec	Egg Shell		Crushed	Bleach	24	-	-	1	Ethanol	3	7.52	Stirred	22	27	No

Table 10 shows a summary of the bench-scale reactions involving a calcined egg shell catalyst. A more in depth discussion of these trials can be found in the Results and Discussion section under “Egg Shells”. Calcining details, reactants, and reaction details are all included in the table, along with a column to say whether or not the reaction was successful in passing the qualitative test that was employed. The following figures are examples of the reactions.



Figure 26: Eggshells, 900°C, 6 hours, 1.0 g catalyst, MeOH, Stirred, 24 hours.

2 phases present, reaction considered “passed”



Figure 27: Eggshells, 400°C, 6 hours, 900°C, 6 hours, 1.5 g catalyst, MeOH, Stirred, 22 hours.

2 phases present, reaction considered "passed"



Figure 28: Eggshells, 900°, 1.0 g catalyst, MeOH, Oscillating Water bath (35°C), 19 hour.

1 phase present, reaction considered "failed."

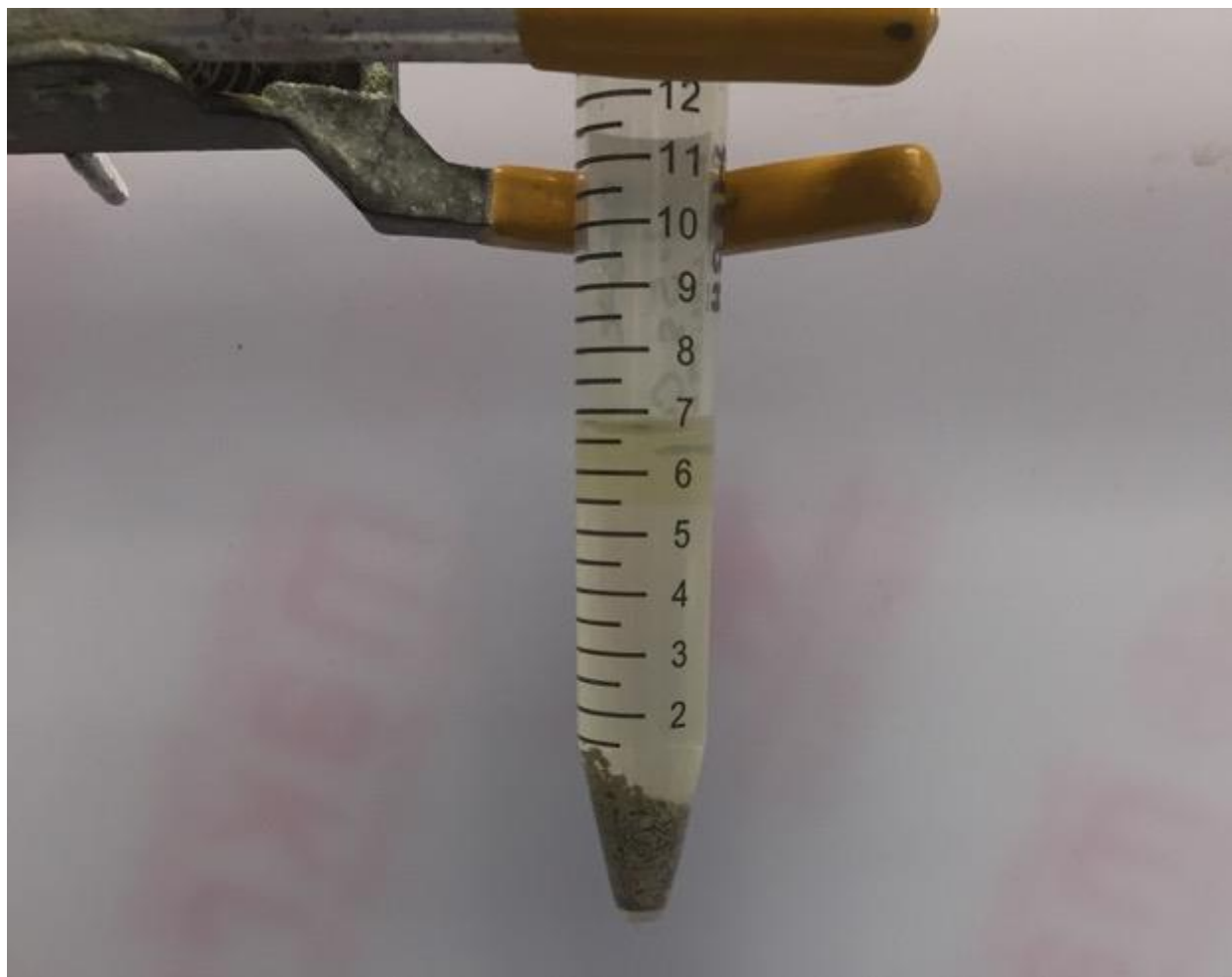


Figure 29: Eggshells, 400°, 6 hours, 1.5 g catalyst, MeOH, Oscillating Oscillating Water bath (35°C), 19 hours.

