OBJECTIVE:
The objective of this experiment is to gain experience in the operation of a small and large scale chemical processing unit, to practice sampling and analysis techniques, to investigate imperfect mixing and mass transfer limitations and relate them to kinetic data.

THEORY: In this lab, we center our attention on processing raw fuel, Triglycerides, into a more refined product, Fatty-Acid- Methyl-Esters (FAME’s), which are better suited to the currently available energy conversion technologies.

![Diagram of transesterification reaction](image)

Figure 1. Simplified schematic diagram of the transesterification reaction to produce methyl ester biodiesel. The methanol is shown as methoxide, the form after deprotonation by strong base.

The reaction pictured above in Figure 1 is carried out by mixing Potassium Hydroxide, KOH, with Methanol to form the strong organic base Methoxide. Methoxide solution is added to Triglycerides with heating and stirring to complete the reaction. The reaction proceeds via the following nucleophillic substitution mechanism.
Figure 2: Diagram of the nucleophilic substitution mechanism.

All chemical reactions are theoretically reversible and this one lends a significant reality to that theory.

Figure 3: Diagram of the reverse reaction between Methyl Esters and Glycerol.

Thus, there are three linked equilibrium relationships that can be modeled. This leads to six Arrhenius dependent kinetic parameters as one would expect from reaction kinetics class. Using experimental data to determine the kinetic parameters, the following reaction model can be derived.
This model gives an idealized optimal reaction time given perfect mixing, the first assumption of the several modeling assumptions used. The trans-esterification reaction is mildly exothermic but given the fact that we are heating the reaction, an isothermal approach to the kinetics is a valid assumption. MG is monoglycerides, DG is diglycerides, TG is triglycerides, ME is methyl esters, Glycl is Glycerol. Time is in minutes, the gas constant is in cal/K mol, temperature is in Kelvins. The temperature and the final time are meant to be changed.

SAFETY PRECAUTIONS:

1) The mixing of Methanol and KOH is highly exothermic and must be carried out in a hood or specifically designated area without heating. Do not mix Methanol and KOH without supervision from a T.A. or instructor.
2) Appropriate eye protection and chemical resistant gloves are required at all times.
3) Familiarize yourself with both the small scale and the large scale apparatuses. Pay attention to pressure/temperature gauges; be careful with glassware.
4) Look up safety sheets for all chemicals that will be used, since many of them are flammable and can be hazardous to your health. Due to the solvents used to prepare samples for Gas Chromatography, sample preparation should be done inside of a chemical hood with appropriate Personal Protection Equipment worn at all times.
5) Familiarize yourself with the usage of a syringe. Do not use the Gas Chromatograph unless supervised by a faculty member or a Biodiesel Lab staff member.
6) Concentrated Hydrochloric Acid is very corrosive, use with caution.

PRELIMINARY PREPARATIONS:

Using a basis of 500 mL Oil, and 50 gallons of Oil, and given the following physical properties, determine the amount of Methanol and KOH that need to be mixed for a stoichiometric conversion for each volume of Oil. The density of Methanol and Vegetable Oil are 0.79 g/mL and 0.9 g/mL respectively. Use 885 g/mole as an average molecular weight for the Vegetable Oil. From your stoichiometric calculations, double the amount of Methanol. Be prepared to explain why this is necessary. A typical catalyst loading for this reaction is 2 wt% with respect to triglycerides. The experiment cannot be started without the T.A. or lab staff checking over these calculations, so make sure to have them ready when you get to lab.

Small Scale Reaction:

Equipment and Materials:

1. Vegetable Oil – 500 mL
2. Methanol – _______g
3. KOH – _______g
4. Scale / Balance
5. 1 L 3-neck round bottom flask
6. Ring stand
7. Plastic syringe
8. Reflux Condenser
9. 2 – 200 mL Erlenmeyer Flasks (measuring Vegetable Oil, preparing Methoxide)
10. 4 sample vessels
11. Ice Bath
12. Water Bath
13. Temperature Controlled Hot Plate
14. Magnetic Stirring Bar
15. Pipettes and Bulbs
16. 12 M HCl
17. Stopwatch

Procedure

1. Set up experimental apparatus; place the water bath on the temperature controlled hot plate, and put the 3-neck round bottom flask into the water bath. Secure the 3-neck round bottom flask using a ring stand and a clamp. Insert the thermometer, stopper, and reflux condenser into the necks of the flask.
2. Set the temperature on the hot plate to 40 °C so that the water bath will begin to warm up. Make sure the thermocouple is placed in the water bath.
3. Add Vegetable Oil and a magnetic stirring bar into the 3-neck round bottom flask. Set the stirring to 700 rpm and allow for the Vegetable Oil to heat up to temperature.

