

UC BERKELEY COLLEGE OF CHEMISTRY

CHEMISTRY 4AL

GENERAL CHEMISTRY AND QUANTITATIVE ANALYSIS

Synthesis and Characterization of Biofuels

Author:

Jonathan MELVILLE

Lab Partners:

Giulio ZHOU and Kevin WU

November 27, 2012

1 Abstract

In this lab, we had multiple goals which we reached using a variety of experiments and analysis techniques. We wanted to investigate the synthesis, ecotoxicity, and other characterizations of various biofuels. We synthesized both biodiesel and ethanol; biodiesel by transesterifying waste cooking oil to combine triglyceride groups with alcohols, and ethanol by fermenting and distilling white grape juice. We measured the ecotoxicities of these biofuels, along with 2-butanol and methanol, by analyzing the effects of the biofuels on radish seed germination with respect to concentration. Finally, we analyzed our biofuels by determining their densities, viscosities, and (perhaps most importantly) their heats of combustion. We found that biodiesel was the least toxic of all the substances, and also had the highest heating value, making a strong case for its use as a biofuel.

2 Introduction

The first portion of our lab consisted of the synthesis of ethanol and biodiesel fuels. We synthesized biodiesel using a reaction of triglycerides and alcohol, once with an acid catalyst and once with an alkaline catalyst (Figure 1), a process known as *transesterification*. In our lab, we utilized waste

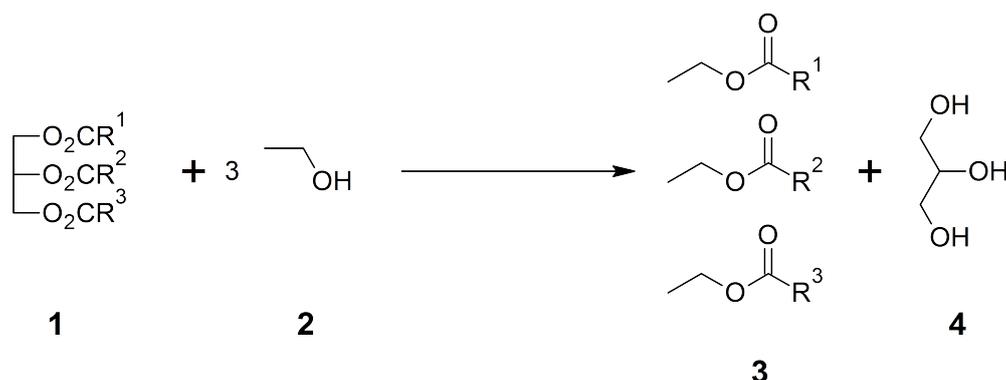


Figure 1: Transesterification. Triglyceride + 3 alcohol \rightarrow 3 esters (biodiesel) + glycerol

cooking oil from Crossroads Dining for our triglycerides, and used both methanol (catalyzed basically by NaOH) and propanol (catalyzed acidically by H_2SO_4) as our alcohols. After synthesis, we used NaCl and MgSO_4 samples to remove water from the sample, and subsequently evaporated off the excess organic solvents to isolate the biodiesel. The major advantage to using this process was that it is a comparatively easy way to synthesize biofuels using fairly common ingredients.

Ethanol, on the other hand, was synthesized in an extremely different manner. By applying yeast's ability to convert raw sugars into ethanol and carbon dioxide in an anaerobic environment (also known as brewing or *fermentation*), we were able to create a solution containing a relatively high percentage of ethanol (approximately 20 proof, where yeast begins to die out). The process by which yeast turns common table sugar (sucrose) into ethanol is detailed in Figure 2. This process is incredibly easy to operate, and in fact has been used by man for millenia. However, it is not by itself a suitable process for producing high-purity ethanol; the resulting mixture contains mostly water, with a small fraction ethanol and large amounts of grape juice and yeast impurities.