2 phases present, reaction considered "passed"

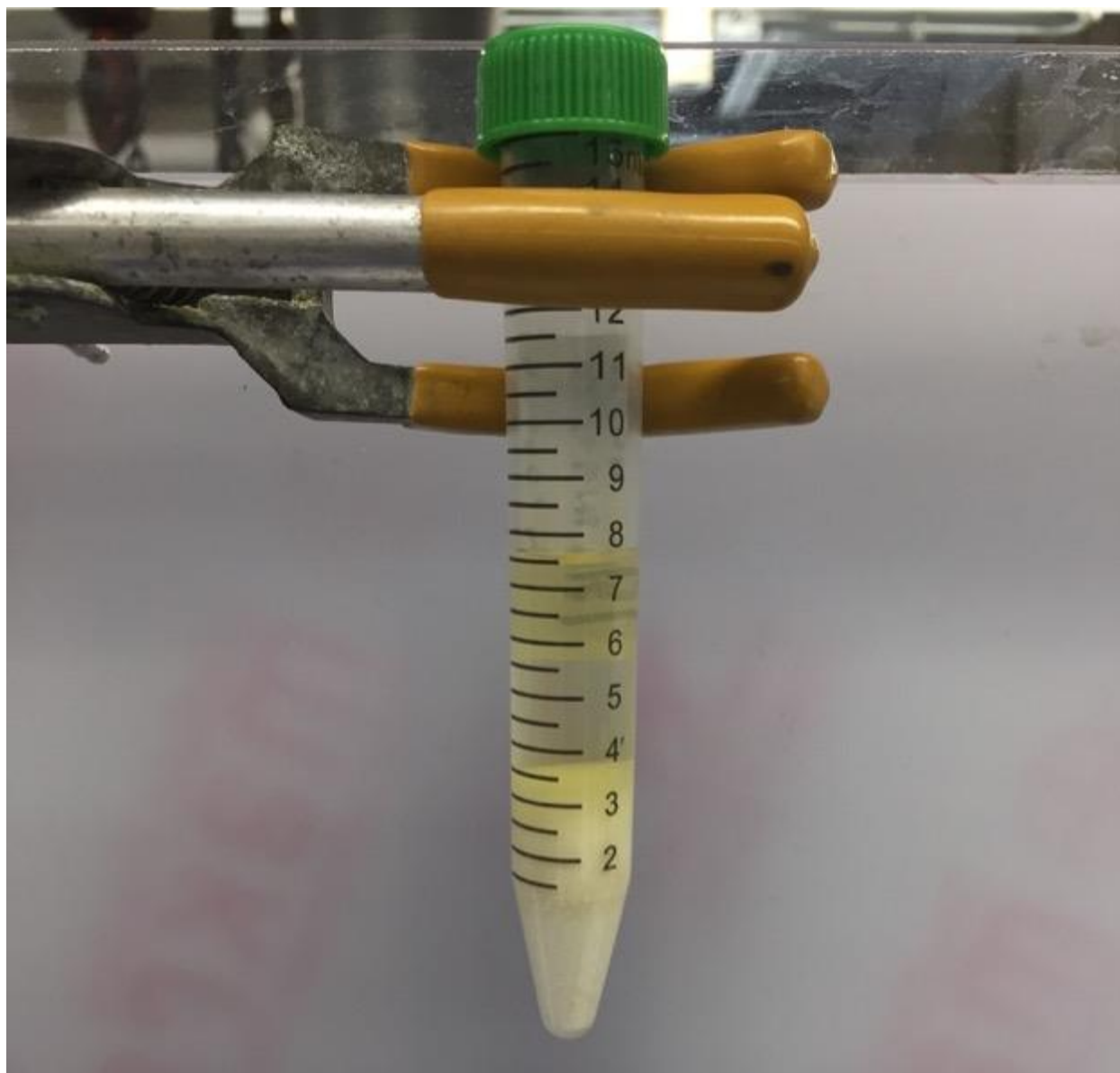


Figure 30: Eggshells, 900°, 3 hours, 1.5 g catalyst, MeOH, Oscillating Water bath (35°C), 19 hours

1 phase present, reaction considered "failed."