4. Place 4 sample vessels into an ice bath and allow the vessels to cool; Have 12 M HCl with pipettes and bulbs ready next to the ice bath.

5. Measure the correct amounts of Methanol and KOH; mix to make the Methoxide solution. This reaction is highly exothermic. When mixing, first measure the appropriate amount of Methanol, and then add the KOH. DO NOT POUR METHANOL ON TOP OF KOH PELLETS.

6. When the KOH has fully dissolved, and Vegetable Oil is at 40 °C (turn stir bar off and check with a thermometer), add the Methoxide into the 3-neck round bottom flask and start the stop watch.

7. Using the plastic syringe, take AT LEAST 4–10 mL timed samples during the reaction at the following time intervals; 4,15,60,120 minutes. More samples can be taken for a more complete kinetics study, but make sure to keep G.C. analysis time in mind.

8. Inject samples into sample vessels in the ice bath. Immediately upon injecting the sample, add 3 drops of 12 M HCl using the pipette and bulb. Give the sample vessel a little shake and allow it to sit in the ice bath until the end of the reaction.

9. At the end of the 90-minute reaction period, take your last sample, quench it and place on ice. Then, shut down the hot plate, condenser, and stir bar. Allow your reaction mixture to cool; it is added to the large scale reaction mixture after the large scale oil has undergone its first pass through the reactor.

10. Make sure to record the mass and volume of each phase.

**Large Scale Reaction**

**Equipment and Materials:**

1. Vegetable Oil – 50 gallons
2. Methanol – ______ Liters
3. KOH – ______ kg
4. Scale / Balance
5. Water Heater (large batch reactor)
6. Mixing Tank (methoxide reactor)
7. 55-gallon drum of methanol
8. positive displacement 55-gallon drum pump
9. 2 centrifugal pumps
10. 5 sample vessels
11. Ice Bath
12. Pipettes and Bulbs
13. 12 M HCl
14. Stopwatch
15. valves and hosing
16. large beaker or jar to transport KOH
17. 55-gallon drum for Hazardous waste.
18. Temperature Gauge
19. Pressure Gauge

Procedure

1st Lab Period

For the large scale experiment the large batch reactor (water heater) is utilized. The basis for your calculations is 50 gallons of used Vegetable Oil from various UCONN and Pratt & Whitney food establishments. The Methoxide is mixed in the designated area beside the water heater (Methoxide reactor), and has to be done under the supervision of a T.A. or Biodiesel Lab Staff.

1. A 55-gallon drum of Methanol is in front of the experimental apparatus. The appropriate amount of Methanol (check with the T.A.) is first added to the Methoxide reactor before addition of any KOH. One student pumps the Methanol with the manually operated drum pump, and the other student holds the hose in place. A large lab coat should be worn along with protective goggles and neoprene gloves.
2. Once the Methanol is added to the Methoxide reactor, the impeller can be plugged-in.
3. Potassium Hydroxide is weighed out and added into the reactor. Potassium Hydroxide is added in increments no larger than 250 grams every 30 seconds, and is done by the T.A. or Biodiesel Lab Staff.
4. Once all of the Potassium Hydroxide is added to the reactor, allow mixing for 20-30 minutes before beginning the main reaction.
5. Mixing in the large reactor is accomplished only via the circulating pump (what is the flowrate?), and the valves must be configured accordingly to allow this. Once the Oil has reached 120°F, Methoxide is added to the reactor when the circulating pump is on by closing the valve from the reactor to the pump and simultaneously opening the valve from the Methoxide reactor to the pump. While Methoxide is being pumped into the reactor, the vent on top of the water heater should be open.
6. The stopwatch is started at this point, and samples are to be taken in line with the small scale experiment, at 4,15,60,120 minutes AT LEAST. The samples are taken by slowly opening a sampling valve on the top of the reactor. Samples are quenched with HCl and put on ice.
7. At the end of the reaction turn the heating element off by turning off the switch next to the water heater. Open the vent and pump the contents of the reactor over to the large separatory funnel by opening and closing the appropriate valves. Phase separation occurs over the next ten minutes.
8. The waste phase is transferred to the appropriately labeled 55 gallon drum beside the spill palettes by placing the exit hose into the drum and opening the valve on the bottom of the separatory funnel. Be careful not to lose any biodiesel. The centrifugal pump is used for this transfer.
9. Once the waste is successfully removed and the mixture from the small scale reaction is added to the large batch, the total volume can be sent back to the water heater for a
second pass. Due to the equilibrium nature of the reaction, the reaction mixture is probably only 75 – 85 % converted based on the recipe utilized. To attain biodiesel fuel of ASTM quality, the reaction mixture is processed a second time with stoichiometric KOH and Methanol via the same procedure.