To isolate the ethanol to a much higher-purity state, filtration followed by *distillation* (Figure 3) of the filtrate is our process of choice, heating the mixture at a temperature of about 80°C such that only 95% pure ethanol vapors boil off and are condensed off in a separate container. Distillation is an

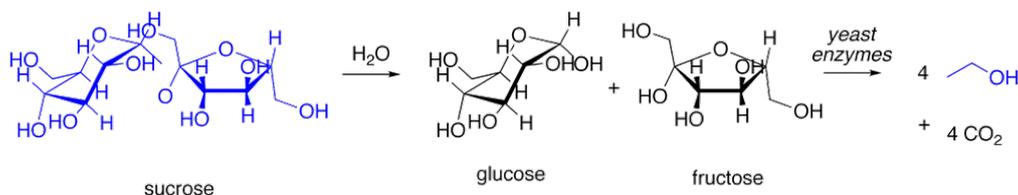


Figure 2: Fermentation. Sucrose \rightarrow glucose + fructose \rightarrow 4 ethanol + 4 carbon dioxide

easy way to separate water and alcohol to a relatively high degree (it is very difficult to pass 95% pure ethanol because of the formation of an azeotrope with water), which pairs very well with fermentation and filtration.



Figure 3: Our distillation apparatus.

Finally, we had synthesized several samples of ethanol (based on condensation temperature) and two samples of biodiesel (one acid- and one base-catalyzed). We calculated the density of ethanol by using a syringe to evaluate the mass of a known volume. We determined the viscosity of the biodiesel by measuring the time it took a set volume of biodiesel to drip out of a pipette. Heat of combustion, however, was much more difficult to quantitatively measure, and required the construction of both a combustion apparatus (Figure 4) and a calorimeter (which we calibrated using 95% ethanol, a substance whose heat of combustion was known to us). We combusted each of our biofuels in our apparatus until the temperature of the calorimeter had risen approximately $10^{\circ}C$, at which point we measured the change in the mass of the fuel and applied the equation $q = mc\Delta T$ to find the heat of combustion of each fuel.

In our final experiment, an ecotoxicity assay, we attempted to germinate radish seeds in petri dishes containing concentrations of biofuels (we tested biodiesel, 2-butanol, ethanol, and methanol), then observed the percentage of radish seeds that successfully germinated, under the hypothesis that more toxic biofuels would hamper radish seed germination more than less toxic biofuels.

As a whole, our experiments helped to show what some of the broader impacts of biofuel usage could be. Biofuels, as we demonstrated with synthesis experiments, can be created renewably using crops like corn or waste like used cooking oil, unlike fossil fuels that are almost completely nonrenewable. Depending on the fuel, biofuels may burn cleaner or more efficiently than fossil fuels, and they may be less harmful for the environment as a whole (seen in our ecotoxicity and calorimetry experiments).



Figure 4: Our combustion apparatus.

3 Methods

3.1 Biodiesel

3.1.1 Synthesis

We began our biodiesel synthesis by warming about 20 mL of waste cooking oil to about 50°C, whereupon we added 5 mL of 0.4 M NaOH in a methanol solution to act as an alcohol reagent. This mixture was stirred for about 45 minutes, then left by itself for a week to settle out into its constituent parts. We also repeated this synthesis using 8 mL of 0.33 M H₂SO₄ in a propanol solution as well.

3.1.2 Purification

After a week, the reaction had completed and formed biodiesel and glycerol, which had, over time, settled into different layers. We separated them as follows: first, we pipetted out as much of the glycerol as we could, measuring the amount of biodiesel left. Next, we added 7 mL of 1 M NaCl, which drew out a water layer from solution (this is known as breaking the biodiesel's emulsion). We repeated the salt water rinse twice more to ensure that little water remained. Next, we added MgSO₄, a compound that naturally absorbs water from its surroundings (known as a desiccant). We added MgSO₄ until it no longer clumped when added, indicating that no water remained in the solution. We filtered out the hydrated MgSO₄ clumps, then heated the filtrate to 80°C for 20 minutes in the fume hood. Finally, we measured the end volume of biodiesel, and stored it for later analysis.

3.2 Ethanol

3.2.1 Fermentation

We began our synthesis of ethanol with nothing but grape juice, yeast, and an overpowering thirst of alcohol (in a purely platonic, chemically ideal sense). We added 1.5 grams of yeast to 100 mL of our grape juice and stirred it well. We poured this into a side-arm flask, which we connected to a tube

with a vial containing 20mL saturated $\text{Ca}(\text{OH})_2$, which acted to absorb CO_2 . We left this apparatus in our lab drawer for a week to ferment.

3.2.2 Filtration

After our sample had had time to ferment, we mixed in 5g of Celite (we had 100 mL of solution) and used a vacuum pump to filter out the mixture. We originally planned to use a Buchner funnel with filter paper, but this process was not efficient and would have necessitated repeated filtrations. Instead, we opted to use smaller Gooch filtering crucibles, which were slower to filter the mixture but far more efficient in doing so; we set up three vacuum filters and divided the fermented solution between the three of them to maximize the speed of the filtration. By the end of our filtration, our mixture was a light yellow but contained no visible particulates.

3.2.3 Distillation

Finally, we took the filtrate and ran it through our distillation apparatus (Figure 3). We placed the filtrate in a round-bottom flask which was partially immersed in a sand bath to ensure even heating. We carefully monitored the temperature of the vapors to ensure that they remained at the optimal temperature to boil off mostly ethanol and not water. As the ethanol boiled off, it rose up and flowed into a section of tubing cooled by ICW, where it condensed and trickled down into a collection vial. We collected about 2 mL of ethanol at various temperature ranges, representing the different stages of the distillation; however, as our density analysis later showed, all but one of these were almost certainly contaminated and therefore not as close to pure as we would have liked. Even disregarding that, the process is fundamentally not perfect; an azeotrope between water and ethanol that forms ensures the even at the optimal temperature, the resulting distillate will be 95% ethanol and 5% water.

3.3 Characterization

Finally, having obtained our samples of biodiesel and ethanol, we proceeded to characterize the biofuels by viscosity, density, and heat of combustion. We calculated the viscosity of biodiesel (only) by measuring the time it took for 1 mL of the biodiesel to drip completely out of a Pasteur pipet. Density was calculated by taking approximately 0.5 mL of ethanol in a plastic syringe, calculating the mass of the ethanol (the mass of a syringe with ethanol minus the mass of an empty syringe), then dividing the ethanol mass by the ethanol volume. To calculate heat of combustion, we created small lamp-like apparatuses that contained a biofuel in a small reagent bottle, covered with aluminum foil and with a protruding wick soaked in the fuel (see Figure 4). We created one of these lamps for each fuel, and created a calorimeter consisting of a soda can filled with water; we used a known sample of 95% ethanol (whose heat of combustion we also know) to find the specific heat of the calorimeter, then combusted each of our fuels until the temperature increased by 10°C and measured the amount of fuel that was burned off.

3.4 Ecotoxicity

For our ecotoxicity assay, we used serial dilution to create samples of biofuel of progressively halving concentration: 10%, 5%, 2.5%, 1.25%, and .625%. We took these samples and added them to petri dishes containing 30 radish seeds and a sheet of filter paper apiece. After giving the seeds a week to

germinate, we counted the number that had germinated in each dish, and used it to estimate the relative toxicities of the various fuels.

4 Data and Calculations

4.1 Final Volumes Synthesized

4.1.1 Biodiesel A: 6.0 mL

$$20 \text{ mL} * \frac{0.895g}{1mL} * \frac{1mol}{885.43g} = 0.02 \text{ mol oil}$$

$$5 \text{ mL} * \frac{0.79180g}{1mL} * \frac{1mol}{32.04g} = 0.1236 \text{ mol methanol}$$

$$\frac{0.02 \text{ mol oil}}{1 \frac{\text{mol}}{\text{rxn}}} < \frac{0.1236 \text{ mol methanol}}{3 \frac{\text{mol}}{\text{rxn}}}$$

Therefore oil is the limiting reactant.

$$0.02 \text{ mol oil} * \frac{3 \text{ mol biodiesel}}{1 \text{ mol oil}} * \frac{294.472g}{1 \text{ mol}} * \frac{1 \text{ mL}}{0.8747g} = 20.20 \text{ mL biodiesel (theoretical yield)}$$

$$\frac{6.0 \text{ mL}}{20.20 \text{ mL}} * 100\% = 30.\% \text{ yield}$$

4.1.2 Biodiesel C: 6.5 mL

$$20 \text{ mL} * \frac{0.895g}{1mL} * \frac{1mol}{885.43g} = 0.02 \text{ mol oil}$$

$$8 \text{ mL} * \frac{.80340g}{1 \text{ mL}} * \frac{1 \text{ mol}}{60.095g} = 0.1070 \text{ mol propanol}$$

$$\frac{0.02 \text{ mol oil}}{1 \frac{\text{mol}}{\text{rxn}}} < \frac{0.1070 \text{ mol propanol}}{3 \frac{\text{mol}}{\text{rxn}}}$$

Therefore oil is the limiting reactant.

$$0.02 \text{ mol oil} * \frac{3 \text{ mol biodiesel}}{1 \text{ mol oil}} * \frac{294.472g}{1 \text{ mol}} * \frac{1 \text{ mL}}{0.8747g} = 20.20 \text{ mL biodiesel (theoretical yield)}$$

$$\frac{6.5 \text{ mL}}{20.20 \text{ mL}} * 100\% = 32\% \text{ yield}$$

4.1.3 Ethanol

Yeast has a theoretical ethanol tolerance of about 10%^[2], so this is approximately the maximum concentration of ethanol in the fermented grape juice.

$$200 \text{ mL} * 0.10 = 20. \text{ mL ethanol}$$

4.2 Ethanol Density

4.2.1 Data

Ethanol	Fraction 1			Fraction 2			Fraction 1		
Mass (g)	0.5425	0.5408	0.5401	0.5103	0.5098	0.5121	0.4101	0.4099	0.4093
Volume (mL)	0.50			0.50			0.50		
Density ($\frac{g}{mL}$)	1.1	1.1	1.1	1.0	1.0	1.0	0.82	0.82	0.82

4.2.2 Sample Density Calculation

$$\text{Density } (\rho) = \frac{\text{mass}}{\text{volume}} = \frac{0.4101g}{0.50mL} = 0.82 \frac{g}{mL}$$

$$(0.789 \frac{g \text{ EtOH}}{mL \text{ EtOH}})(0.89 \frac{g \text{ EtOH}}{mL \text{ total}}) + (1.000 \frac{g \text{ H}_2\text{O}}{mL \text{ H}_2\text{O}})(0.11 \frac{g \text{ H}_2\text{O}}{mL \text{ total}}) = 0.82 \frac{g}{mL}$$

4.2.3 Percent Ethanol by Fraction

Fraction	% Ethanol by Mass
1	0%
2	0%
3	89%

4.3 Viscosity Test Data

Substance	Time to Empty (sec)
Biodiesel A	80
Biodiesel C	85
Methanol	2.5
1-Propanol	4.3
Waste Oil	91

4.4 Heat of Combustion

4.4.1 Combustion Data

Fuel	Vol. H ₂ O (mL)	I. Mass (g)	F. Mass (g)	ΔMass (g)	I. Temp. (°C)	F. Temp. (°C)	ΔTemp. (°C)
95% EtOH	200.	18.3792	17.6082	0.7710	22.0	32.2	10.2
Biodiesel A	200.	17.6901	17.2443	0.4458	22.0	32.0	10.0
Biodiesel C	200.	16.7758	16.5004	0.2754	23.0	30.0	7.0
Ethanol D	200.	17.3342	16.5006	0.8336	21.3	30.5	9.2
Cooking Oil	200.	21.2607	20.9993	0.2614	22.0	29.0	7.0

4.4.2 Class Calorimeter Data

Δm (g)	ΔT (°C)	Heat Capacity ($\frac{\text{kJ}}{^\circ\text{C}}$)
-0.6895	12.0	1.5995
-0.6650	11.1	1.6678
-0.7895	10.0	2.1978
-0.8173	11.2	2.0314
-0.7384	10.3	1.9957
-0.6087	10.4	1.6293
-0.771	10.2	2.1042
Mean (μ):		1.8894
Std. Dev. (σ):		0.2495

$$\mu = \frac{x_1 + \dots + x_n}{n}$$

$$\sigma = \sqrt{\frac{1}{N-1} \sum_{i=1}^N (x_i - \bar{x})^2}$$

4.4.3 Calorimetry Calibration Calculation

95% EtOH by volume: $Q = c\Delta T$

$$\frac{0.771\text{g} * \frac{(0.95)(0.789 \frac{\text{g}}{\text{mL}})}{(0.95)(0.789 \frac{\text{g}}{\text{mL}}) + (0.05)(1.000 \frac{\text{g}}{\text{mL}})} * \frac{1 \text{ mol}}{46.069\text{g}} * \frac{1368 \text{ kJ}}{1 \text{ mol}}}{10.2^\circ\text{C}} = 2.104 \frac{\text{kJ}}{^\circ\text{C}}$$

4.4.4 Sample Heat of Combustion Calculation

$$Q = mc\Delta T$$

$$1.890 \frac{\text{kJ}}{\text{°C}} * 10.0^{\circ}\text{C} * \frac{1}{0.4458\text{g}} = 42.40 \frac{\text{kJ}}{\text{g}}$$

$$(1.890 \frac{\text{kJ}}{\text{°C}} \pm (1.97996)(0.2495)) * 10.0^{\circ}\text{C} * \frac{1}{0.4458\text{g}} = [32.01 \frac{\text{kJ}}{\text{g}}, 52.74 \frac{\text{kJ}}{\text{g}}]$$

*95% of a normal distribution is within 1.97996 standard deviations of the mean.

4.4.5 Calculated Heat Capacities of Biofuels

Biofuel	ΔH ($\frac{\text{kJ}}{\text{g}}$)	95% C.I. ($\frac{\text{kJ}}{\text{g}}$)
Biodiesel A	42.40	[32.01, 52.74] (± 10.37)
Biodiesel C	48.04	[36.27, 59.76] (± 11.75)
Ethanol D	20.86	[15.75, 25.95] (± 5.10)
Cooking Oil	50.61	[38.21, 62.96] (± 12.38)

4.5 Ecotoxicity Data

4.5.1 Biodiesel Percent Germinated

Concentration (% volume)	Biodiesel Trial 1	Biodiesel Trial 2	Average
0%	40%	97%	68.5%
0.625%	58%	90%	74%
1.25%	59%	93%	76%
2.5%	42%	93%	67.5%
5%	41%	83%	62%
10%	77%	77%	77%

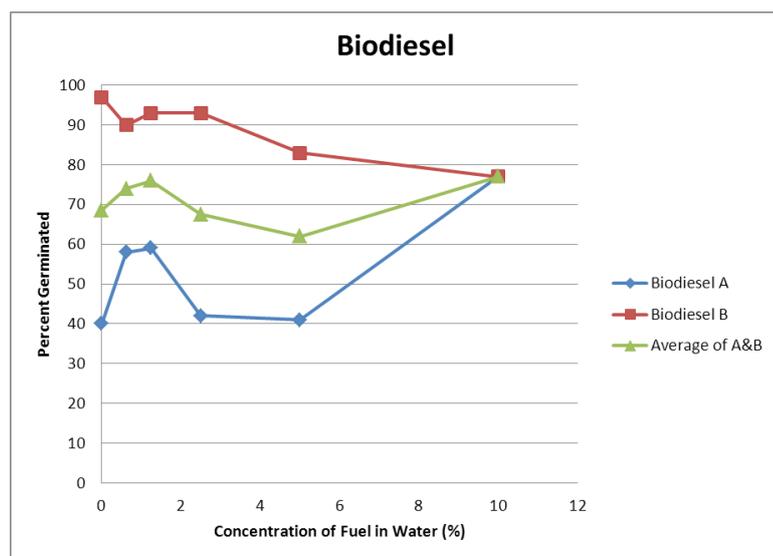


Figure 5: Concentration of biodiesel vs. percent of radish seeds germinated.

4.5.2 2-Butanol Percent Germinated

Concentration (% mass)	2-Butanol Trial 1	2-Butanol Trial 2	Average
0%	93%	100%	96.5%
0.625%	87%	100%	93.5%
1.25%	77%	87.5%	82.25%
2.5%	56%	75%	65.5%
5%	34%	50%	42%
10%	0%	0%	0%

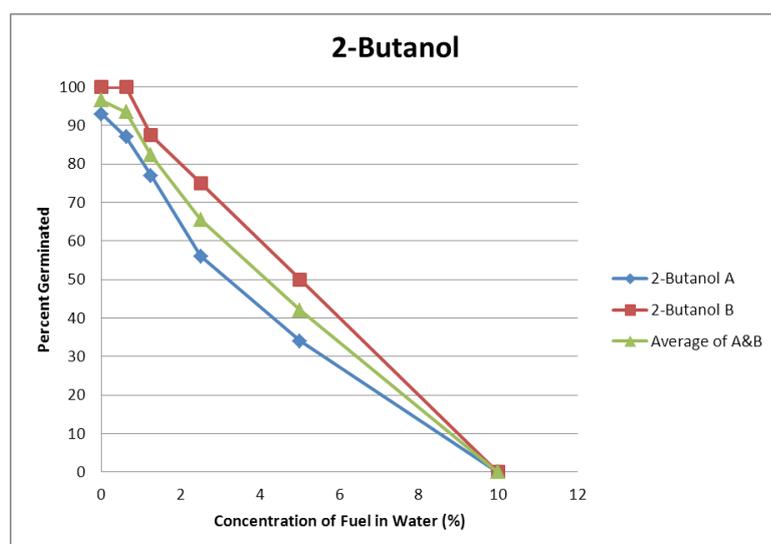


Figure 6: Concentration of 2-butanol vs. percent of radish seeds germinated.

4.5.3 Ethanol Percent Germinated

Concentration (% mass)	Ethanol Trial 1
0%	93%
0.625%	93%
1.25%	80%
2.5%	27%
5%	0%
10%	7%

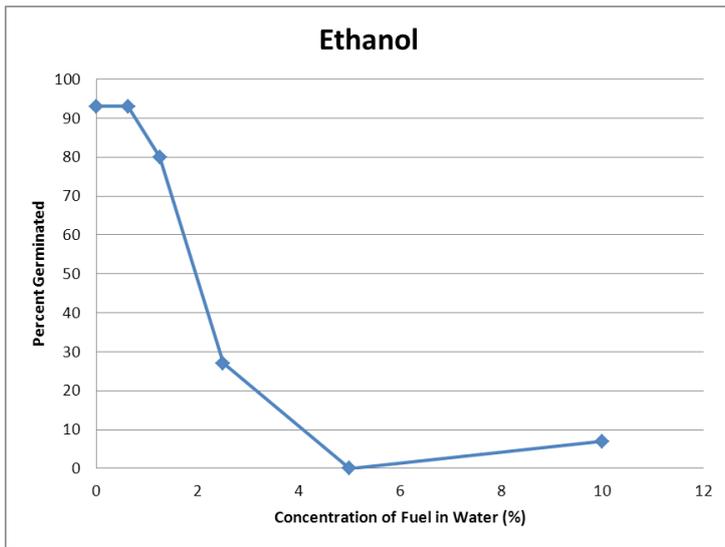


Figure 7: Concentration of ethanol vs. percent of radish seeds germinated.

4.5.4 Methanol Percent Germinated

Concentration (% mass)	Methanol Trial 1
0%	63%
0.625%	73%
1.25%	60%
2.5%	83%
5%	78%
10%	71%

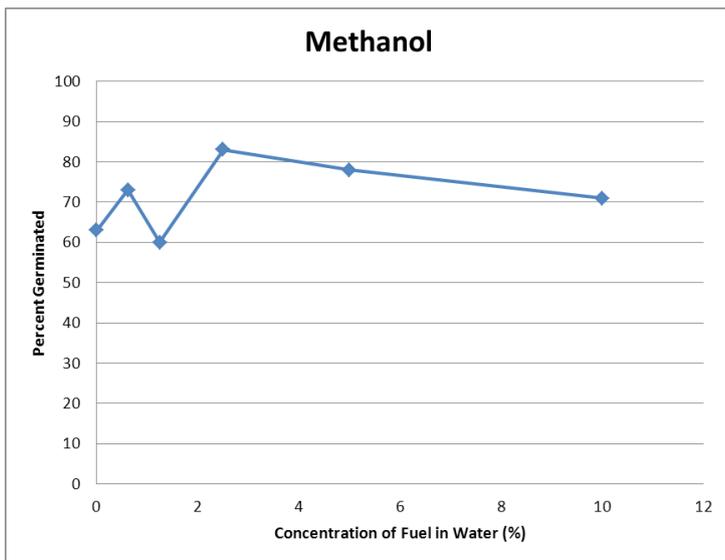


Figure 8: Concentration of methanol vs. percent of radish seeds germinated.

5 Summary and Analysis of Results

In this lab, our goal was to analyze biofuels and several factors that affect their relevancy to the modern energy crisis; ecotoxicity, heating value, ease of production, and other properties are incredibly important for determining whether biofuels will be able to conceivably replace fossil fuels as a major source of energy. We were able to create samples of biodiesel and ethanol, as planned, and successfully measured several key metrics of the biofuels (such as heat of combustion and density); however, there were several major sources of error in our experiments. While several of our data points for our ecotoxicity assay have high correlation and are tight fits, some of them (particularly methanol) appear to be subject to serious experimental error; else, a solution of 10% by mass methanol is less toxic to radish seeds than pure water. Methanol, having one of the highest LD₅₀ (median lethal dose) of any of our biofuels, should severely impair radish germination, especially at high concentrations.

While the errors in our ecotoxicity assay are probably due merely to small sample size and experimental error, the distillation process used to create our ethanol samples had several serious flaws as well. While the distillation process is inherently imperfect (the formation of a 95:5 EtOH-H₂O azeotrope prevents further distillation), our density analysis of the “distilled” 95% ethanol revealed the troubling fact that our first two ethanol samples were far too dense – one, as dense as water, the other somehow even more so. Because it is impossible for a mixture of ethanol and water to be denser than either alone, we hypothesized that the ethanol sample was not pure, and may have somehow been contaminated with compounds dissolved in the filtered grape juice. Another possibility is that there was a measurement error when calculating the density: it is plausible that the syringes used to measure out exactly 0.50 mL of EtOH may have held slightly more than that, due to a small amount of volume between the tip of the syringe and the ‘zero’ mark. If this was the case, we would have underestimated the amount of mass of EtOH, and our densities could be significantly lower.

While this experiment was extremely enjoyable, there are several ways in which it could have been improved. Using a ‘lamp’ to combust biofuels to heat a calorimeter seems like it may have a high rate of heat loss; using a bomb calorimeter to run the combustion reactions would ensure that the effects of heat dissipation would be minimized. Another suggestion would be using (more pure) vegetable oil as opposed to (possibly contaminated) waste cooking oil for biodiesel synthesis; while the latter provides a more realistic metric for the economical value of biodiesel, using vegetable oil could provide an upper bound to the energy efficiency of biodiesel, and could provide more detail on the applications of filtration and purification of waste oil prior to biodiesel processing. In fact, creating biodiesel from both samples would allow for a direct analysis of the effects of impurities in waste oil.

6 Discussion

The two major biofuel properties we measured in this lab are heating value and ecotoxicity; the two major fuels we analyzed were ethanol and biodiesel. Biodiesel proved to be significantly less toxic than ethanol (the slope of concentration vs. germination rate is less negative), and also had a higher heating value (20.86 ± 5.10 vs. 50.61 ± 12.38 , well above 95% confidence).

For the last century or so, the dominant fuels have been gasoline and diesel fuel. Both fuels are created via the fractional distillation of crude petroleum. Biodiesel is created by the reaction of alcohols with lipids, and ethanol can be created by the fermentation of sugars.

The most obvious difference between biofuels and traditional fuels is their availability in the future; petrol byproducts are considered nonrenewable: there is a functionally finite quantity. Global oil production debatably hit ‘peak oil’ (after which it will only decline) in 2005[3], and estimates predict that world oil reserves will last from as late as 2030[4] to as soon as 2015[6]. As world supplies of oil dwindle, petroleum will become increasingly scarce, making the sustainability case for Team Biofuels. However, biofuels have their own problems. In the US, a large amount of ethanol is produced from corn, mostly due to huge subsidies to corn farmers in the Midwest; however, corn is massively inefficient as a source for ethanol, especially compared to other plants like sugarcane[7]. These huge subsidies are largely responsible for the advances in ethanol ‘flex-fuel’ and the ability for all modern cars to take E15. However, ethanol from crops creates direct competition with the world food production, and if implemented inefficiently (such as with corn) can actually be *worse* for the environment than fossil fuels due to the inefficiencies in the mass production of crops like corn. A proposed response to this would be increasing the amount of world farmland – which would cause deforestation, and impact greenhouse gas levels even further.

A number that represents the efficiency of fuel production techniques is also known as the *energy balance*. This value for ethanol can range from as low as $1.3 \frac{\text{Joules output}}{\text{Joule input}}$ for corn ethanol in the US to as high as 8 for sugarcane ethanol in Brazil[8]; experimental cellulosic ethanol can reach as high as 20. Biodiesel has an average value of about $2.5 \frac{\text{Joules output}}{\text{Joule input}}$, while fossil fuels produce a net loss, with diesel at about 0.83 and gasoline at 0.80. This shows that production of biofuels has the potential to be far more energy efficient than current fossil fuels.

Studies have also shown that biofuels like ethanol and biodiesel are less harmful than counterpart fossil fuels when burned, producing fewer emissions and fewer carcinogenic compounds[9].

The major staying power of petroleum-based fuels, besides their existing infrastructure, is pure cost-value: A gallon of gasoline costs between \$0.30 and \$0.60, while an equivalent amount of ethanol is about \$1.00. Petroleum diesel costs around $\frac{\$0.70}{\text{gal}}$, while biodiesel costs almost \$2.50 a gallon.

7 Conclusion

Biofuels are an increasingly important topic in the near future, with oil reserves decreasing and almost certainly gone within the century (an extremely liberal estimate). While not all methods for creating biofuels are efficient[7], several of them, such as sugarcane or cellulosic ethanol, show incredible amounts of promise. Biofuel combustion byproducts are probably less harmful for both the environment and people than their petroleum alternatives; however, because of their higher production costs, it is unlikely that we will see biofuels until the production process becomes more efficient and cheap enough to surpass petroleum. Biofuels are slowly gaining traction, and as new methods for their production and synthesis are developed, we will probably see them more and more.

References

- [1] UC Berkeley bSpace: *Synthesis and Characterization of Biofuels*.
<https://bSpace.berkeley.edu/access/content/group/4cc7b769-c78a-4044-9771-d138014adc8d/Lab>
- [2] Wasungu KM, Simard RE. Growth characteristics of bakers' yeast in ethanol. *US National Library of Medicine National Institutes of Health* [online] 1982 May;24(5):1125-34.
<http://www.ncbi.nlm.nih.gov/pubmed/18546405> (Accessed November 24, 2012)
- [3] US Energy Information Administration: *International Energy Statistics*.
<http://www.eia.gov/cfapps/ipdbproject/iedindex3.cfm?tid=5&pid=57&aid=1&cid=regions&syid=2005&eyid=2011&unit=TBD> (Accessed November 24, 2012)
- [4] Monbiot, George. The Guardian: *We Were Wrong on Peak Oil*.
<http://www.guardian.co.uk/commentisfree/2012/jul/02/peak-oil-we-we-wrong>. July 2nd, 2012 (Accessed November 23, 2012).
- [5] International Energy Agency. *Oil Market Report*.
<http://omrpublic.iea.org/omrarchive/18jan07tab.pdf>. January 18, 2007 (Accessed November 24, 2012).
- [6] Campbell Colin, Laherre Jean. *The End of Cheap Oil*. <http://dieoff.org/page140.htm>. Scientific American, March 1998 (Accessed November 23, 2012).
- [7] Pineiro, et al. *Set-Asides Can Be Better Climate Investment than Corn Ethanol*.
http://gea.unsl.edu.ar/pdfs/Pineiro_et_al%20_set_asides_vs._corn_ethanol_Ecol_%20Appl_2009.pdf. Ecological Applications, 19(2), 2009, pp. 277282 (Accessed November 21, 2012).

- [8] Green Dreams J.K. Bourne JR, R. Clark National Geographic Magazine October 2007 p. 41.
<http://ngm.nationalgeographic.com/2007-10/biofuels/biofuels-interactive.html> (Accessed November 23, 2012).
- [9] CCoOHaS. *Health effects of Gasoline*.
http://www.ccohs.ca/oshanswers/chemicals/chem_profiles/gasoline/health_gas.html (Accessed November 23, 2012).