**Biodiesel production protocol[[1]](#footnote-1)**

**Background**

Alcohol

A variety of alcohols can be used, ranging from 1-4 carbons. Water content should be less than 0.08% to maximize rates and quality of transesterification. Although it is toxic and highly flammable, methanol is most commonly used because: 1) it is less expensive ($0.61/gallon vs. $1.45 for ethanol); and 2) it is easier to recover after transesterification because it distills more easily from water than does ethanol. Ethanol and water form an ‘azotrope’ that complicates separation by distillation. While the transesterification reaction requires a 3:1 molar ratio of alcohol:triacylglycerol, a higher molar ratio (often 6:1) is often used to drive the reaction forward. The excess methanol is later recovered to prevent fouling of the biodiesel and because it can be used again to reduce costs.

Base

Either sodium or potassium hydroxide can be used as the transesterification base, aka catalyst. Both are hygroscopic (they absorb water) so they must be stored to prevent contact with water. *[If the feedstock oil contains significant amounts of free fatty acids it may be better to use an acid catalyst in place of the base. For these materials, transesterification can be done in a two-step process: acid catalyzed conversion of FFAs followed by base catalyzed conversion of TAGs.]*

**Chemistry**

1. An acid-base reaction between methanol and sodium hydroxide to form sodium methoxide.
2. Nucleophilic attack by methoxide on each of triacylglycerol’s three carbonyl carbons to produce unstable tetrahydral intermediates. TAG is ‘cracked’ and the glycerol backbone is eliminated.
3. The products are glycerol and three fatty acid methyl esters (FAME), aka biodiesel.

If too much sodium hydroxide is used the glycerol will be converted into soap that may take on a jelly-like appearance.

**Materials**

pure virgin vegetable oil beakers test tubes

UFO (used fryer oil) Erlenmeyer flasks centrifuge

sodium or potassium hydroxide graduated cylinders ring-stand

anhydrous methanol burets pH paper

isopropanol (2-propanol) pipettes & rollers balance

phenolphthalein or phenol red thermometers funnel

acetic acid heating stir-plate & bars coarse filter paper

distilled water separatory funnel

**Protocols**

Lab 1: Drying and titration of oil or UFO

1. Filter the UFO through a coarse paper filter to remove any particulate matter.
2. Heat the filtered UFO to 70°C for several hours to cause any water and additional food particles (aka ‘crumb’) settle to the bottom of the container. Let the oil cool and then decant the clean oil from the upper part of the container, leaving residual water and crumb behind.

Lab 2: Titration to determine free fatty acid concentration

1. Prepare the titrating base (aka the titrant): 0.0100 M NaOH. (Dissolve 0.40 g of NaOH in 1 L of distilled water.)
2. Fill a titration buret with the hydroxide titrant.
3. Use a graduated cylinder to measure out 20 mL of pure isopropyl alcohol and transfer it to a 50-mL flask.
4. Add 2-3 drops of phenolphthalein (or phenol red) to the alcohol & swirl to mix.
5. Use a pipette to add 1 mL of filtered and ‘dried’ UFO to the flask. Swirl thoroughly to mix the oil and alcohol. The mixture should transform from distinct ‘bubbles’ to a milky solution.
6. Place the oil/alcohol sample below the buret of hydroxide solution. Record the initial volume of the buret and then titrate slowly until the color turns a very light pink and persists. Record the final volume of hydroxide solution.
7. Repeat for a total of three titrations for each sample of UFO.
8. Calculate the amount of hydroxide solution used to titrate the sample: T = Vo – Vf. Calculate the mean value of T. The value of T (in mL) is equal to the number of grams of *additional* hydroxide that will be needed in the transesterification reaction.
9. Calculate volumes of oil, methanol and base for the transesterification reaction:  
   \_\_\_ L of UFO or oil feedstock  
   \_\_\_ mL of methanol = (0.2)(L of oil)(1000)  
   \_\_\_ base: g KOH = (5.0 + T)(L of oil) or g NaOH = (3.5 + T)(L of oil)

Lab 3: Transesterification reaction [micro-scaled for 100 mL of oil]

1. Place 20 mL of pure, anhydrous methanol (MeOH) into a 250-mL Erlenmeyer flask containing a magnetic stir bar.
2. Add 0.35 g of finely ground anhydrous NaOH and stir vigorously until all of the NaOH is dissolved. The reaction is exothermic – it produces heat – and continuous stirring dissipates the heat. The reaction forms sodium methoxide, an extremely powerful base that must be handled with care! Avoid splashing!
3. In a 250-mL beaker, warm 100 mL of pure vegetable oil to about 40°C in order to increase the rate of the transesterification reaction.
4. Pour 100 mL of the warm oil into the methoxide solution while stirring continuously. The mixture should appear cloudy but soon separate into two layers. Stir for 15 – 30 minutes at a fairly high speed.
5. Stop stirring and pour the reaction into a separatory flask sitting in a ring-stand. The mixture of glycerol and biodiesel should phase separate with an hour or two. Seal the flask and sit in in a beaker until the next lab.

Lab 4: Separation of biodiesel and glycerol

1. Two layers should have formed in the separatory flask: 1) glycerol on the bottom; and 2) biodiesel on top.
2. Sit the separatory flak into a ring-stand. Open the stopcock and drain the glycerol layer into a beaker. Be sure not to contaminate the glycerol with the interface layer.
3. Use a large pipette to transfer the upper biodiesel layer to a small clean beaker. Be sure to leave the interface layer in the separatory funnel.
4. Drain the interface layer into a third beaker.

Alternate rapid but small-scale separation & washing technique

1. Fill two centrifuge tubes with completed transesterification reaction mixture.
2. Balance the masses of pairs of tubes using a pan balance.
3. Centrifuge for 5 minutes to separate the biodiesel and glycerol layers.
4. Use a pipette to harvest biodiesel from the top of the tubes and transfer it to clean centrifuge tubes. [Harvest the glycerol as well, leaving the interface layer behind and transferring the glycerol to clean tubes.
5. Extract with acid by adding 1 mL of 0.1 M acetic acid to each nearly full tube of biodiesel. Cap the tube and invert it gently five times to mix the acid and biodiesel.
6. Centrifuge for five minutes, harvest the upper, biodiesel layer and move it to a clean centrifuge tube.
7. Extract with water by adding 1 mL of water to each nearly full tube of biodiesel. Cap the tube and invert it gently five times.
8. Centrifuge for 5 minutes. Harvest the upper, biodiesel layer and move it to a clean and pre-weighed bottle of flask.
9. Heat the biodiesel at 70 – 80°C for 10-15 minutes to evaporate any residual water.
10. Weigh the flask and calculate the mass of the dried biodiesel.

Lab 5: Biodiesel testing

* Determine pH of the biodiesel:
  + Add 5 drops of biodiesel to 1 mL of water.
  + Mix thoroughly.
  + Use pH paper to determine the pH of the biodiesel.
* Combustion test
  + Roll a bit of cotton wool around the end of a glass spatula or rod to form a ‘torch’.
  + Dip the torch into the biodiesel.
  + Stand the torch up in a test tube or beaker.
  + Light it with a match and make observations.
* Freezing point
  + Place 3 mL of biodiesel in a test tube.
  + Place the test tube in a freezer for 15-20 minutes.
  + Does the sample gel?
  + Quickly place a thermometer into the biodiesel gel and stir slowly.
  + Record the temperature at which the biodiesel melts back to a liquid.
* Viscosity
* Heat content

1. Adapted from Waste vegetable oil & titrations: teacher manual, Loyola University of Chicago www.luc.edu/media/lucedu/sustainability-new/pdfs-biodiesel/Biodiesel%20Curricula%20-%20Waste%20Oil%20Biodiesel.pdf] and Wen et al.’s Making your own biodiesel [https://biology.mit.edu/sites/default/files/Make\_Biodiesel.pdf] and Green chemistry in the curriculum: biodiesel module, Fisher Science Education. [↑](#footnote-ref-1)