10. The second reaction runs for sixty minutes. After completion, a fifth sample should be taken, quenched and placed on ice.

2nd Lab Period

1. Water is used as a stripping agent in the cone tank to remove any water-soluble components (what are they?) from the Methyl-Ester product. Using the sprinkler and water from the sink, add an equivalent volume of water to the product. The water looks like white rain falling through the Ester layer.

2. Once enough water is added, it is pumped or gravity drained off into the sink taking care not to pump Methyl-Ester product out of the cone tank. This process is repeated until the water in the tube is clean enough to see through.

3. At this point the fuel is clean, but wet. Use the same sprinkler and compressed air to cause air to bubble through the Methyl-Ester product to dry the fuel. This takes 1-2 days to complete. The more bulk water removed prior to initiating the air, the sooner the fuel will be dry enough for use.

As your fuel dries, a few outcomes are possible. Good starting material yields good clean fuel. Fuel with high fatty acid content precipitates fatty acids as the emulsion is broken by drying the fuel. This precipitate must be removed prior to use as it will clog fuel filters. The second possible complication is a raised cloud point which indicates the possibility of solidifying in its holding tank. This is caused if the oil was thermally abused in the cooking process and is fully saturated rather that partially unsaturated. Both phenomena have been observed here with university oil.

ANALYSIS:

I. Gas Chromatography:

Do not run or prepare any samples for G.C. without supervision or approval by a Biodiesel Lab Staff Member.

Review ASTM Test Method Sections 10 through 12 (procedure, calculation and report, and precision and bias). The copyright on this document prevents the lab from being able to provide copies to students. However, it is recommended that every student come in prior to the start of lab to review the analysis method packet.

Also, review the procedures attached prepared by Robert Fusco and J.D. Stuart.

How to Prepare G.C. Samples

Materials:
1. 8 – 10 mL Volumetric Flasks
2. 100 uL syringe
3. 500 uL syringe
4. Tricaprin Stock Solution (Int Std #2)
5. Butanetriol Stock Solution (Int Std #1)
6. MSTFA; Derivatising Agent
7. Heptane
8. Balance Scale
9. Pipettes and Bulbs
10. Stopwatch

G.C. Sample Preparation Procedure

1. Thirty minutes before preparing the biodiesel samples, make sure to remove internal standard #1 (butanetriol), internal standard #2 (tricaprin), the derivatizing agent (MSTFA), and standard solution #5 from the refrigerator to allow them to come to room temperature.
2. Add sodium sulfate to each of the centrifuge tubes equal to an eighth of the total sample volume. Shake gently and use the centrifuge to help the phases separate. This removes any water that might be in the sample.
3. Using forceps, move a clean 10mL volumetric flask, unstoppered, to the balance. Shut the side door, and proceed to tare (zero) the balance.
4. Using a Pasteur pipette, transfer as close to 0.1 grams of the top oil phase of the sample to the volumetric flask. (This is equal to about seven drops of oil.)
5. Record the weight of the sample to 0.0001 grams, remove the flask from the balance, and label the volumetric flask with the sample name, your initials, date, and the weight of the sample.

The following steps should be carried out in a hood.

6. Using the blue-barreled, white-taped, 100µL-syringe carefully add 100µL of butanetriol (internal standard #1) to the volumetric flask. First, clean the appropriate syringe three times with heptane. Continue cleaning by drawing a small amount of butanetriol into the syringe and then pulling the plunger up to 100µL, thereby cleaning the inside entirely. Then, draw 100µL of butanetriol into the syringe and carefully deliver the full 100 µl of the butanetriol (internal standard #1) to the volumetric flask. When finished, clean the syringe with heptane three times and return it to its proper location.
7. Follow the same procedure for adding 100µL of tricaprin as in step #5, using the second blue-barreled, yellow-taped, syringe.
8. Make sure to record the concentration (mg/mL) and date of preparation for both butanetriol and tricaprin which is located on each amber vial.
9. Follow the same procedure for adding 100µL of MSTFA, but note that the syringe holds a larger volume. (MSTFA is a highly toxic effective derivatizing agent added to react with all free hydroxyl groups, i.e., in the glycerol, and the various mono- and di-glycerides so that they are more volatile and can be better gas chromatographed.)