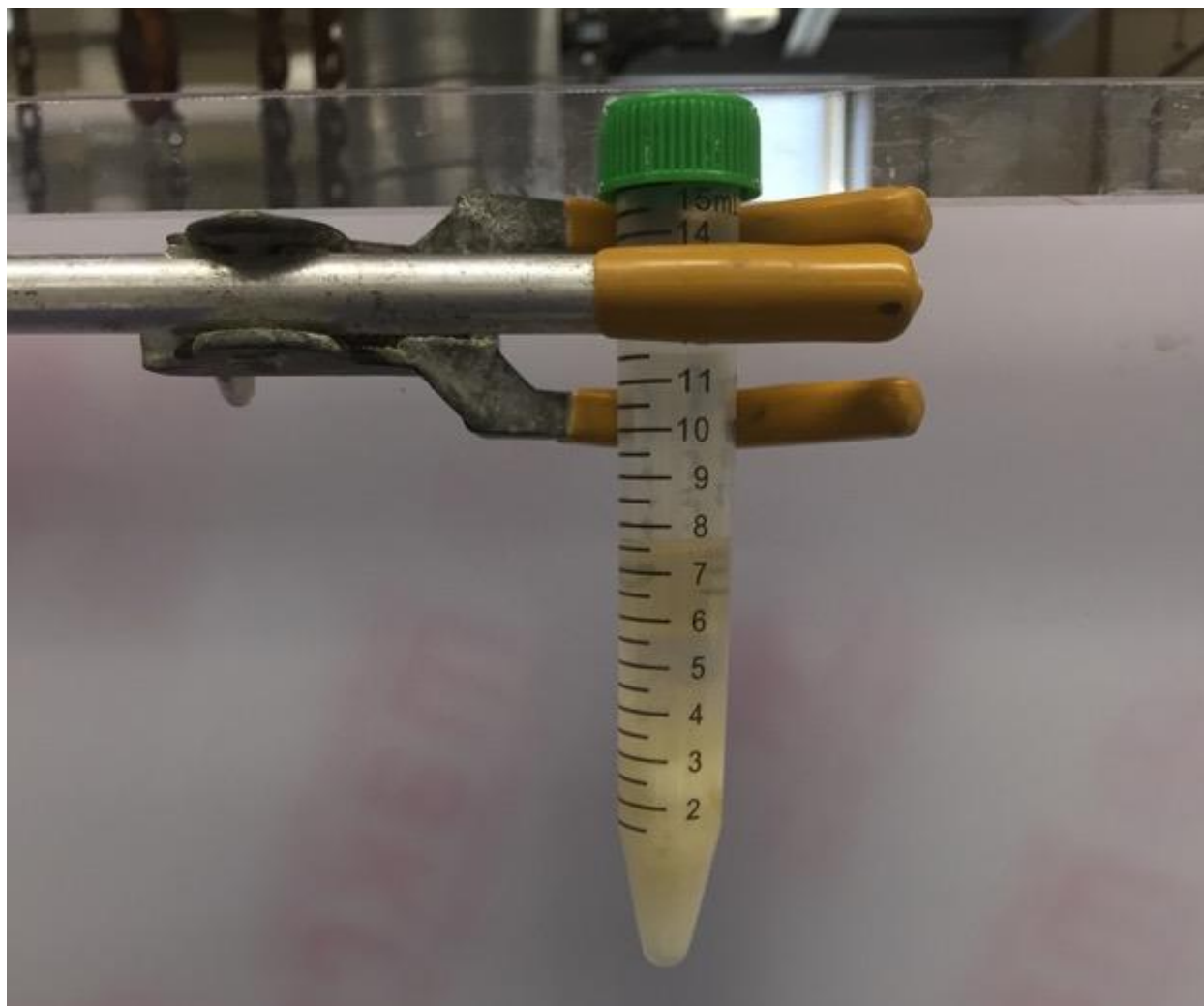


Figure 31: Eggshells, 900°C, 3 hours, 0.5 g catalyst, MeOH, Oscillating Water bath (35°C), 19 hours

1 phase present, reaction considered "failed."



Figure 32: Eggshells, Bleach, 24 hours, 1.0 g catalyst, MeOH, Stirred, 27 hours.

1 phase present, reaction considered "failed."



Figure 33: Eggshells, Peroxide, 24 hours, 1.0 g catalyst, EtOH, Stirred, 27 hours

1 phase present, reaction considered "failed."



Figure 34: Eggshells, Bleach, 24 hours, 1.0 g catalyst, EtOH, Stirred, 27 hours.

1 phase present, reaction considered "failed."



Figure 35: Eggshells, Peroxide, 24 hours, 1.0 g catalyst, MeOH, Stirred, 27 hours

1 phase present, reaction considered "failed."



Figure 36: Eggshells, 900°C, 3 hours, 1.0 g catalyst, EtOH, Stirred, 24 hours.

1 phase present, reaction considered “failed.”