10. Cap the volumetric flask and let it sit for 20 minutes.

11. At the end of the wait time, carefully add heptane up to the 10mL graduation.

12. The sample is now ready to analyze with the G.C.

**G.C. Injection and Analysis Procedure**

The G.C. should already be powered on when you arrive in the lab. **ONLY BIODIESEL LAB STAFF MEMBERS ARE ALLOWED TO POWER ON, POWER OFF, OR TOUCH ANY OF THE LARGE COMPRESSED AIR TANKS ATTACHED TO THE G.C. ANY STUDENT CAUGHT TOUCHING ANY OF THE COMPRESSED AIR TANKS OR ATTEMPTING TO POWER ON OR OFF THE G.C. WILL AUTOMATICALLY FAIL THE LAB AND BE ASKED TO LEAVE. THE G.C. IS USED FOR ASTM QUALITY TESTING OUTSIDE OF THE UNDERGRADUATE LAB AND IS VERY SENSITIVE. IF A PROBLEM OCCURS AT ANY STAGE OF YOUR ANALYSIS, FIND A STAFF MEMBER, T.A., OR PROFESSOR ANDERSON.**

1. ChemStation software is the software that is interfaced with the G.C. It should be open when you arrive in the lab. Click on “View” and scroll down to “Method and Run Control.” This will start up a new file page for you. Now, click on “Run Control” and scroll down to “Sample Info.” Increment the file number, give the file a new name, and add any additional comments.

2. After clicking “Run Method” the screen should say “Run in progress, waiting for injection.”

3. At this point you may inject the sample. To inject sample into the G.C. first mix your sample in the 10 mL volumetric flask by inverting the flask several times. Rinse out the 1µl syringe located on the top of the G.C. with Heptane several times to ensure it is clean of residue. Next, fill the syringe several times with your sample to get a uniform sample in the syringe. Once the syringe is filled beyond the 1µl graduation, inspect the syringe to ensure there are no gas bubbles in the barrel of the syringe. Carefully advance the plunger until it reaches the 1µl graduation then wipe the tip on a kimwipe so no external sample can enter the G.C.. Carefully guide the needle through the septum and quickly inject the sample while simultaneously pushing the start button on the G.C.. Withdraw the needle and rinse it with Heptane several times to prepare it for the next use.

4. Each run takes 31.5 minutes to complete. When the run is done, click on “View” and scroll down to “Data Analysis.”
5. Click the printer button in the top right corner to print the entire report. To print a larger copy of the chromatograph go to “File”, “Print”, “Selected Window.”

6. The G.C. will now have a red light on letting know that it is cooling down and is not yet ready. When the injector temperatures and the oven temperatures cool down the red light will turn off and the green light will come on the computer screen. This process takes about 10 minutes. The G.C. will then be ready to start a new file.

7. From the chromatograph printout the areas of peaks that fall within certain residence time windows are added to come up with the total peak area for that compound. Five calibration plots have been prepared using known amounts of prototype molecules and are available in the biodiesel lab. Based on calibrations done by the Biodiesel Staff, a list of retention times has also been developed and is available in the lab.

8. Using the printout from the G.C. the areas are summed up for the peaks in the appropriate time ranges. There is a small amount of latitude that can be applied to the interpretation of the peaks. The standards should be large single peaks and should be easily identifiable. There can be slight variation however in the timing of the peaks. Should you see the standards appearing 0.1 minutes earlier or later, you should adjust your other ranges accordingly. Once you have the totals for the 5 types of molecules, Glycerol, Monoglycerides, Diglycerides, Triglycerides, and the Methyl Esters, you can compare the ratio of the peak area of the compound against the internal standard it is calibrated against. The 5 calibration plots are available for you to reference in the biodiesel lab. The X and Y axis’ are ratios.