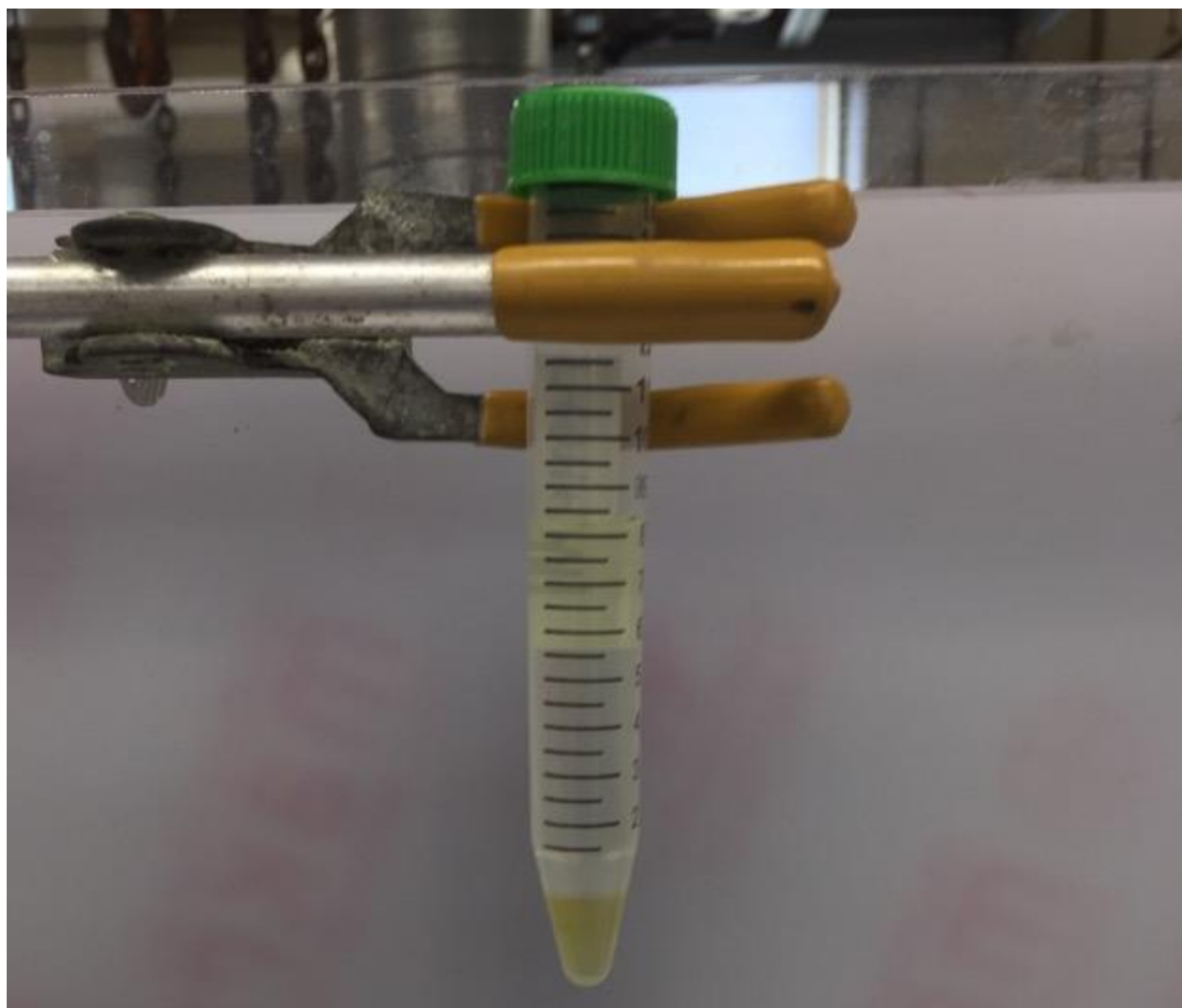


Figure 37: KOH, 1.0 g catalyst, MeOH, Stirred, 24 hours.

2 phases present, reaction considered "passed"

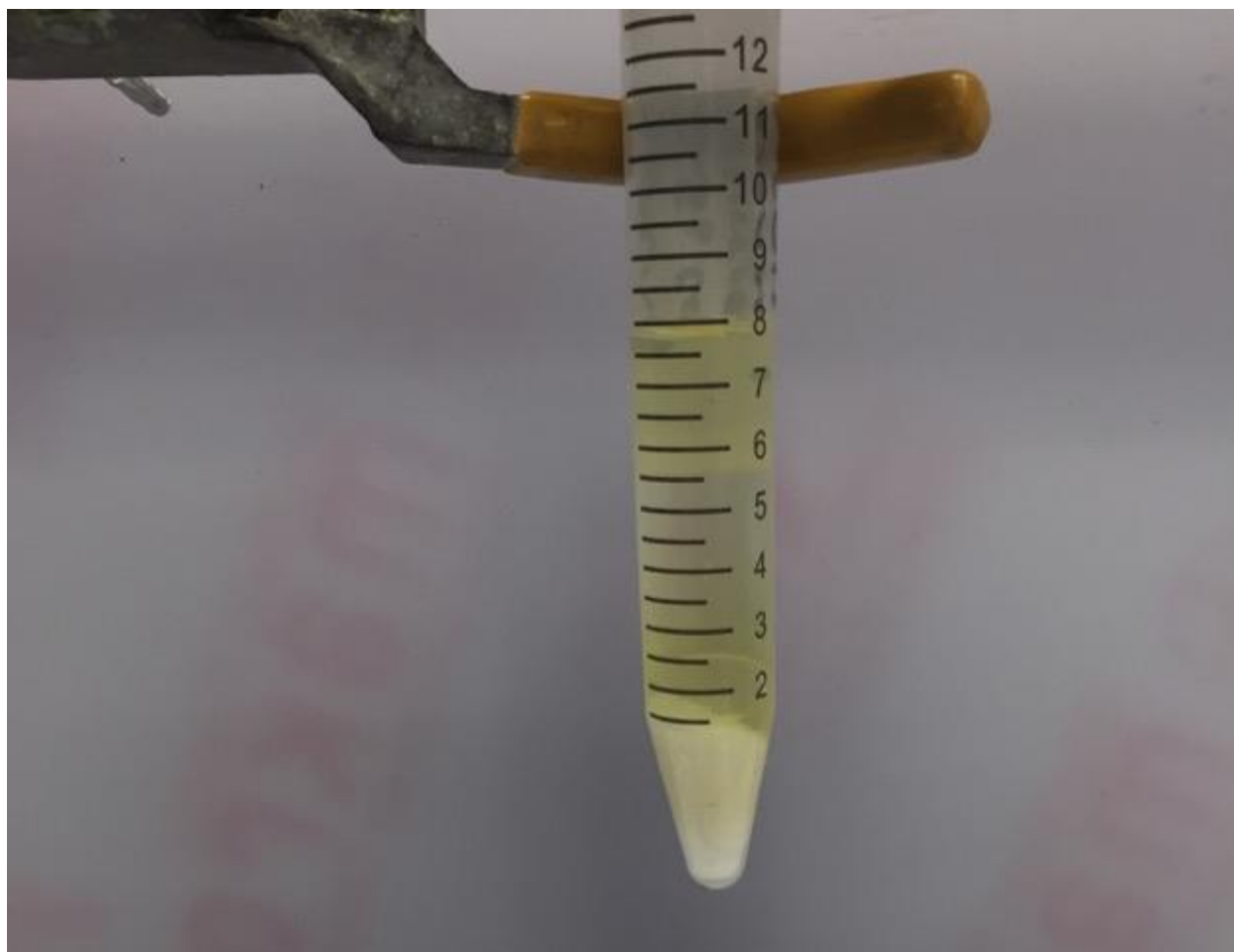


Figure 38: Eggshells, 400°C, 6 hours, 900°C, 6 hours, 0.5 g catalyst, MeOH, Stirred, 22 hours.

2 phases present, reaction considered “passed”

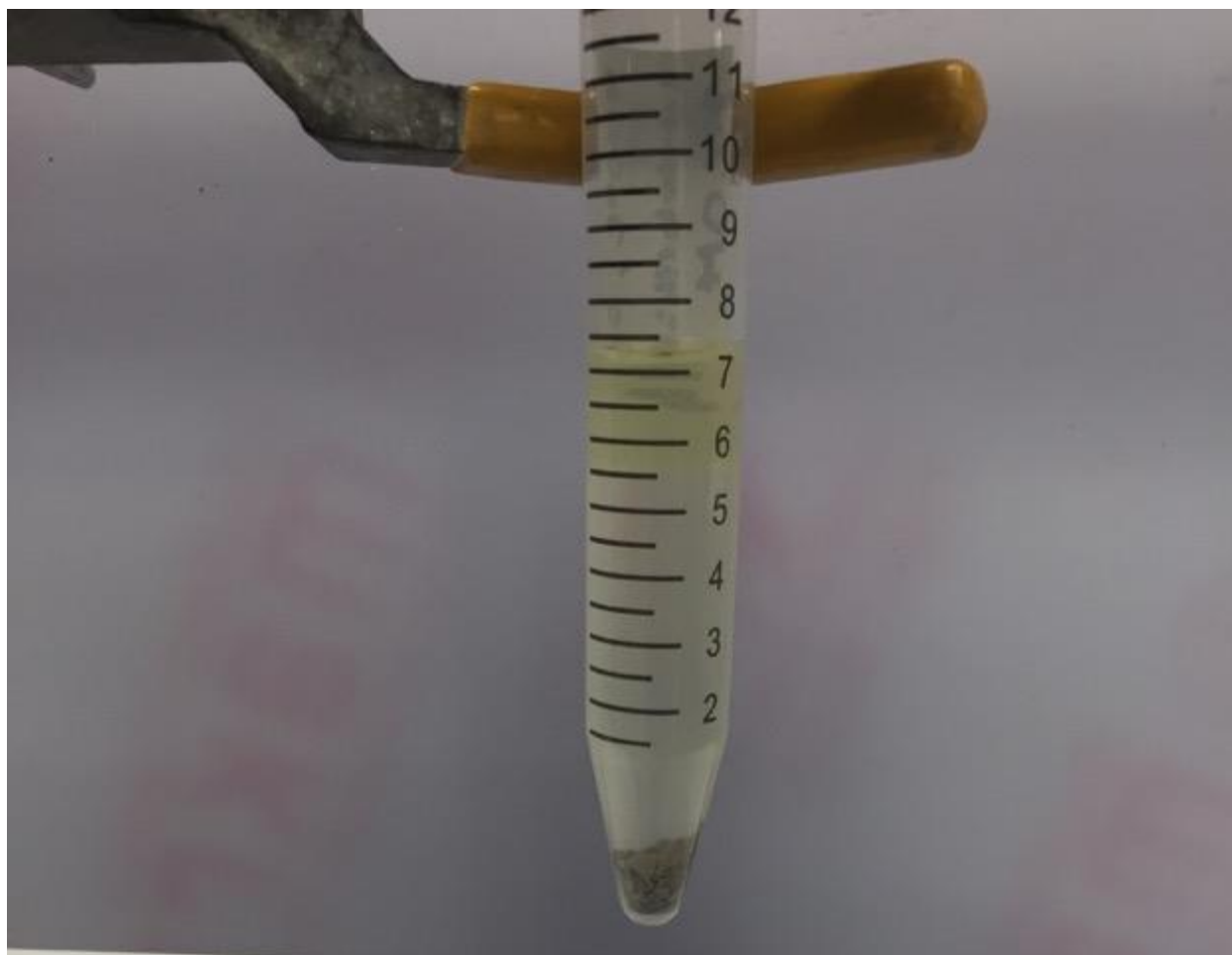


Figure 39: Eggshells, 400°C, 6 hours, 0.5 g catalyst, MeOH, Oscillating Water bath (35°C), 19 hours.

1 phase present, reaction considered "failed."



Figure 40: Eggshells, 400°C, 6 hours, 900°C, 6 hours, 1.0 g catalyst, MeOH, Stirred, 22 hours.

2 phases present, reaction considered "passed"

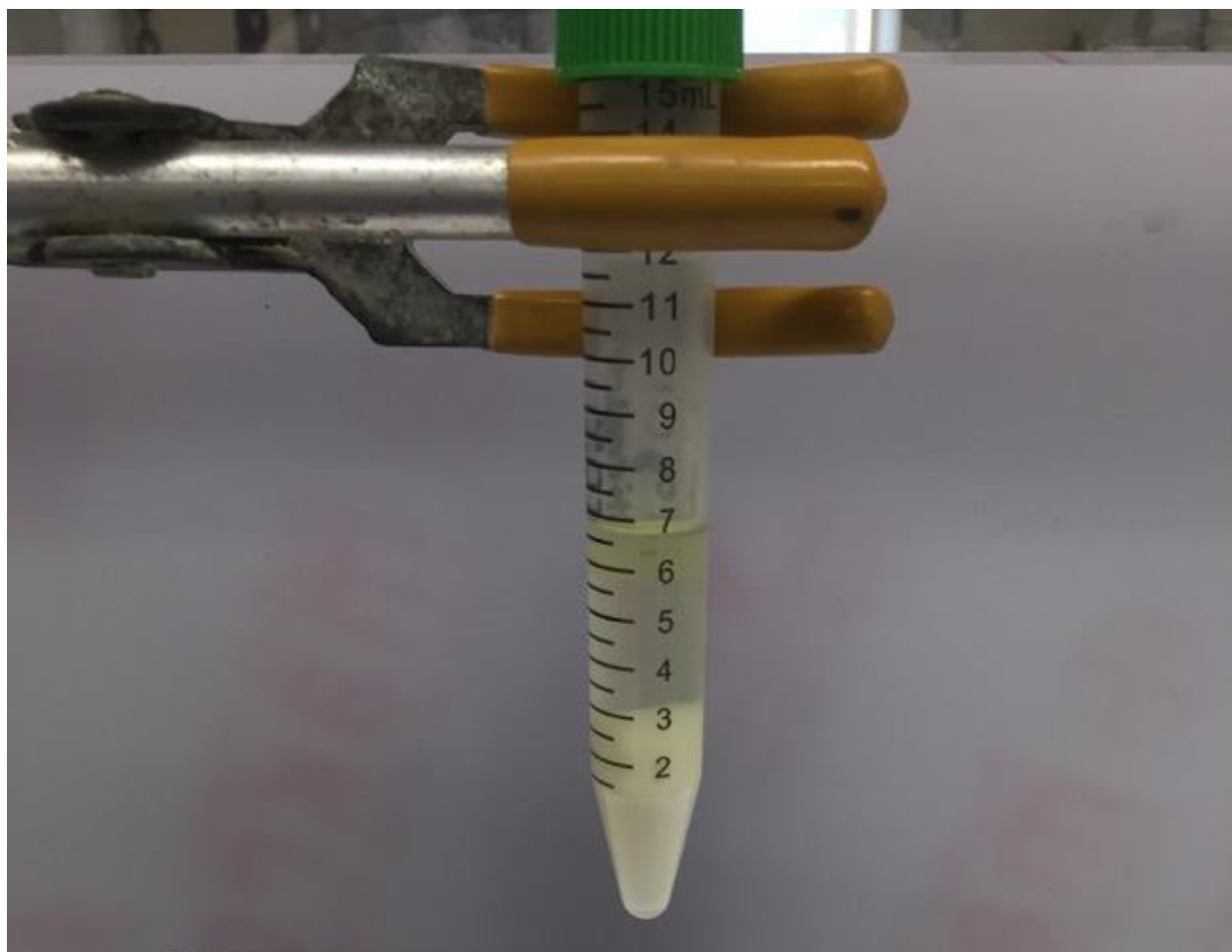


Figure 41: Eggshells, 900°C, 3 hours, 1.0 g catalyst, MeOH, Stirred, 24 hours

2 phases present, reaction considered “passed”



Figure 42: Eggshells, 400°C, 6 hours, 1.0 g catalyst, MeOH, Oscillating Water bath (35°C), 24 hour.

2 phases present, reaction considered "passed"

F. Calculations/Equations

Equation 1

Calcined Catalyst Price per Liter of Biodiesel

$$= \text{Price Uncalcined Catalyst} \left[\frac{\$}{\text{kg}} \right] * \frac{m_{\text{Catalyst oven,in}} [\text{kg}]}{m_{\text{Catalyst oven,out}} [\text{kg}]}$$

$$* \text{Catalyst per Liter Methanol} \left[\frac{\text{kg}}{\text{L}} \right] * \frac{V_{\text{MeOH,Reactant}} [\text{L}]}{V_{\text{Biodiesel,Product}} [\text{L}]}$$

Equation 2

$$\frac{V_{\text{Biodiesel}}}{\text{L MeOH}} = \frac{\rho_{\text{MeOH}}}{MW_{\text{MeOH}}} * \frac{1 \text{ mole Biodiesel}}{1 \text{ mole MeOH}} * \frac{MW_{\text{Biodiesel}}}{\rho_{\text{Biodiesel}}}$$

$$\frac{V_{\text{Biodiesel}}}{\text{L MeOH}} = \frac{792 \text{ g/L}}{32.04 \text{ g/mol}} * \frac{1 \text{ mole Biodiesel}}{1 \text{ mole MeOH}} * \frac{297.1 \frac{\text{g}}{\text{mol}}}{880 \frac{\text{g}}{\text{L}}} = 8.21 \text{ L}_{\text{Biodiesel}} / \text{L}_{\text{MeOH}}$$

Density Values from Clark, 2016

Equation 3

$$\text{Electricity Price per kg of uncalcined catalyst} = kW_{\text{Oven}} * 6\text{hr} * \frac{\$0.12}{\text{kwh}} * \frac{333\text{g}}{\text{cycle}} * 3 \frac{\text{cycle}}{\text{kg}}$$

$$\text{Electricity Price per kg of uncalcined catalyst} = 1.8\text{kW} * 6\text{hr} * \frac{\$0.12}{\text{kwh}} * \frac{333\text{g}}{\text{cycle}} * 3 \frac{\text{cycle}}{\text{kg}} = \frac{\$3.89}{\text{kg}}$$

\$0.12/kwh based on national average

Equation 4

Electricity Price per Liter of Biodiesel

$$= \text{Electricity Price per kg of uncalcined catalyst} * \frac{m_{\text{Catalyst oven,in}} [\text{kg}]}{m_{\text{Catalyst oven,out}} [\text{kg}]}$$

$$* \text{Catalyst per Liter Methanol} \left[\frac{\text{kg}}{\text{L}} \right] * \frac{V_{\text{MeOH,Reactant}} [\text{L}]}{V_{\text{Biodiesel,Product}} [\text{L}]}$$

Equation 5

Total Cost = Electricity Price per Liter of Biodiesel + Calcined Catalyst Price per Liter of Biodiesel

Equation 6

$$\% = \frac{m_{\text{Initial}} - m_{\text{Final}}}{m_{\text{Initial}}} * 100$$

Table 12: Catalyst Lost through Calcining

Catalyst	Initial Amount (g)	Final Amount (g)	Amount Lost (g)	Percentage Lost (%)
KOH	-	-	-	-
CaO	-	-	-	-
CaCO ₃	8.91	5.23	3.68	0.41
Eggshells	10.84	5.81	5.03	0.46
Clams	10.06	5.44	4.62	0.46
Seashells	15.70	8.56	7.14	0.45
Snails	13.54	7.12	6.42	0.47

G. Pass/Fails Results

Table 13: Biodiesel Reactor Pass/Fail

	Reactor	CaO	Days since calcining	CaCO ₃	Days since calcining	Clam Shells	Days since calcining	Seashells	Days since calcining	Snails Shells	Days since calcining
Run 1	Small	P	-	P	7	NP	1	NP	0	P	0
Run 2	Small	P	-	P	8	P	2	P	1	P	2
Run 3	Small	P	-	P	9	F	3	P	2	P	4
Run 4	Large	P	-	F	16	P	4	F	3	P	0
Run 5	Large	P	-	F	17	P	5	P	4	F	2
Run 6	Large	P	-	P	0	P	6	P	5	P	4
Pass%		100%		66.7%		75%		75%		83.3%	

P: Pass NP: Near Pass F: Fail

Table 14: Biodiesel Reactions

Catalyst	Reactor	Oven		Reactor		Sep. Funnel		3/27 Test	ID
		In	Out	In	Out	In	Out		
CaCO ₃	Small	1/18/2017 11:00	1/18/2017 5:00	1/25	1/26	1/27	1/27	CaCO ₃ -1	
CaCO ₃	Small	"	"	1/26	1/27	1/28	1/28	CaCO ₃ -2	
CaCO ₃	Small	"	"	1/27	1/28	1/30	1/30	CaCO ₃ -3	
CaCO ₃	Large	"	"	2/3	2/4	2/5	2/5	CaCO ₃ -4	
CaCO ₃	Large	"	"	2/4	2/5	2/6	2/6	CaCO ₃ -5	
CaCO ₃	Large	2/5/2017 15:00	2/6/2017 10:00	2/6	2/7	2/8	2/8	CaCO ₃ -6	
CaO	Small	-	-	1/30	1/31	2/1	2/1	CaO-1	
CaO	Small	-	-	1/31	2/1	2/2	2/2	CaO-2	
CaO	Small	-	-	2/1	2/2	2/3	2/3	CaO-3	
CaO	Large	-	-	1/31	2/1	2/2	2/2	CaO-4	
CaO	Large	-	-	2/1	2/2	2/3	2/3	CaO-5	
CaO	Large	-	-	2/2	2/3	2/4	2/4	CaO-6	
Clam Shells	Small	2/1/17 10:00	2/1/17 16:00	2/2	2/3	2/4	2/4	Clam-1	
Clam Shells	Small	"	"	2/3	2/4	2/5	2/5	Clam-2	
Clam Shells	Small	"	"	2/4	2/5	2/6	2/6	Clam-3	
Clam Shells	Large	"	"	2/7	2/8	2/9	2/9	Clam-4	
Clam Shells	Large	"	"	2/8	2/9	2/10	2/10	Clam-5	
Clam Shells	Large	"	"	2/9	2/10	2/13	2/13	Clam-6	
Seashells	Small	2/7/2017 9:30	2/7/2017 16:00	2/7	2/8	2/13	2/13	Sea-1	
Seashells	Small			2/8	2/9	2/13	2/13	Sea-2	
Seashells	Small			2/9	2/10	2/13	2/13	Sea-3	
Seashells	Large			2/10	2/11	2/13	2/13	Sea-4	
Seashells	Large			2/11	2/12	2/13	2/13	Sea-5	
Seashells	Large			2/12	2/13	2/14	2/15	Sea-6	
Snail Shells	Small	2/16/2017 15:00	2/17/2017 10:00	2/17	2/18	2/19	2/19	Snail-1	
Snail Shells	Small	"	"	2/19	2/20	2/21	2/21	Snail-2	
Snail Shells	Small	"	"	2/21	2/22	2/23	2/23	Snail-3	
Snail Shells	Large	"	"	2/17	2/18	2/19	2/19	Snail-4	
Snail Shells	Large	"	"	2/19	2/20	2/21	2/21	Snail-5	
Snail Shells	Large	"	"	2/21	2/22	2/23	2/23	Snail-6	

H. Methanol MSDS

Hazards Identification:

Potential Acute Health Effects: Hazardous in case of skin contact (irritant), of eye contact (irritant), of ingestion, of inhalation. Slightly hazardous in case of skin contact (permeator). Severe over-exposure can result in death.

Potential Chronic Health Effects: Slightly hazardous in case of skin contact (sensitizer). CARCINOGENIC EFFECTS: Not available.

MUTAGENIC EFFECTS: Mutagenic for mammalian somatic cells. Mutagenic for bacteria and/or yeast.

TERATOGENIC EFFECTS: Classified POSSIBLE for human.

DEVELOPMENTAL TOXICITY: Not available. The substance is toxic to eyes. The substance may be toxic to blood, kidneys, liver, brain, peripheral nervous system, upper respiratory tract, skin, central nervous system (CNS), optic nerve. Repeated or prolonged exposure to the substance can produce target organs damage. Repeated exposure to a highly toxic material may produce general deterioration of health by an accumulation in one or many human organs.

First Aid Measures:

Eye Contact: Check for and remove any contact lenses. Immediately flush eyes with running water for at least 15 minutes, keeping eyelids open. Cold water may be used. Get medical attention.

Skin Contact: In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Cover the irritated skin with an emollient. Cold water may be used. Wash clothing before reuse. Thoroughly clean shoes before reuse. Get medical attention immediately.

Serious Skin Contact: Wash with a disinfectant soap and cover the contaminated skin with an anti-bacterial cream. Seek immediate medical attention.

Inhalation: If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention immediately.

Serious Inhalation: Evacuate the victim to a safe area as soon as possible. Loosen tight clothing such as a collar, tie, belt or waistband. If breathing is difficult, administer oxygen. If the victim is not breathing, perform mouth-to-mouth resuscitation. WARNING: It may be hazardous to the person providing aid to give mouth-to-mouth resuscitation when the inhaled material is toxic, infectious or corrosive. Seek immediate medical attention.

Ingestion: If swallowed, do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Loosen tight clothing such as a collar, tie, belt or waistband. Get medical attention immediately.

Fire and Explosion Data:

Flammability of the Product: Flammable.

Auto-Ignition Temperature: 464°C (867.2°F)

Flash Points: CLOSED CUP: 12°C (53.6°F). OPEN CUP: 16°C (60.8°F).

Flammable Limits: LOWER: 6% UPPER: 36.5%

Products of Combustion: These products are carbon oxides (CO, CO₂).

Fire Hazards in Presence of Various Substances: Highly flammable in presence of open flames and sparks, of heat. Non-flammable in presence of shocks.

Explosion Hazards in Presence of Various Substances: Risks of explosion of the product in presence of mechanical impact: Not available. Explosive in presence of open flames and sparks, of heat.

Fire Fighting Media and Instructions: Flammable liquid, soluble or dispersed in water.

SMALL FIRE: Use DRY chemical powder.

LARGE FIRE: Use alcohol foam, water spray or fog.

Special Remarks on Fire Hazards: Explosive in the form of vapor when exposed to heat or flame. Vapor may travel considerable distance to source of ignition and flash back. When heated to decomposition, it emits acrid smoke and irritating fumes. CAUTION: MAY BURN WITH NEAR INVISIBLE FLAME

Special Remarks on Explosion Hazards: Forms an explosive mixture with air due to its low flash point. Explosive when mixed with Chloroform + sodium methoxide and diethyl zinc. It boils violently and explodes.

Accidental Release Measures:

Small Spill: Dilute with water and mop up, or absorb with an inert dry material and place in an appropriate waste disposal container.

Large Spill: Flammable liquid. Poisonous liquid. Keep away from heat. Keep away from sources of ignition. Stop leak if without risk. Absorb with DRY earth, sand or other non-combustible material. Do not get water

inside container. Do not touch spilled material. Use water spray to reduce vapors. Prevent entry into sewers, basements or confined areas; dike if needed. Call for assistance on disposal. Be careful that the product is not present at a concentration level above TLV. Check TLV on the MSDS and with local authorities.

Handling and Storage:

Precautions: Keep locked up. Keep away from heat. Keep away from sources of ignition. Ground all equipment containing material. Do not ingest. Do not breathe gas/fumes/ vapor/spray. Wear suitable protective clothing. In case of insufficient ventilation, wear suitable respiratory equipment. If ingested, seek medical advice immediately and show the container or the label. Avoid contact with skin and eyes. Keep away from incompatibles such as oxidizing agents, metals, acids.

Storage: Store in a segregated and approved area. Keep container in a cool, well-ventilated area. Keep container tightly closed and sealed until ready for use. Avoid all possible sources of ignition (spark or flame).