9. By the time you have analyzed your data, you will have 3 of the 4 pieces of information that comprise the 2 ratios that are plotted. When the evaluation of the 5 molecules is complete, perform a mass balance to validate your interpretation of the G.C. output.

   Possible questions for this portion of the lab could include what could lead to a low mass balance, what could lead to a high mass balance, and why do some compounds have single residence times while others have ranges? If your sample had Methanol or Water in it what would be your reported conversion compared to the actual conversion? How could you figure this out? Why is the area of the Glycerol peak different compared to what one would expect based in a kinetic model? How would free fatty acids that remained in the ester layer affect the G.C. results? We usually run a pure Heptane sample when the G.C. is first turned on. If you see peaks above the baseline what does this tell you about the status of the column?

II. Cloud Point:

   The second ASTM test you perform is a cloud point test. The cloud point is the temperature where crystals first begin to form. The significance of this point is that Diesel engines have fuel filters and fuel injection systems that operate with pressures as
The presence of crystals in the fuel system would be detrimental in both situations. The cloud point tester employs an optical sensor that measures light transmittance through the fluid. As crystals form, some of the light beam is diffracted, decreasing the signal. To operate the cloud point tester, turn it on and follow the on-screen prompt for the self test. Once the internal test is passed, place the cup under the overflow tube and fill the provided syringe with clean and dry sample. Ensure that there are no bubbles in the syringe and inject 10 mL into the port on the top of the tester. Press the start button, and the tester will decrease the temperature until crystals begin to form. Testing time can take a few minutes for good clean fuel to 15 minutes for samples with contaminants.

Questions for this portion of the lab include how does your cloud point compare to what is reported in the literature? How does the cloud point of Biodiesel compare to that of Petroleum derived Diesel? Do you see any problems with the use of Biodiesel based on the information you found? Is Biodiesel a homogeneous or heterogeneous compound? To increase the cloud point, which way would you want to move towards homogeneity or heterogeneity? What if you wanted to decrease the cloud point? What effect does the degree of saturation in the Methyl-Esters have on the cloud point? How does this information affect your choice for starting materials?

Items for Discussion:
1. You must discuss the mass balance around the each reactor. Make sure you take enough data during the lab to allow you to attempt this. Clearly identify any assumptions or errors in this calculation.
2. Is reactor conversion controlled by equilibrium, kinetics, or mass transfer? How do you know? Could the experiment be better designed to allow these effects to be analyzed separately?

Additional Questions for contemplation: What type of unit operation is the washing step?
Why does air dry the fuel?
Would the fuel dry without compressed air?
Describe the mass transfer that is occurring in the reaction, the washing, and the drying operations?
How does mass transfer affect the overall kinetics of the reaction?
Is the final equilibrium conversion a function of mass transfer?
Explain in terms of Gibbs energy why we see two phases.
What is the catalyst being used in the experiment?
How does the presence of a catalyst affect the conversion?
How does the presence of a catalyst affect the kinetics?
What part of Methoxide, Methanol or KOH, determines the equilibrium conversion?
What part of Methoxide, Methanol or KOH, is responsible for its catalytic effect?
To make this process commercially viable you would need to recover excess Methanol from the product and discarded phases, how can you do this?
To make this process commercially viable you would need to make this a continuous process. How could this be done?
Can reaction and separation occur in the same unit operation? Could this be advantageous?

This reaction can be run with ethanol but it must be 99% anhydrous. Ethanol has an azeotrope with water at 95%. What is an azeotrope? How can we obtain 99% pure Ethanol?

In the analytical section of the Senior Lab Biodiesel experience, you are introduced to gas chromatography as an analytical tool. Using the species concentration vs. time data, graphs like the one shown below, and the differential equations seen in the Polymath model, how can the kinetic parameters be determined?

**Figure 5:** Typical graph of the mass fraction of Methyl-Esters, Triglycerides, Diglycerides, and Monoglycerides as a function of time.

**REPORT:**
Using your cloud point and G.C. results, conversion, yield, selectivity and a mass balance can be completed. Describe the design of your experiment and your results (including a discussion of their precision and accuracy). Provide thoughtful and quantitative discussion of results, explain trends using physical principles and relate your experimental results to accepted empirical values from literature or predicted from theory. Calculate kinetic parameters as well as mass-transfer parameters wherever possible. Express any discrepancies between observed and expected results in terms of quantified experimental uncertainties or limitations in published values or theory. You may find it useful to research scientific publications.
REFERENCES: