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Development of a Kinetic Model for the Transesterification of Glycerol Fatty Acid Triesters Using ^1H (1D and 2D) NMR

Leonard W. Sprague

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**Development of a Kinetic Model for the
Transesterification of Glycerol Fatty Acid Triesters
Using ^1H (1D and 2D) NMR**

Leonard W. Sprague

Commonwealth Honors Thesis in Chemistry

Submitted: Spring 2016

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Abstract

Fossil fuels dating back to the first coal mine dug from the Earth have been vital to society's advances, but have come at a significant cost. Rise in pollutants such as CO₂ and particulate matter due to combustion byproducts of these fuels (coal, oil, gasoline, diesel) continue to negatively impact the environment, harming various ecosystems by disrupting precarious balances. Attempts have been made to "clean" fossil fuels, but only to temporary degrees of success. Alternative energy sources such as solar, wind, or hydroelectric are increasing in viability, but are often a difficult transition for fossil fuel reliant individuals. Biodiesel (Fatty Acid Methyl Esters, FAME) is an alternative "cleaner" fuel made from renewable vegetable oils that can be used in diesel vehicles with few to no engine modifications, easing consumer transition to alternative energy sources. Biodiesel is a possible replacement for petroleum diesel due to reduced greenhouse gas emissions, unburned hydrocarbons, carbon monoxide, particulate matter and nitrogen oxides. A diesel engine can run on an 80/20 (B20) mix of petroleum diesel to biodiesel, and with adjustments (to avoid possible clogging) can run 100% (B100) biodiesel. Biodiesel produced from vegetable (soybean) oil through a base catalyzed transesterification with methanol is, however, an inefficient and wasteful process.

Our research group is applying green chemistry principles to improve the efficiency of small-scale (bench top) biodiesel production, but this work has been hindered by the lack of detailed information about the mechanism behind the transesterification reaction. Our research goal was to develop a detailed kinetic model that would identify whether rate limiting transesterification occurs at C1/3 or C2 as the glyceryl triester is converted into the di- and mono- ester, and finally free glycerol. Transesterification reactions were conducted on a 25mL scale at 60°C to test our methodology for quenching the reaction with acetone d-6, and at 25°C to acquire a time zero data point. Reaction aliquots were analyzed using quantitative NMR, employing an internal reference standard of maleic acid for concentration calculations, and integrated data was used to analyze and obtain time course data. All controls and standards gave unambiguous NMR spectra with <5% error. 2D spectroscopy was also performed and will be assessed for future usefulness in determining C1/3 or C2 ester branches leaving as the limiting step. The results of this research will help narrow the options for improvements to the reaction efficiency, for example in catalyst design. This theses' secondary goal is to develop an introduction and guidebook to cleaner fuels research and kinetic experimentation using NMR instrumentation.

Preface

New researchers, especially those who understand the vast wealth of information truly floating around out there, begin their career on a new topic floundering for relevant details on which to gain a foothold. This thesis, presented to the Department of Chemistry in partial fulfillment of the Bachelor of Science degree with Commonwealth Honors in Chemistry (Environmental and Professional concentrations), is not just a description of biodiesel kinetics research that will be used as a new project at some later point. This thesis is meant as a guide for new researchers, a resource that they may examine and peruse to their heart's content, to find those relevant details when entering renewable energy (e.g., biodiesel) research, as well as kinetics experiments.

Part 1, the Introduction, resolves to outline fossil fuels' and energy's importance to society and its progression throughout history, leading up to the work being done today on cleaning fossil fuels and alternative energy sources. Part 2 contains research on the analysis of transesterification kinetics, from methodology to results and discussion, culminating my research done at Bridgewater State University with Dr. Brush. This brings new information to the kinetic and mechanistic studies of biodiesel synthesis, as well as invites newer researchers into the realm of reading scientific writing. Finally, part 3 is a pure reference material for researchers new to the field of kinetics studies, and to using Nuclear Magnetic Resonance instrumentation. Guides range from running scans and changing parameters to properly handling error messages, assembled for quick reference, in a more welcoming setting than a multi-hundred page manual.

So, to all of those who use this thesis for its intended (albeit unusual) purpose, I hope it helps in your endeavors. For all of those that don't...

well, you probably didn't read this part anyway.

Thesis Committee Approval (as to style and content):

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Acknowledgements

Within a small section, a mere page, I will attempt to thank all of those wonderful individuals and groups that have helped me achieve my goals. I will attempt to properly thank them for their aid, advice, and assurances, as well as their welcomed critiques and questions, all of which has improved by magnitudes the work I have done. Before I do, however, please know that these words are not enough to do justice to the appreciation I have for each person, but are intended as a representation of this in a physical form.

First I'd like to thank my current mentor, Dr. Edward Brush, for guiding me through the realm of chemical research and scientific writing, whilst also mentoring me in the time management of crazy workloads that a chemist ends up carrying around. Without his guidance I would have been stumped more often than not; his incredible patience and simultaneous devotion to our work pushed me to succeed, and for that I am grateful.

I would also like to thank my first and continuing mentor Dr. Ward Heilman, who gave me my first opportunity in the research field, and who taught me my most important lesson about it: to enjoy finding not answers but more questions, and learning how to approach them one by one. Without him, I may never have begun research nor been confident enough to write this thesis. His support and advice were invaluable to my prior research and my work moving forward; for that I am eternally grateful.

All of the faculty I have ever interacted with, from the humanities to the sciences, that pushed me in some way, shape, or form to chase my passions. Many if not all of them gave me motivation to pursue my dreams, and all of them helped me to achieve them (whether it was through patiently listening to ramblings or offering constructive criticism). I cannot thank them all enough, and wish I could properly describe each and every individual's contribution in greater detail.

My colleagues in the chemistry department – thank you for the camaraderie during even the most difficult times. I couldn't have asked for better friends; your support was unending and critical in moving my lazy self forwards.

My loved ones, both family and friends – thanks for listening to all of my rants, my long winded explanations, and for your unrelenting support.

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1. Introduction

1.1 *Understanding Fuels*

1.1.1 Origins and Uses of Fossil Fuels

In order to properly assess the importance of all energy sources and their effects in present day society, their origins must be fully understood. Over 300 million years ago, during the Carboniferous period and before the dinosaurs we all know and love, Earth was populated by an abundance of plant species.¹ Swamps covered the surface, which was plentiful with algae and large leafy plants. Dead plants sank to the bottom of the swamps as peat (plant debris) and were slowly compressed over time by settling sand and other decaying organisms. Water was pressed out while heat began chemically modifying this peat layer, transforming the peat into coal, oil and natural gas.¹ Biological matter composition prior to deposition determines the resulting fossil fuel: coal is mostly plant matter from a terrestrial environment, whereas oil and natural gas are generated from deposited diatoms or plankton among aquatic plants (Figure 1). By burning these fuels and their derivatives, and harnessing the gas/steam force through turbines or explosive force through pistons, electrical and mechanical energy are generated.

¹ California Energy Commission. *Energy Story*, EnergyQuest; California, 2012.
<http://www.energyquest.ca.gov/story/index.html> (accessed February 15, 2016)

OIL AND NATURAL GAS FORMATION

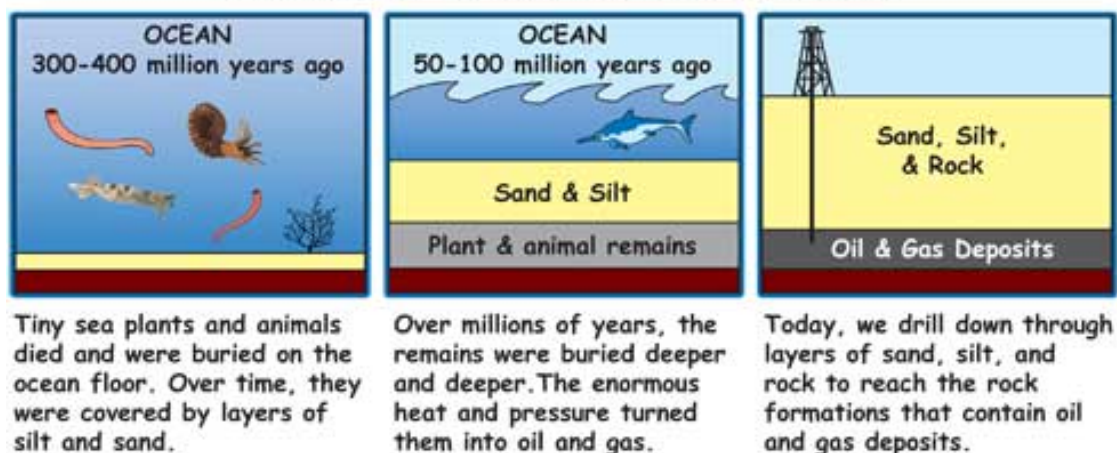


Figure 1 – Illustration from NASA of oil and gas formation, which is also representative of how coal is formed with different peat compositions (terrestrial versus aquatic organisms, the prior for coal and the latter, such as plankton, for oil).

Four stages of peat compression and heating over time lead to the formation of coal (Figure 2), and are ranked by carbon content. Low-rank (low carbon content) coal consists of the first two stages: lignite, the soft and reddish first stage, and sub-bituminous, a darker and more compact form. Both contain lower energy content with more moisture than high-rank coals. High-rank coal consists of bituminous, used for sealing wooden boats or canals²; and anthracite, where a final chemical change creates the hard and vitreous coal used for generating electricity.³

² Sams, Joseph; Herd, Janice; Marcus, Lawrence; Terrell, Ellen; Wilson, Angela; and Joan Sullivan. The Oil & Gas Industry. *BERA*. [Online] **2006** (updated **2013**), 5/6.

https://www.loc.gov/rr/business/BERA/issue5/issue5_main.html (accessed February 20, 2016)

³ Kentucky Geological Survey. Classification and Rank of Coal. *University of Kentucky*. 2016.
<https://www.uky.edu/KGS/coal/coalkinds.htm> (accessed February 24, 2016)

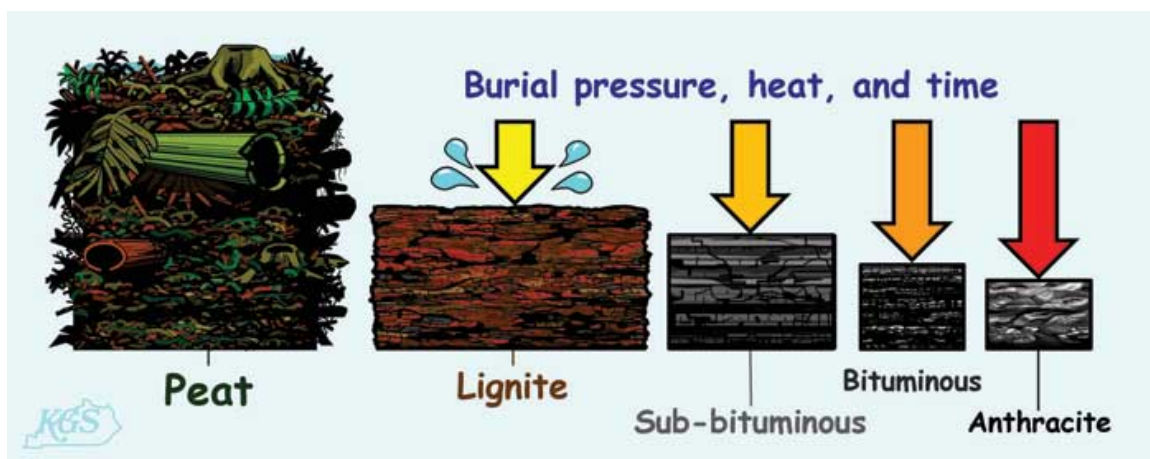


Figure 2 – Peat transitional stages to anthracite coal, with added pressure and heat over time.²

Anthracite coal, with its high carbon content, inherently high energy content and a physical state that is easy to transport, has been in use “since the caveman” for heating and cooking.⁴ Since then, evidence of coal usage has been discovered in China over three thousand years ago for smelting bronze¹, and as early as 100-200 A.D., where Romans used it for similar purposes along with cooking and heating. It is also seen commonly in Native American pottery ovens around 1300.³ Wood charcoal was still prominent for the early parts of the coal era, but phased out during the Industrial Revolution; the immense need for energy to produce materials such as steel (which required consistent high temperature furnaces) made coal’s energy to size ratio more favorable, and it became the driving force of new industry around the 1700’s. Coke is another coal derivative, used in the production of steel, which is burned at upwards of 1100°C in anaerobic conditions to concentrate the carbon content further.⁵ Soon trains and boats converted

⁴ United States Department of Energy. A Brief History of Coal Use. 2013.

http://www.fossil.energy.gov/education/energylessons/coal/coal_history.html (accessed February 19, 2016)

⁵ Valia, Hardarshan S. Coke Production for Blast Furnace Ironmaking. *American Iron and Steel Institute*, 2015.

<http://www.steel.org/making-steel/how-its-made/processes/processes-info/coke-production-for-blast-furnace-ironmaking.aspx> (accessed March 10, 2016)

to coal for its increased transportability and energy density, and by the end of the 1800s, coal was being used to create electricity directly.⁶

Coal was not the only fossil fuel in use though; oil's applications also date back thousands of years, mainly as a sealant or light source and only recently as a source of energy.² Oil seeps, locations where underground oil presses its way to the surface, and skimming oil from lakes were the first places oil was collected from. Asphalt, another form of oil, was used by “[a]ncient Sumerians, Assyrians, and Babylonians [...] from seeps along the Euphrates as mortar and for waterproofing,” and Egyptians used liquid oil “for medicinal purposes and embalming.”⁷ Native Americans used tar as an adhesive. Lubrication, fueling torches, waterproofing, and caulking ships are other applications in use far before being used in energy and electricity generation.⁷

Oil consists of saturated and unsaturated highly variable carbon chains, and exists in its own pockets beneath the earth's folds, paired with natural gas, and/or trapped within sediment pores.¹ Liquefied coal can be converted to oil under the proper conditions as well, but is used only when the demand for oil products is higher than that for coal supplies.⁸ Crude oil is pumped from these pockets or concentrated oil reservoirs underground using hydraulic pumps after locating them by drilling. Fractional distillation of the crude oil, a process in which oil is heated into the gas phase and run through a temperature gradated column, separates the components by boiling point and therefore by carbon chain length (Figure 3). Each of these mixtures is used in

⁶ The NEED Project. Coal. 2013. <http://www.switchenergyproject.com/education/CurriculaPDFs/SwitchCurricula-Intermediate-Coal/SwitchCurricula-Intermediate-CoalFactsheet.pdf>

⁷ Extreme Oil. The evolution of a valuable resource. PBS. 2004. <http://www.pbs.org/wnet/extremeoil/history/> (accessed March 15, 2016)

⁸ U.S. Energy Information Administration. Today in Energy: Crude oil distillation and the definition of refinery capacity. 2012. <http://www.eia.gov/todayinenergy/detail.cfm?id=6970> (accessed March 15, 2016)

different ways: gasoline for automobiles, kerosene for lamps, and distillate for diesel and heating oil (low to high boiling points, respectively).⁸

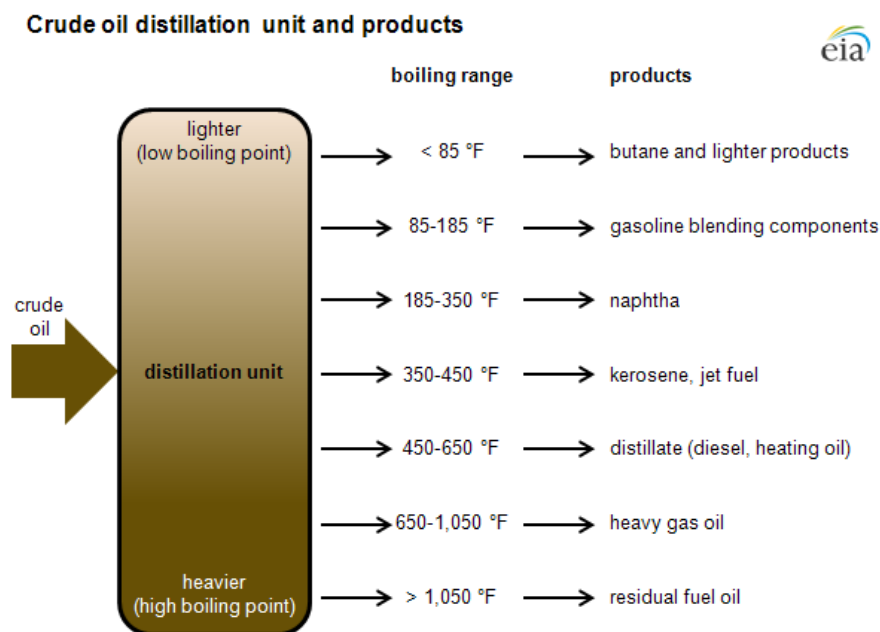


Figure 3 – Crude oil distillation process, where the darker shading is higher temperatures and the light shading is a lower temperature within the column (U.S. Energy Administration). Boiling point ranges are shown to the right of each fraction, with respective product.

Natural gas is the final fossil fuel, and is often found near oil reservoirs. In fact, natural gas was also first discovered in seeps similar to oil seeps, and may be accidentally ignited by lightning.¹ Decay of biomatter before complete compression and/or heating of peat into the gas phase creates natural gas (mostly methane). Natural gas can be burned as is and transported via pipelines to storage facilities for later.¹

1.1.2 Drawbacks, “Cleaning,” and Alternatives

Fossil fuels are an energy dense resource with multiple applications, and have successfully been woven into the backbone of society. Worldwide, nearly 80% of all energy used

is generated by the burning of fossil fuels in one of its three forms (Figure 4).⁹ However, each fossil fuel contributes to a growing problem: global warming, where excessive release of chemicals known as greenhouse gases (e.g., water, methane, ozone, carbon dioxide and nitric oxide) build up in the atmosphere and over time wreak havoc on Earth's temperature dependent ecosystems.¹⁰ Most notable and problematic of the greenhouse gases are carbon dioxide (CO₂) and nitric oxide (NO_x), because of their long life span in the stratosphere and ability to reflect and contain ultraviolet radiation.^{11,12} In tandem, toxic pollutants reside in some of these fossil fuels, and are released upon burning, creating thick smogs, acid rain, and organismal death.^{4,13}

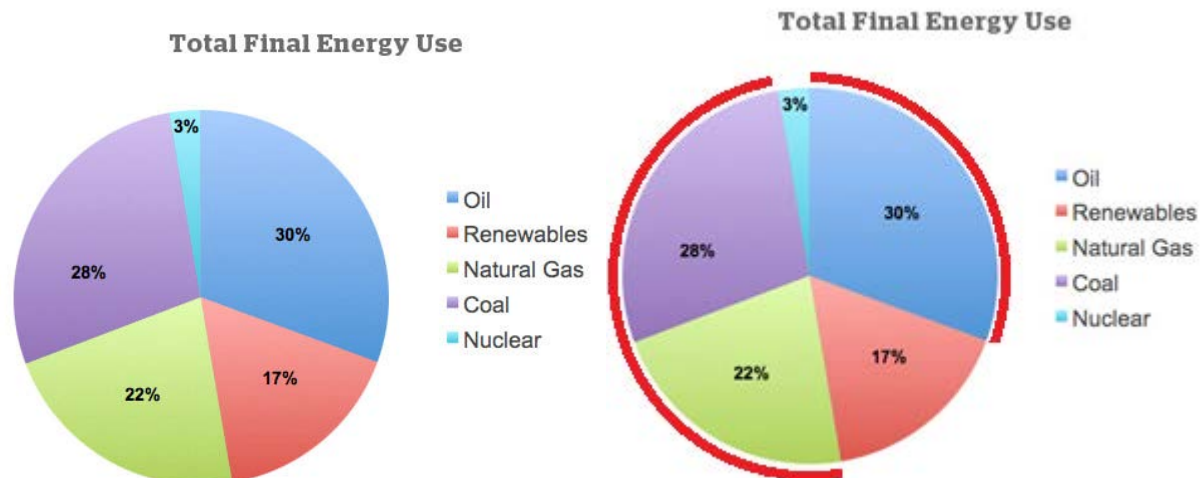


Figure 4 – Percentage of worldwide energy usage (2013) sustained using fossil fuels⁹ (highlighted with red arcs).

⁹ Friday, Leslie. Breaking the Fossil Fuel Habit: The Promise, and Challenge, of Shifting to Alternative Energy. *BU Today Science and Tech*. [Online] <http://www.bu.edu/today/2013/the-climate-crisis-breaking-the-fossil-fuel-habit/> (accessed January 10, 2016)

¹⁰ Stanford SOLAR Center. Global Warming. <http://solar-center.stanford.edu/sun-on-earth/glob-warm.html> (accessed January 15, 2016)

¹¹ Carbon Dioxide Absorbs and Re-emits Infrared Radiation. UCAR. 2012. [online] <http://scied.ucar.edu/carbon-dioxide-absorbs-and-re-emits-infrared-radiation> (accessed February 25th, 2016)

¹² Greenhouse Gases: Nitrous Oxide (N₂O). *University of Hawai'i at Manoa*. 2006. [online] http://www.soest.hawaii.edu/mguidry/Unnamed_Site_2/Chapter%202/Chapter2C3.html (accessed February 25th, 2016)

¹³ Lemkau, Karin. Tracking Toxic Chemicals In Oil Spills: Does out of sight mean into the air or into fish? *Oceanus Magazine*. [Online] <http://www.whoi.edu/oceanus/feature/tracking-toxic-chemicals-in-oil-spills> (accessed January 17, 2016)

Two notable elements reside trapped in coal and oil, sulfur and nitrogen, and can cause acid rain and contribute to the greenhouse effect. During combustion, carbon binds to oxygen and generates carbon dioxide and water, along with releasing trapped sulfur, nitrogen, and fine particulate matter (dirt).⁴ Sulfur and nitrogen also bind with oxygen during combustion (high temperatures and close proximity to oxygen atoms), becoming gaseous oxides, and rise upwards through the troposphere and stratosphere, where clouds and ozone reside, respectively.¹⁴ Condensation with water vapor in the troposphere forms sulfuric and nitric acid, acidifying precipitation and resulting in acid rain.^{15,16} Figures 5 and 6 illustrate alternative nitrogen and sulfur pathways, showing the many impacts that excess of either may induce in the system^{17,18}. Built up particulate matter, ash or soot found after burning, creates a hazardous breathing environment. Soot and ash can also follow sulfur dioxide into the stratosphere, creating the black smog observed after a volcano's eruption.¹⁹ However, there are some techniques to counter release of damaging sulfur, nitrogen, and/or particulate matter from burning coal and oil: flue-gas desulfurization units, gasification processes, carbon capture and storage (CCS), and coal-to-hydrogen conversion.

¹⁴ Wigand, Rob. Stratification. *Stanford University: NOVA*. 1999. <http://nova.stanford.edu/projects/mod-x/id-strat.html> (accessed December 15, 2016)

¹⁵ Walter, Thomas. In *the City University of New York lectures*, The Sulfur Cycle. New York, NY. 2015. http://www.geo.hunter.cuny.edu/tbw/soils.veg/lecture.outlines/ecology.chap.23/sulfur_cycle.htm (accessed January 20, 2016)

¹⁶ Carnegie Mellon University. Nitrogen Cycle. Environmental Decision Making, Science, and Technology. <http://environ.andrew.cmu.edu/m3/s4/cycleNitro.shtml> (accessed January 5, 2016)

¹⁷ The Marine Biology Laboratory in Woods Hole: Ecosystems Center. Introduction to the Nitrogen Problem. <http://dryas.mbl.edu/Research/Clue/nproblem.html> (accessed February 9th, 2016)

¹⁸ Davidson, Eric A., et al. *Excess Nitrogen in the U.S. Environment: Trends, Risks, and Solutions*. Issues in Ecology. ESA; Winter 2012.

¹⁹ Takle, Eugene S. Nitrogen Cycle, Sulfur Cycle; Tropospheric Ozone. *Iowa State University*. 2002. <http://www.meteor.iastate.edu/gccourse/alumni/chem/nitro/text.html> (accessed January 26, 2016)

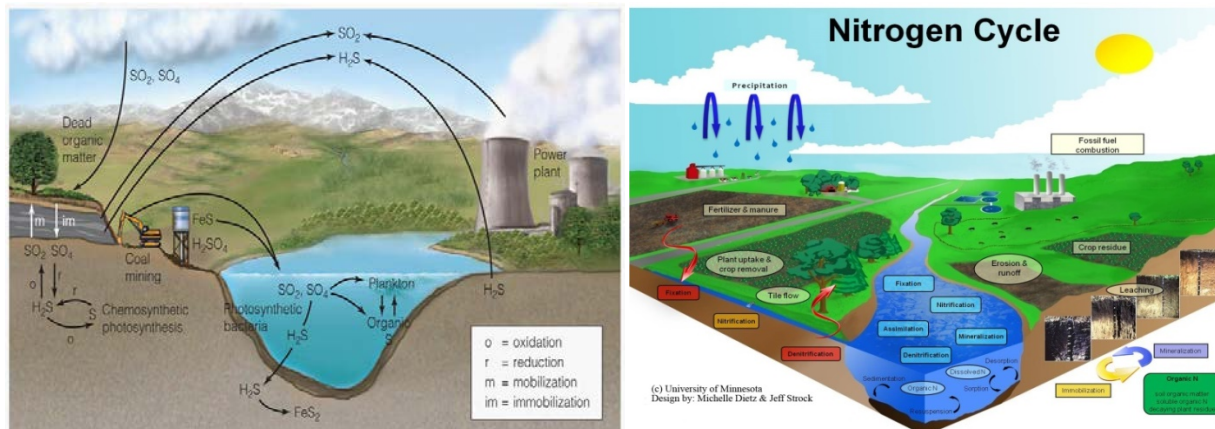


Figure 5 (Left) and 6 (Right) – Sulfur and nitrogen cycles, respectively, with form and process of each chemical species. Images from the City University of New York and the University of Minnesota.

Flue-gas desulfurization units, more commonly known as scrubbers, were developed to remove sulfur contaminants from coal after burning. Coal gas containing sulfur dioxide is vented into a separate chamber containing a mixture of water and a sulfur absorbing compound, such as limestone.²⁰ Gaseous sulfur dioxide reacts with limestone (calcium carbonate) to produce synthetic gypsum (calcium sulfate), a solid precipitate that is easily removed.²¹ Gypsum is the main compound in drywall and plaster materials, as well as a useful fertilizer for replenishing low calcium and/or sulfate levels.²² Variations of a scrubber's basic design (Figure 7) exist for different environments, such as pressurized and non-pressurized fluid bed compression, and fluid bed boilers.²³ Fluidized bed boilers suspend burning coal in the limestone/water mixture, causing a tumbling effect that increases the limestone-coal surface interactions and improving sulfur

²⁰ Olivares, Hely. The Future of Clean Coal. *University of North Carolina at Chapel Hill: Powering a Nation*. 2014. <http://news21.jomc.unc.edu/index.php/stories/335.html> (accessed February 12, 2016)

²¹ Claes Tullin and Evert Ljungstroem. Reaction between calcium carbonate and sulfur dioxide. *Energy Fuels*. **1989**, 3 (3), 284-287.

²² University of Kentucky Center for Applied Energy Research. What are coal combustion by-products? Synthetic Gypsum. *Kentucky Ash Education Site*. 2015. <http://www.caer.uky.edu/kyasheducation/gypsum.shtml> (accessed February 22, 2016)

²³ Bonk, Donald L.; Freir, Mark D.; Buchanan, Thomas L.; Goldstein, Harvey N.; and Jay S. White. First-Generation Circulating Pressurized Fluidized Bed (CPFB) Combustor Power System with Industrial Components. *US Department of Energy*. [Online] <http://physics.oregonstate.edu/~hetheriw/energy/topics/doc/elec/natgas/cc/> (accessed February 22, 2016)

capture.⁴ Pressurized scrubbers have follow-up chambers that send clean gas through a narrowing tube, increasing its velocity to turn a turbine on the other end at a faster rate, and also have several variations in design. Scrubber technology also allows coal burning to be done at lower temperatures, below those that would trigger synthesis of NO_x with air.⁴

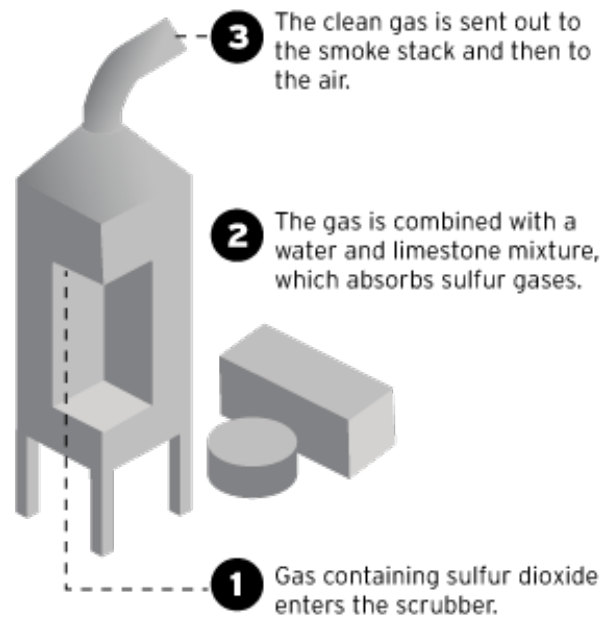


Figure 7 – Basic design of scrubber system.

Gasification uses the gases produced when burning coal in the presence of steam or air at high temperature and pressure, breaking the coal into constituent gaseous compounds (CO_2 , H, etc...) which are pressed through a turbine to generate electricity.¹⁵ Sulfur is removed easily from the gas phase that gasification creates, using scrubbers. Simultaneously, excess heat given off by the steam traveling through the first turbine is used to heat a secondary source and generate more steam, turning a secondary turbine. Figure 8 illustrates the block structure of this design, which can be attached directly to other cleaning techniques such as scrubbers, described above.

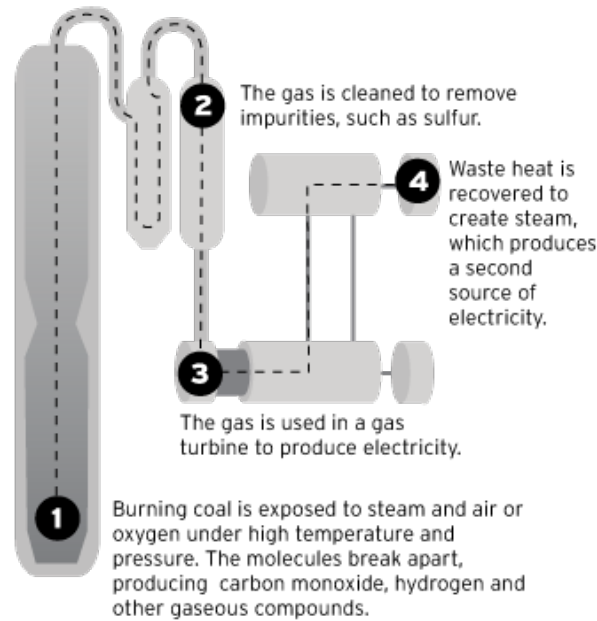


Figure 8 – Basic design of gasification setup.

CCS captures carbon dioxide either during the boiling process (before burning, in the fluidized bed boiler, for example) or after the burning process.¹⁵ CO₂ is captured with certain chemicals based on the type of fuel burned, and pumped as a gas deep below ground, either onto the seabed or under large terrestrial locations (Figure 9 shows simple design plans). Increased pressure with increased depth changes carbon dioxide into its liquid state, forming a pool or pocket of CO₂. Stores of carbon dioxide, if properly handled and made accessible, can be used as a chemical feedstock for other processes such as urea and methanol synthesis.²⁴ A process called CCS oxy-combustion implements an oxygen rich environment through burning, resulting in high levels of carbon dioxide, plus an air separation unit and re-circulation of flue gas to remove nitrogen and sulfur pollutants. Oxy-combustion makes application of post-combustion CCS simpler with high levels of carbon dioxide instead of a mixture of gases to separate carbon dioxide out of. Many capture systems exist, such as chilled ammonia and advanced amines processes, for optimum capture in differing conditions (pressure, temperature, and gas content).¹⁹

²⁴ Alstom. CO₂ Solutions. *Alstom Power*. 2014. [Online] (accessed March 31, 2016)

CCS is also used in tandem with scrubbers and gasification techniques, in order to both remove sulfur and nitrogen pollutants and contain carbon dioxide release.

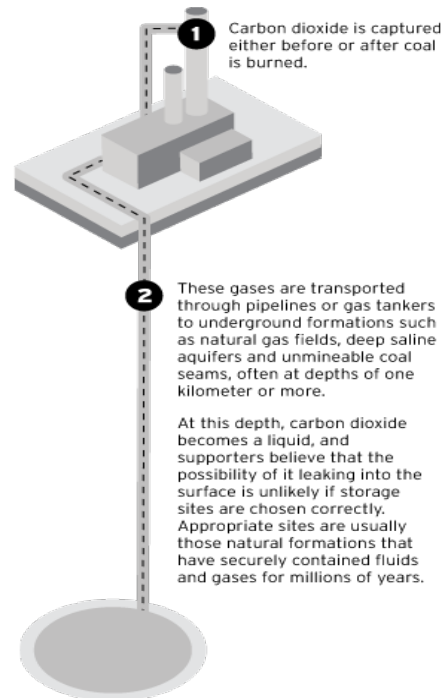


Figure 9 – Basic design of CCS system.

Coal-to-hydrogen is a system that is a direct add-on to the other aforementioned techniques, directing the hydrogen gas remaining after gasification, scrubbing, etc... into storage cells, batteries, for later energy production.¹⁵ Figure 10 illustrates the steps involved in arriving at stored fuel.

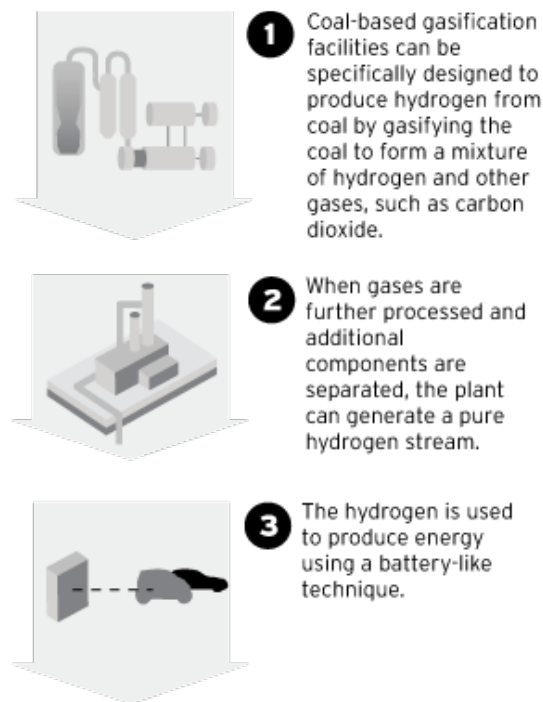


Figure 10 – Basic concept map behind coal cleaning techniques being used to generate hydrogen fuel for battery run systems.

Similar methods to those above can be applied to oil after fractional distillation and burning of fuel fractions (at least at an industrial scale). However, even though there are multitudes of cleaning techniques to remove some of the harmful toxins, some problems persist: not only are each of these applications costly to install and maintain to some significant degree, but an abundance of carbon dioxide is still introduced into the environment (albeit contained, leeching into surrounding soil is possible), carbon capture systems must be replenished with chemicals from outside the system (ruining a closed loop), and scrubbers remove sulfur into a waste salt that must be neutralized. A true solution to the inherent problems of fossil fuels and even their cleaning techniques must be accomplished with a renewable (short turnover of resource availability) and sustainable system (e.g. able to generate all necessary energy consistently with no harm to Earth's tenuous cycles), whether through alternative energy sources and/or other closed loop processes.

Natural gas, the third and final fossil fuel, functions differently than coal and oil processes. Combustion of the gas is of course still necessary, but no prior stages are required to reach the gas phase (no initial heating, boiling, or gasification). Natural gas, being mostly methane, follows the most basic combustion reaction pathway, combining with oxygen to produce water and carbon dioxide. Filtering systems to assure any non-carbon gases, other hydrocarbons, water vapour, etc... are removed are used, and then ignition of natural gas is performed.²⁵ Filtration systems consist most commonly of amine sweetening (use of amines to remove acid gas), dessicant and/or glycol dehydration (removal of water vapour). CCS techniques can then be used to trap the inherently high concentration of carbon dioxide, and deposit it for storage or feedstocks. Due to methane's low carbon:hydrogen ratio (1:4), more water is produced which could then be converted into storable fuels (e.g., hydrogen gas, oxygen, or hydrogen peroxide). Even storage of natural gas itself gives it the ability to be used directly as a fuel source for specially designed vehicles.²⁶ Drawbacks to direct injection in vehicles include high costs to produce specialized vehicles, limited refueling locations, longer fueling times, and shorter driving range, to only note a few. However, natural gas' major drawback (other than low to nonexistent renewability as a fossil fuel) is during mining and retrieval from gas pockets near oil reservoirs using a technique known as fracking.

Hydraulic fracturing (fracking) is the process by which a solution of water, various chemicals, and a propping agent (such as sand, added after injection) are pumped to a high

²⁵ Parker. *Natural Gas Solutions Applications, Features, Specifications*. Parker Hannifin Ltd; Maidstone, Kent UK, 2007. [online] (accessed March 24, 2016)

²⁶ Messersmith, David T. Natural Gas Vehicle Basics. *Penn State Extension*. <http://extension.psu.edu/natural-resources/natural-gas/issues/natural-gas-vehicle-basics> (accessed March 20, 2016)

pressure into a well, over seven thousand feet below ground to reach a viable resource deposit.²⁷ Intense pressure, once it has exceeded the external rock's strength surrounding the well, will create or widen fractures several hundred feet away. Pressure difference then pushes the wastewater used back up the well as the natural gas pockets escape out of the fractures. Figure 11 illustrates the process described.

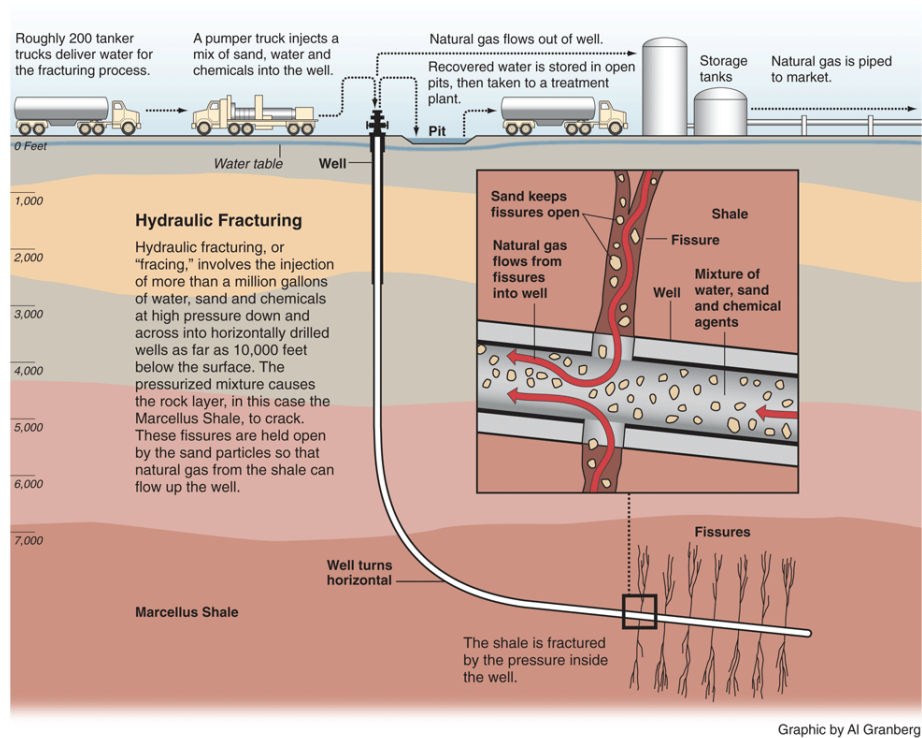


Figure 11 – Hydraulic fracturing process, laid out left to right.

Greenhouse gases are necessary for Earth's survivable climate, but it is the rate at which they are introduced in today's present day era of constant consumption. Natural cycling of these compounds, CO₂ and NO_x, is required to sustain the greenhouse effect in harmony with an organism-friendly atmospheric composition (keeping it warm and breathable), but is a tenuous balance severely disrupted by human's exponential reliance on fossil fuels.⁹ Attempts to "clean" these resources of pollutants have been made with varying degrees of success, but the impact on

²⁷ Hydraulic Fracturing in Lehigh Valley. Lafayette. 2011. <http://sites.lafayette.edu/egrs251-fall1-fracking/> (accessed April 12, 2016)

greenhouse gas levels is an inherent trait of fossil fuels which can only be counteracted through alternative energy: renewable resources that do not increase the greenhouse effect.⁹ Alternative energy in the continued pursuit of mankind's technological advances and desires, whilst leaving the precarious balance of greenhouse gases alone.

Solar, wind, and hydroelectric alternative energy resources each rely on a natural phenomena to trigger movement (of an electron, propeller blades, or water wheels), which in turn can be used to produce electricity from a preexisting source without combustion. Removing the need for non-renewable feedstocks (coal, oil, natural gas, etc...), and feedstocks that upon use release and form pollutants and greenhouse gases, results in a renewable and sustainable set of systems to replace fossil fuels. Many studies have been performed to assess how effective a complete transition to renewable resources would be, and all have found it to be plausible and realistic (albeit perhaps difficult in some ways).^{28,29,30, 31} However, each of these resources currently comes with its share of drawbacks.

Solar energy conversion is achievable in two forms: photovoltaic cells and thermal systems.³² Photovoltaic cells use n-type (negative) and p-type (positive) conductors, usually silicon doped with phosphorous and boron, respectively, layered on top of each other. As

²⁸ Jacobson, Mark Z. et al. Examining the feasibility of converting New York State's all-purpose energy infrastructure to one using wind, water, and sunlight. *Energy Policy*. **2016**, 57, 585-601

²⁹ MIT Energy Initiative. The Future of Solar Energy: An Interdisciplinary MIT Study led by the MIT Energy Initiative. MIT, **2015**. [online] <https://mitei.mit.edu/futureofsolar> (accessed April 20th, 2016)

³⁰ U.S. Department of Energy. *20% Wind Energy by 2030 Increasing Wind Energy's Contribution to U.S. Electricity Supply*. DOE: Energy Efficiency and Renewable Energy. 2008. [online] http://energysystems.princeton.edu/EnergyResources/20percent_wind_energy_report_revOct08.pdf (accessed March 29th, 2016)

³¹ Bailey, Tim. *Hydroelectric Feasibility Study: An Assessment of the Feasibility of Generating Electric Power Using Urban Stormwater in Oregon City*. Oregon Institute of Technology. 2009. [online] (accessed March 23rd, 2016)

³² Penn State College of Agricultural Studies. *Renewable and Alternative Energy: Solar Energy*. PennState Extension. 2016. [online] <http://extension.psu.edu/natural-resources/energy/solar-energy> (accessed April 15th, 2016)

photons strike the p-type conductor, electrons are released into the n-type layer, causing an electron flow (Figure 12).

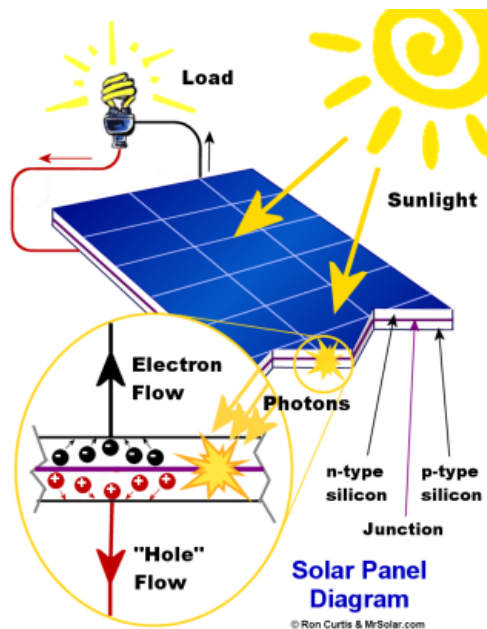


Figure 12 – Illustration of solar panel layering, n-type and p-type layers, and how photons trigger electron to jump from p to n-type, driving current. (image courtesy of McDowell at Blogs.cas.suffolk.edu)

Thermal systems, however, utilize heat from the sun. Heat is stored either actively or passively; heating water tanks is a form of an active system, while methods of storing the heat to be released during the cooler nights is a passive system. Yet while solar energy needs no initial or continuous feedstock, it relies on an intermittent source; cloudy days and nighttime cut the source, and would leave devices reliant on solar unable to function. Storage systems such as batteries, synthesis of green fuels, or pumping water uphill can help with these gaps by storing excess energy over time, but these techniques must be improved further.³³

³³ *Engineers Develop New Grid-Level Energy Storage Technology*. Drexel University. 2012 [online] <http://drexel.edu/now/archive/2012/July/Engineers-Develop-New-Grid-Level-Energy-Storage-Technology/> (accessed April 21st, 2016)

Wind power harnessing the motive force of air currents by directing them to rotate turbines (Figure 13). Turbine cycling generates an electric field, which in turn creates current for electricity.³⁴ Viable air currents lie hundreds of feet above the surface onshore, or the same offshore. Drawbacks for wind as a resource revolve largely around the structures that must be built; large structures, hundreds of feet tall, must be erected to reach these air currents, which hold an arsenal of problems to manufacture, along with a general eye sore to the public (albeit some myths have spread negative opinions on the matter).³⁵ Designs have been improved over the years.

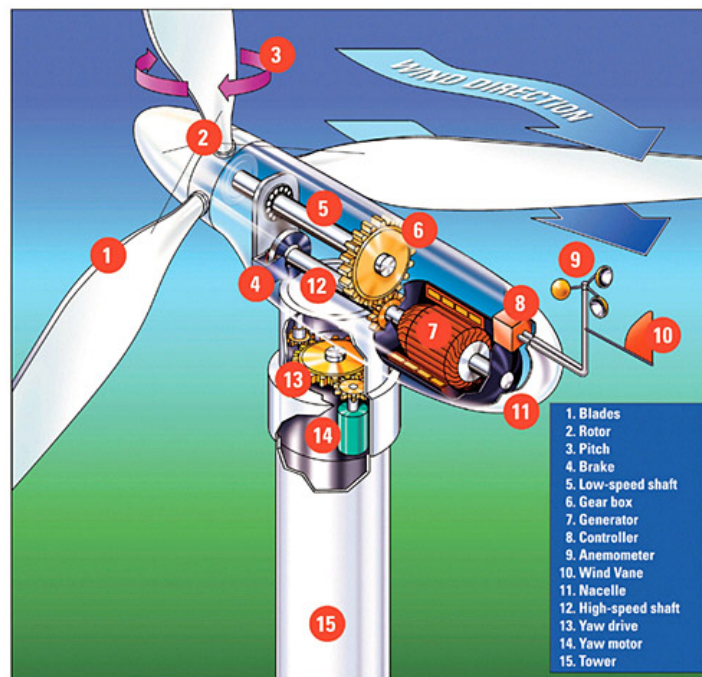


Figure 13 – Schematic slice of a wind turbine.

³⁴ North Carolina Wind Energy. *Wind Turbines*. Appalachian State University. 2016 [online] <http://wind.appstate.edu/wind-power/wind-turbines> (accessed March 28th, 2016)

³⁵ Wind Powering America. *Wind Energy Myths*. GE Wind Energy. 2005. [online] [http://wind.jmu.edu/documents/Wind%20Energy%20Myths%20\(2005\).pdf](http://wind.jmu.edu/documents/Wind%20Energy%20Myths%20(2005).pdf) (accessed April 11th, 2016)

Hydroelectric systems perform using the same turbine design as wind power, and use either man-made canals or dams (Figure 14).³⁶ Hydroelectric systems in various forms have been around for thousands of years, dating back to 250 B.C.³⁷ Drawbacks for hydroelectric revolve around the impacts to upstream aquatic and terrestrial life, as damming can cause rapid flooding to occur, washing away soil and vegetation.³⁸

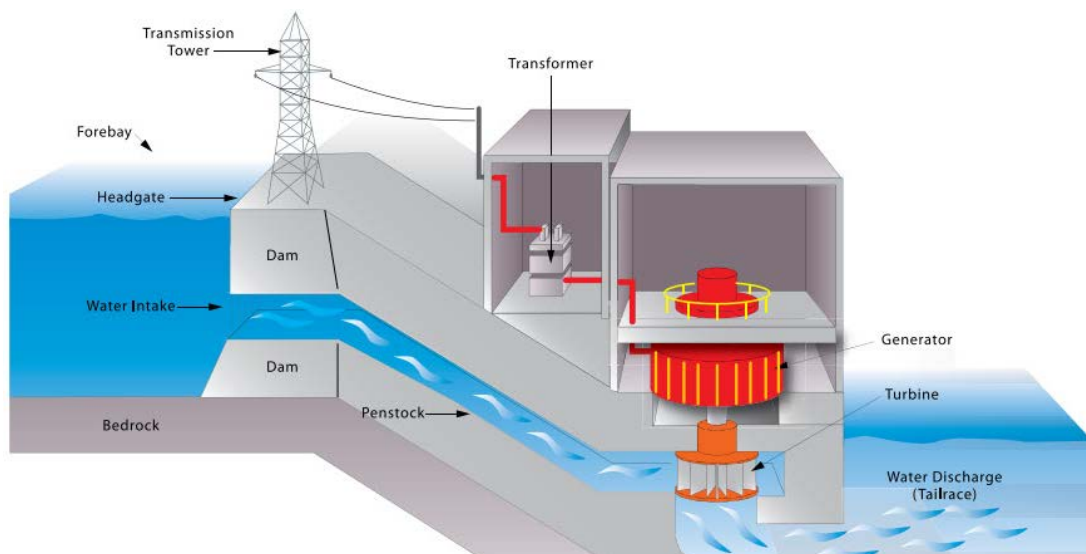


Figure 14 – Schematic slice of hydroelectric dam.

Each alternative energy resource does require a significant amount of initial investment, both time and money, in order to obtain the long term benefits, and many groups and individuals may have difficulty supplying this investment. In order to create a smoother transition from nearly complete reliance on fossil fuels to a complete reliance on combined solar, wind, and hydroelectric sources, an intermediary fuel source should be used. Instead of a drastic change from fueling a vehicle with gasoline to leaving it in the sun or remembering to plug it in during

³⁶ U.S. Energy Information Administration. *Hydropower Basics: Energy from moving water*. EIA. 2008. [online] https://www.eia.gov/kids/energy.cfm?page=hydropower_home-basics-k.cfm (accessed April 11th, 2016)

³⁷ Jeske, Jacob. *Hydroelectricity*. Hesston college. [online] http://www2.hesston.edu/Physics/hydroelectricityjeske/research_paper.htm (accessed March 25th, 2016)

³⁸ Levitan, Dave. *As Small Hydropower Expands, So Does Caution on its Impacts*. Yale: Environment360. 2014. [online] http://e360.yale.edu/feature/as_small_hydropower_expands_so_does_caution_on_its_impacts/2790/ (accessed May 1st, 2016)

cloudy days, biodiesel would only require making an adjustment of a few engine valves before continuing a normal pattern of filling up at the gas station. Now, however, it would be a more environmentally friendly energy source supplying your early morning commute. Government agencies and industries are already moving towards implementation of biodiesel (or biofuels, more generally) as a transitional step between removing fossil fuel dependency and using solar, wind, or hydroelectric.^{39,40,41,42}

1.2 *Biodiesel (FAME)*

1.2.1 Overview

Dr. Rudolph Diesel invented the diesel engine in the 1890s to improve upon “inefficient, cumbersome and sometimes dangerous steam engines,” and had originally been designed to run on fuels ranging from “kerosene to coal dust.”^{43,44,45} Diesel engines were made on the principle of compression ignition, where fuel is injected into a piston pressurized cylinder and ignited to cause retreat of the piston, generating mechanical energy. Vegetable oils had been envisioned as the fuel for “remote areas of the world” and were seen in use as early as 1900 (at the World Fair,

³⁹ U.S. Environmental Protection Agency. *Renewable Fuel Standard Program*. EPA. 2015. [online] <https://www.epa.gov/renewable-fuel-standard-program/program-overview-renewable-fuel-standard-program> (accessed April 27th, 2016)

⁴⁰ Sparks, Caitlyn and Prasino Group. *Bioenergy/Biofuels Environmental Assets Strategies and Tactics*. EPA. 2015. [online] https://www.epa.gov/sites/production/files/2015-09/documents/presentation_sparks.pdf (accessed April 5th, 2016)

⁴¹ European Biofuels Technology Platform. *Biofuels Policy and Legislation*. EBTP-SABS. 2014. [online] <http://biofuelstp.eu/biofuels-legislation.html> (accessed April 7th, 2016)

⁴² *Biofuels*. European Energy Commission. 2016. [online] <https://ec.europa.eu/energy/en/topics/renewable-energy/biofuels> (accessed May 2nd, 2016)

⁴³ *History of Biodiesel Fuel*. Pacific Biodiesel. 2016. [online] <http://www.biodiesel.com/biodiesel/history/> (accessed May 2nd, 2016)

⁴⁴ *Recognizing Rudolf Diesel's foresight in Celebrating Biodiesel Day*. Biodiesel America's Advanced Biofuel. 2012. [online] <http://biodiesel.org/news/news-display/2012/03/18/recognizing-rudolf-diesel's-foresight-in-celebrating-biodiesel-day> (accessed May 3rd, 2016)

⁴⁵ *Biodiesel: Biodiesel 101*.

using peanut oil), but due to the availability and low costs of petroleum diesel fuel, fuels synthesized from vegetable oils were put aside until only recently.

Development of the biodiesel industry began with greater earnest around the early 1990's, with research in Europe and South Africa leading the charge. Rising oil prices worldwide have sparked an even greater increase in biodiesel alternatives, opening the public's eyes to its benefits as a sustainable fuel.⁴¹

1.2.2 Biodiesel Benefits

Biodiesel, an alternative fuel made from renewable vegetable oils, can be used in diesel vehicles without engine modifications (only slight modifications with gasoline vehicles), and results in reduced emissions of unburned hydrocarbons, carbon monoxide, particulate matter and nitrogen oxides, plus reduction of greenhouse gas emissions.⁴⁶

Diesel engines can run on 80/20 (known as B20) mix of petroleum diesel to biodiesel, and with few mechanical design adjustments can run 100% (B100) biodiesel.⁴⁷ Stocks of diesel at gas stations could simply be replaced with higher levels of biodiesel as engines are slowly manufactured to avoid clogging/clouding with the more viscous fuel. Implementation of that exact process was stimulated by an EPA regulation.³⁷

Biodiesel, unlike the other renewable energy sources, is still a liquid fuel that is ignited to generate an electromotive force in a vehicle. Instead of learning how to properly care for a new type of vehicle run by solar energy, for example, and learning all its quirks, consumers need only

⁴⁶ Kemp, W.H., *Biodiesel Basics and Beyond: A Comprehensive Guide to Production and Use for the Home and Farm*, Aztext Press, 2006.

⁴⁷ King, Angela G. and Marcus W. Wright. "Rudolph Diesel Meets the Soybean: "Greasing" the Wheels of Chemical Education." *Journal of Chemical Education* 84 (2007): 203-206.

to have an engine modification that in some cases might involve replacement of a few valves, allowing for a smoother transition into “cleaner” fuels when compared to buying a brand new vehicle. Of course, not all engines will readily accept certain ratios of biodiesel in all climates (as mentioned, a higher viscosity than gasoline causes clogging and clouding at low temperatures), so variations in fuel supplies would also have to occur.

1.2.3 Combustion Process

Combustion is the process in which fuel is burned, releasing energy and creating new chemical substances categorized as exhaust gas (Figure 15).⁴⁸ Fuels are chosen based on the energy stored within chemical bonds, how effectively they can be burned, and physical state (solid, liquid, or gas). Oxidizers can also be a solid, liquid or gas, although in automobiles it is simply air (21% Oxygen and 79% Nitrogen). Oxygen in the air then reacts with organic fuel, and results in carbon dioxide as an exhaust gas. However, usage of air as an oxidant allows fuels to react with nitrogen content under certain pressure and temperature, resulting in NO_x formation as well. NO_x formation can be avoided in two ways: controlled oxidants, or further control over combustion environment (such as minimizing pressure and temperature).

⁴⁸ Glenn Research Center. *Combustion*. NASA. 2015. [online] <https://www.grc.nasa.gov/www/k-12/airplane/combst1.html> (accessed March 15th, 2016)

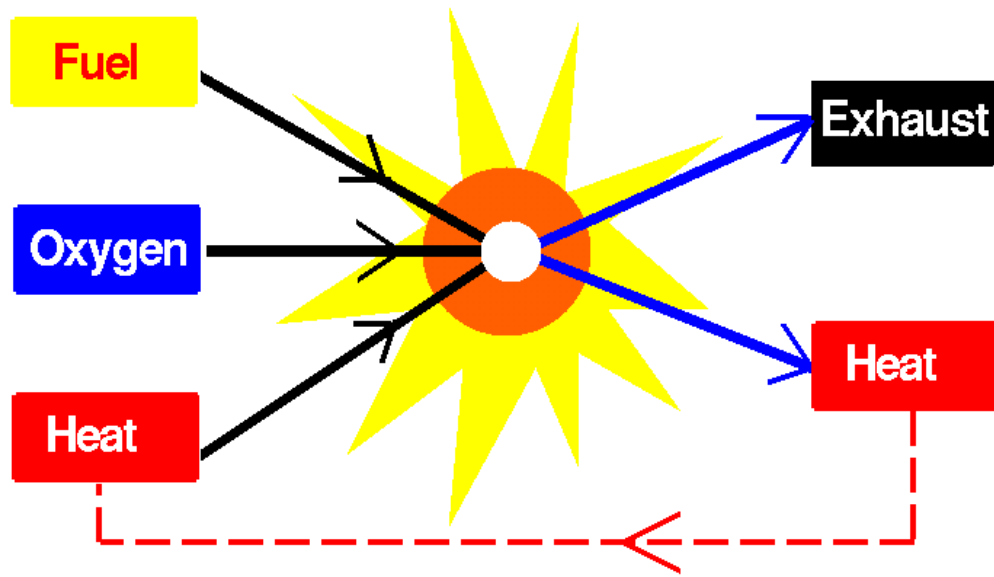


Figure 15 – Diagram of combustion process. Fuel, oxygen and heat combine to trigger burning/ignition, releasing heat and exhaust gases (CO_2 , NO_x , etc...). Image courtesy of NASA.

Low-temperature burning of fuels prevents formation of nitrogen oxides. Engines designed to do this are known as Homogeneous Charge Compression Ignition (HCCI) engines, and involve premixing fuel and oxidant in a lean mix, and then using compression to ignite versus a spark.^{49,50} Other variations of this include diesel low-temperature compression (LTC), or improved designs such as Reactivity Controlled Compression Ignition (RCCI) and Premixed Charge Compression Ignition (PCCI).

1.3 Synthesizing Biodiesel and Constituents Involved

1.3.1 Transesterification

⁴⁹ Dec, John E. Advanced Compression Ignition Engines – Understanding the In-cylinder Processes. *The Combustion Institute. Elsevier*. **2009**. 32, 2727 – 2742.

⁵⁰ McIlroy, Andrew; Greg McRae et al. *Basic Research Needs for Clean and Efficient Combustion of 21st Century Transportation Fuels*. U.S. Department of Energy. [online] http://www.sc.doe.gov/bes/reports/files/CTF_rpt.pdf (accessed April 22nd, 2016)

Transesterification is a simple chemical transformation taught in college organic chemistry (Figure 16)⁵¹, however the transesterification of triacylglycerides is complex. Triacylglycerides of long chain fatty acids have complex conformations, reaction solubility challenges, and uncertainty as to the sequence of tri-ester exchange. It is not clear, for example, if there is rate limiting exchange of the terminal ester groups (1 vs 3) versus the middle or second ester group (C2), Figure 17.

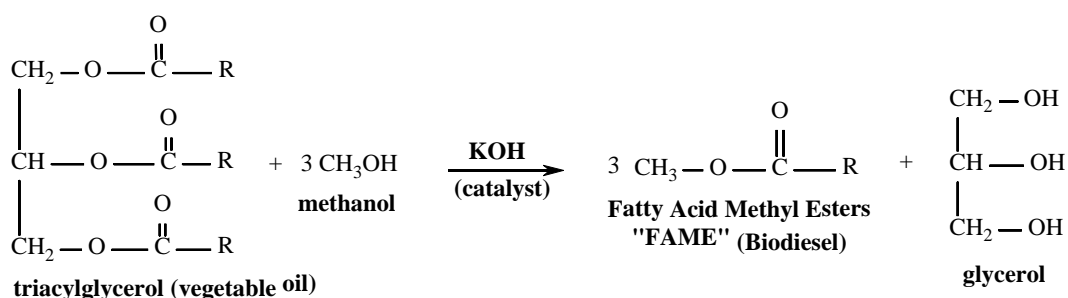


Figure 16: Chemical reaction for the transesterification of vegetable oil to biodiesel.

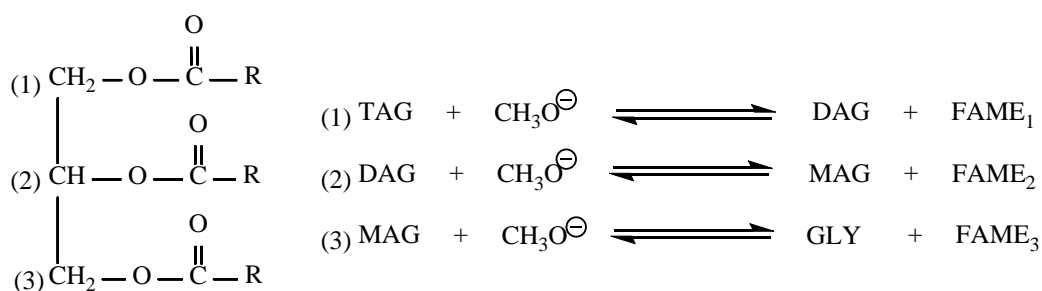


Figure 17. Proposed sequence for triacyl glyceride transesterification with methoxide: TAG = triacylglyceride, DAG = diacylglyceride, MAG = monoacylglyceride, GLY = glycerol, FAME1,2,3 = fatty acid methyl esters (biodiesel).

Via NMR spectroscopy, C1/3 and C2 protons are difficult to separately observe due to overlapping signals in complex triacylglycerides. Analysis of the three separate acyl glyceride

⁵¹ Behnia, M.S., Emerson, D.W., Steinberg, S.M., Alwis, R.M., Duenas, J.A. and Serafino, J.O. (2011), "A Simple, Safe Method for Preparation of Biodiesel," *J. Chem. Ed.*, 88(9):1290–1292.

reactions (Figure 17, mono-, di-, and tri-) would result in a validation or rejection of the proposed sequence of base catalyzed transesterification, by simplifying observable spectra.

1.3.2 Drawbacks

Observed over 10 years of compiled group research, biodiesel's benefits compete constantly with the inefficiency of the overall chemical process. Waste production, energy input, and time are only a few of these inefficiencies.⁵² Transesterification of pure vegetable oil requires at least one hour of heating at high temperatures (using significant amounts of energy), and the glycerol byproduct (essentially waste) becomes exponentially greater as the reaction is scaled up from benchtop to industrial scale. In tandem with that, the catalysts used are caustic (in both base and acid catalyzed reactions, discussed later). A deeper understanding of the time course and mechanism of transesterification would be very beneficial in solving these issues through the application of green chemistry principles.

1.4 *Significance and Purpose*

1.4.1 Understanding Reaction Mechanisms

Chemical reactions are best represented as a step by step sequence of elementary reactions that help describe the overall chemical change occurring. Understanding the vegetable oil transesterification mechanism is important as the mechanism allows a chemist to predict the products of similar reactions, provides information as to how the reaction can be controlled, and

⁵² Agnew, R., Chai, M., Lu, M. and Dendramis, N. (2009), "Making Biodiesel from Recycled Cooking Oil Generated in Campus Dining Facilities," *Sustainability: The Journal of Record*. 2(5): 303-307.

may give insight into catalyst design. The “rate limiting step” controls the rate, or how fast reactants are converted into products. The rate of a chemical reaction is determined using the mathematics of chemical kinetics, a tool that allows us to study how different reaction parameters (temperature, pH, solvent, etc.) affect the rate of the reaction. We can also determine kinetic constants called the “reaction order” and “rate constant” for the reaction. Taken together, these kinetic data can be used to identify the most likely mechanism for a chemical reaction. Generalizations and reasonable conjectures of reaction mechanisms are only a temporary and fragile step towards truly understanding how a set of chemical species interact. By having a clear and concrete electron flow mechanism of the transesterification reaction, proposed catalysts can be assessed before benchtop experimentation, saving time and money while improving biodiesel synthesis.

1.4.2 Tracking Kinetics with Instrumentation

Each instrument has the ability to track the kinetic properties of a chemical reaction, but to differing degrees of accuracy, precision, and usefulness. Many instruments regularly used for quantification purposes are rated to a certain level of detection, such as a gas chromatogram mass spectrometer (GC/MS) or flame atomic absorption spectroscopy (FAAS) which are rated to parts per billion and parts per trillion (ppb and ppt, equivalent to $\mu\text{g/L}$ and ng/L) analyte concentrations. Property of the chemical process’s analyte drives instrumentation choice for kinetic studies. In other words, characteristics that stand out allow for simpler detection and measurement: unique proton signals in the reaction mixture, significantly larger mass than other

compounds in the matrix, or a color that corresponds its intensity directly to the concentration of analyte.

Once an instrument has been chosen, a calibration curve must be generated via external or internal reference standard(s) in order to quantify the intensity of signals that will be received by the instrument's detector. Calibration curves simultaneously demonstrate detection limits and sensitivity of the instrument, and are used to determine concentration levels of analyte (e.g., fatty acid methyl esters and mono-/di-/triacylglycerol). Aliquots from a reaction mixture are retrieved over time (during the reaction) and quenched, creating a "frozen" sample of the reaction at that time point. A kinetic plot may be generated by running samples through chosen instrumentation and calculating concentrations based on calibration curves (illustrated in Results).

1.4.3 Improving Biodiesel's Viability

Over the past 10 years our research group has been studying the chemistry of small-scale biodiesel synthesis by base-catalyzed transesterification of vegetable oils, the traditional method, and determined that this process is highly inefficient, wasteful, and therefore requires improvement. A detailed kinetic model for glyceryl fatty acid transesterification has not been developed; this information could be important for better understanding the chemical process, and in the design of specific catalysts for transesterification.

^1H NMR spectrometry is a rapid, quantitative instrumental method for time-based monitoring of transesterification reactions based on the integration of select proton signals.

Although vegetable oil transesterification into methyl esters has been studied by ^1H NMR^{53,54,55}, very little work has been done studying the reaction progress and kinetics by this method. The goal of this research was to develop a ^1H NMR experimental method to study the time course of vegetable oil transesterification, which may eventually lead to a detailed kinetic model that would identify whether rate limiting transesterification occurs at C1/3 or C2 as the glyceryl triester is converted into the di- and mono- ester, and finally free glycerol. Application of new catalysts or conditions for synthesizing biodiesel could then be performed in a more structured manner, improving efficiency and sustainability of the process at a faster pace.

2. Materials and Methods

2.1 Materials

All reagents were purchased from Sigma-Aldrich or Fisher Scientific and used without further purification. Transesterification reactions were conducted using store bought brand name soybean oil. Methanol was purchased at 99.9% purity. Potassium hydroxide catalyst was purchased in granular form. All glassware was washed with Micro-90 cleaner, and then rinsed with deionized water and acetone before oven drying at 80°C. Syringes were flushed with Micro-90, water, then acetone, and plunger was separated from syringe for air drying overnight (a vacuum system may also be used for proper cleaning).

⁵³ Morgenstern, Mark; Cline, Jessica; Meyer, Sally; and Cataldo, Simon. Determination of the Kinetics of Biodiesel Production Using Proton Nuclear Magnetic Resonance Spectroscopy (^1H NMR). *Energy & Fuels*. **2006**, 20, 1350-1353

⁵⁴ Guillen, Maria; and Ruiz, Ainhoa. High resolution ^1H nuclear magnetic resonance in the study of edible oils and fats. *Trends in Food Science & Technology*. **2001**, 12, 328-338

⁵⁵ Knothe, G. ^1H -NMR Spectroscopy of Fatty Acids and Their Derivatives: Quantification by ^1H -NMR. *National Center for Agricultural Utilization Research, Agricultural Research Service*. **2005**.
<http://lipidlibrary.aocs.org/nmr/1NMRquan/file.pdf> (accessed March 20, 2015)

2.2 *Synthesis of FAME (to equilibrium)*

2.2.1 Procedure for Vegetable Oil Transesterification

Transesterification reactions were run at constant temperature via regulated water bath (60°C or 25°C) using a standard 25 mL batch method (25.1 mmole triglyceride) with 3:1 mole ratios of methanol:ester, and 2.51 mmole potassium hydroxide as catalyst. In order to generate potassium methoxide, potassium hydroxide catalyst was dissolved in 3.2 mL methanol. Reactions reached equilibrium (characterized by a leveling off of kinetic plot), and no excess potassium methoxide was added to attain completion. Aliquots were removed at timed intervals and quenched by dilution in acetone-d₆ containing 0.05% TMS. The molecular mass of the triglyceride (vegetable oil) and biodiesel product were estimated based on the molecular mass of oleic acid as a model fatty acid.

2.2.2 Aliquot and Quenching Technique

Good signal-to-noise ratio was obtained using 1.0 mL of acetone-d₆ to quench 25 µL reaction aliquots. Aliquot volumes were measured with gas-tight syringes and flushed thoroughly between each aliquot to avoid contamination. Aliquot volumes were also measured with Finnipipette F2 (20-200uL) to acquire greater samples in a smaller time frame. Results of each were later compared to assess precision, which was >95%. Immediately after quenching, ¹H NMR spectra were collected, and then scanned again after twenty-four and forty-eight hours. No

changes in NMR spectra were observed over a 48 hour period, suggesting quenching was successful.

2.3 Analytical Methods

2.3.1 Quantitative Nuclear Magnetic Resonance (qNMR)

Nuclear Magnetic Resonance (NMR) spectra were obtained on a JEOL ECX-400 MHz instrument using default parameters and settings for sample spin angle and radio frequency pulse sequences. Number of scans was adjusted to 16, and gain was held below 20 units. Quantitative NMR (qNMR)⁵⁶ was used to determine reaction progress based on theoretical percent yield. In qNMR the quantity of a particular analyte (biodiesel) is determined by comparing the integrated value of a known internal standard signal (normalized to known number of protons, e.g. 2H for maleic acid) to the integrated value of the analyte (scaled by normalization). Equations 1 and 2 demonstrate the calculations done to determine what values were expected and how to back calculate for the concentration of biodiesel in the sample, respectively. Analyte concentration versus time was plotted for reaction rate analysis. Microsoft Excel was used for analyzing the kinetic data.

$$1) \left(\frac{x \text{ mmol Biodiesel}}{y \text{ mmol Maleic Acid}} \right) * 3 = \text{Expected Integration FAME}$$

$$2) \left(\frac{\text{Integration FAME}}{3} \right) * y \text{ mmol Maleic Acid} = x \text{ mmol Biodiesel}$$

⁵⁶ Peterson, J., "1H NMR Analysis of Mixtures Using Internal Standards," *J. Chem. Educ.* **1992**, 69 (10): 843-5.

Equation 1 and 2. Equations used to determine reaction progress by Quantitative NMR. “3H” is the theoretical intensity of proton signals from biodiesel.

2.3.2 Internal Reference and Calibration

Maleic acid (Figure 18) was used as the reference standard (vinyl protons at 6.3 ppm, 2H)⁵⁷, to quantitate the biodiesel formed. Quantification was based on the appearance of the -OCH₃ methyl ester signal at 3.6 ppm (3H) in relation to the vinyl protons of maleic acid normalized to 2H at 6.3 ppm. Maleic acid calibration standards were created by adding incremental volumes (5, 10, 15, 20, 25 uL) of biodiesel to a fixed 25 uL volume of 0.689 M maleic acid. Normalization of the maleic acid signal for each sample was compared to integrations of the -OCH₃ methyl ester signal (3.6 ppm), and that relationship was compared to theoretical integration. A linear relationship with correlation $R^2 = 0.9749$ was obtained (Figure 19).

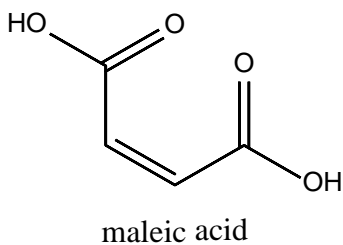


Figure 18 – Maleic Acid Structure. Alkene/vinyl protons are identified at 6.3 ppm.

⁵⁷ JEOL Resonance Application Note NM090009. “What is qNMR (quantitative NMR)?” **2011**. <http://www.j-resonance.com/en/images/application/nmr/nm090009e.pdf> (accessed January 2016).

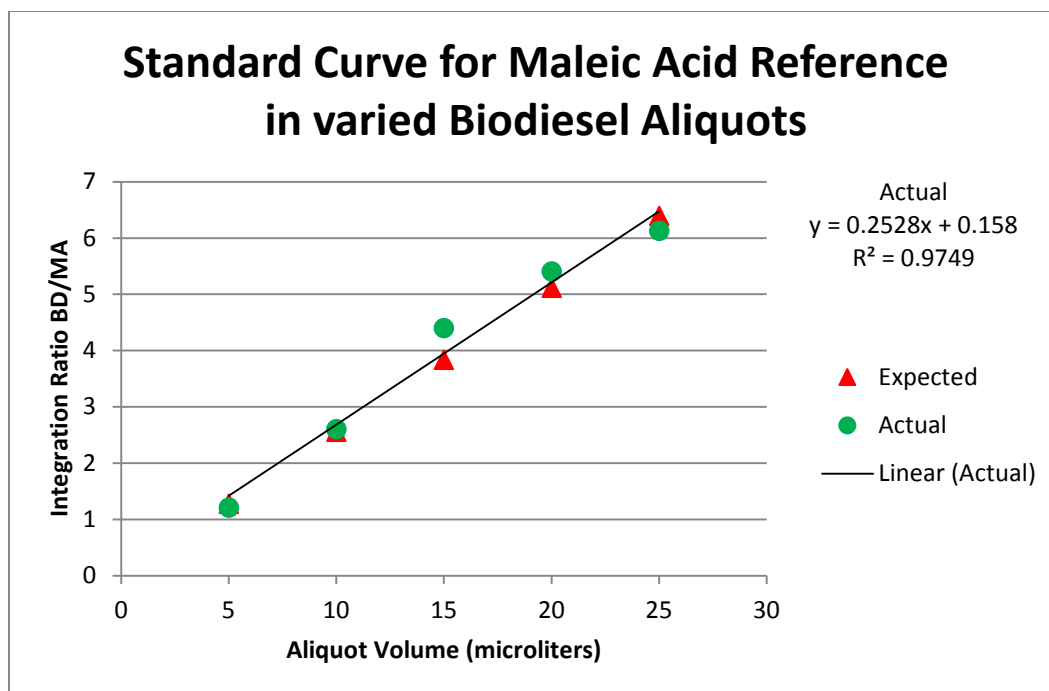


Figure 19 – Aliquot volume of biodiesel versus the ratio between biodiesel and maleic acid integrations (maleic acid at constant 2H). Ratio was used for both expected calculation results (red) and actual scanned data (green), and overlaid here as one plot to determine method precision ($R^2 = 0.9749$, about 97.5% linear relationship).

3. Results

3.1 Quenching and Internal Reference Effectiveness

Acetone-d₆ was chosen based on supply, and found to effectively and completely quench the transesterification reaction, with no evidence of side reactions over a 48 hour period. No other solvents were tested. Also, maleic acid functions as a suitable internal reference for qNMR analysis due to a well separated downfield signal at 6.3 ppm. This resulted in easy and accurate calculations (97.5% correlation to theoretical values) of methyl ester concentrations.

3.2 Temperature Control and Adjustments

The rates of triacylglyceride transesterification were examined at 60°C and 25°C.

Transesterification kinetics studies were initially attempted at 60°C as this is the typical temperature for benchtop and industrial scale biodiesel production. Unfortunately, initial rate of transesterification at 60°C was found to be too fast to measure by our sampling method, and that equilibrium was reached within one minute (data not shown). However, at 25°C a rapid initial rate was clearly observed that was linear for approximately 25-30% of the reaction as shown by the concentration-time data graphed in Figure 20. The rapid initial rate from 0 to 25 minutes was followed by a slower reaction phase which reaches equilibrium in approximately 3 hours.

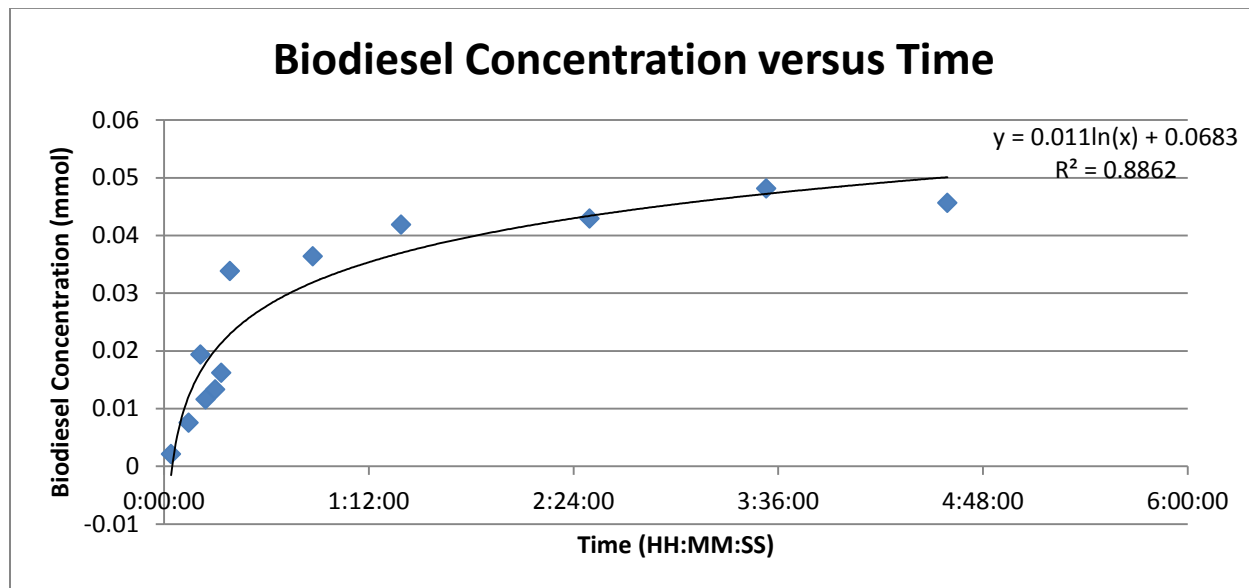


Figure 20 – Original kinetic plot of biodiesel transesterification. R^2 value of 0.8862 implies some discrepancies, likely due to temperature misregulation. Burst phase occurs between 0 to 25 minutes.

Upon further assessment of the reaction, and tightening temperature regulation, equilibrium was reached much more rapidly after potassium methoxide addition, with a burst phase from 0 to 7 minutes that consisted of 45-50% of the reaction. Figure 21 illustrates the most comprehensive tests, with a correlation of 0.9451, illustrating the consistency of the experiment

and removal of previous error, as well as the clear burst phase. Equilibrium was reached within approximately 1.5 hours.

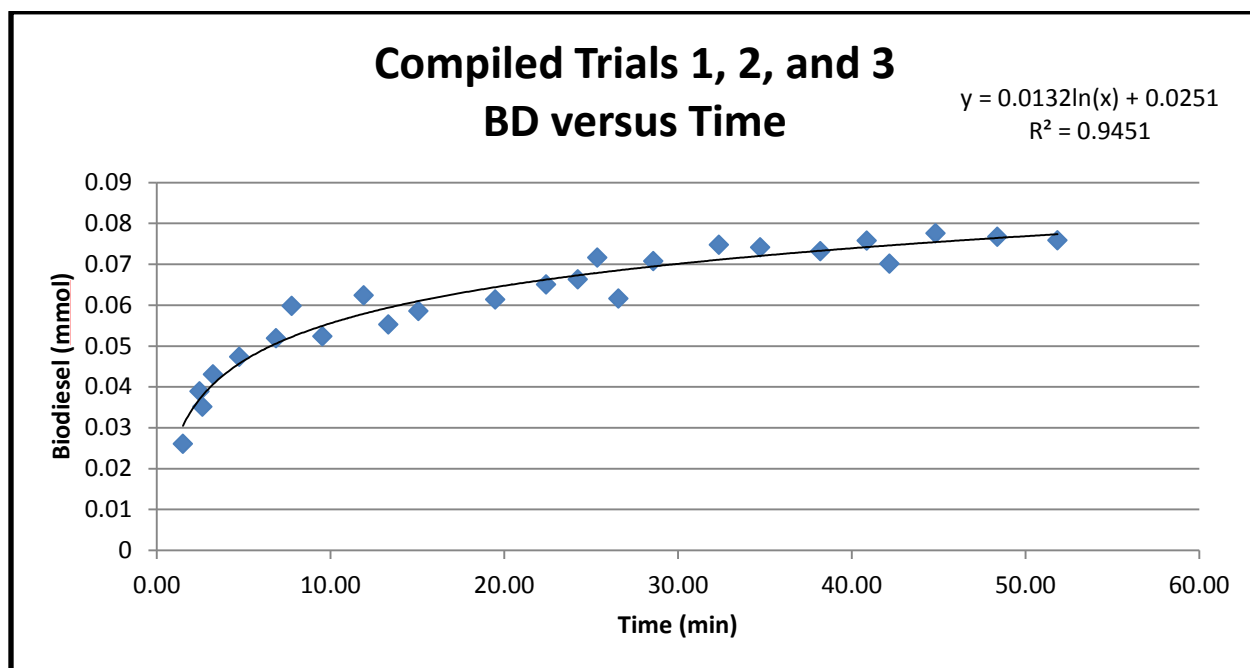


Figure 21 – Cumulative Kinetic Plot of biodiesel transesterification. R^2 value of 0.9451 implies each run was equivalent, and burst phase (<7 minutes) was consistent.

3.3 1D and 2D qNMR Assessment

1D proton qNMR was successfully done for all samples (examples in Appendix), and integrations were converted into usable concentrations of biodiesel using the $-\text{OCH}_3$ methyl ester signal at 3.6 ppm (Table 1). C1/3 and C2 signals were also assessed, with focus on the burst phase described above. Comparison of the C1/3 and C2 proton signal locations (4.1, 4.3, and 5.3 ppm respectively) revealed the clear disappearance of C1/3 during the burst phase, and no significant change in the C2 signal location. Even though other signals overlap the C2 (which is why direct quantification there is not recommended) such as alkene protons on oleic and linoleic

acid chains³⁰⁻³², the relationships between signals appeared consistent, so changes in individual signal intensity were not observed.

Table 1 – Expected calculations obtained through equations 1 and 2, determining expected integrations of methyl ester peaks. Actual integrations were then obtained and plugged in to this setup, and all experimental values were <6% error (excluding one outlier).

Expected Graph						
Volume BD Aliquot (μL)	mmol BD	mmol MA	BD/MA	Expected Integration BD	Ratio Value (per 2H MA)	
5	0.01484	0.0174	0.85286	2.559	1.2792929	
10	0.02968	0.0174	1.70572	5.117	2.5585858	
15	0.04452	0.0174	2.55859	7.676	3.8378787	
20	0.05936	0.0174	3.41145	10.234	5.1171716	
25	0.07420	0.0174	4.26431	12.793	6.3964645	
Actual Graph						
Volume BD Aliquot (μL)	mmol BD	mmol MA	BD/MA	Actual Integration BD	Ratio Value (per 2H MA)	% error
5	0.01484	0.0174	0.85286	2.42	1.21	5.73
10	0.02968	0.0174	1.70572	5.21	2.605	1.78
15	0.04452	0.0174	2.55859	8.8	4.4	12.78
20	0.05936	0.0174	3.41145	10.81	5.405	5.33
25	0.07420	0.0174	4.26431	12.26	6.13	4.35

2D Cosy techniques were applied to intermittent samples: one or two from the initial stages of the burst phase and one or two from the equilibrium afterwards (data not shown). Spectra were compared for disappearance of proton-proton interactions between acylglycerides as each branch was esterified into biodiesel. Hints of weakening intensity were observed at certain locations, although overall no noticeable loss of interaction was recorded. Further experiments with 2D correlations, such as between carbon-13 and proton signals, need to be performed and investigated to fully validate conjectures.

3.4 Determination of Rate Limiting Exchange Ester(s)

Due to a noticeable burst phase present in the kinetic plot, which is undetectable at higher temperatures, there must be a limiting step that slows the reaction down before completion such

as difficulty removing the C1/3 or C2 acylglyceride groups, or abundant biodiesel sterically hindering potassium methoxide from reaching the final acylglycerides. Analysis of 1D proton qNMR revealed that C1/3 signals vanish during the burst phase, suggesting that the terminal chains are more susceptible to esterification than the C2 chain. Also, since the C1/3 signal does not increase in intensity over time or regain any lost intensity, rearrangement from C2 to C1/3 suggested by some computational models can be ruled out from base catalyzed transesterification (although not necessarily syntheses involving other catalysts).⁵⁸

4. Discussion

To our knowledge, this is the first time a rapid initial rate phase has been reported for vegetable oil transesterification. Since our data suggests that this burst phase during the first 15 minutes or less after KOH/MeOH addition accounts for approximately 45-50% of maximum yield, it is tempting to speculate that this implies a rapid exchange of a C1 glyceryl methylene ester group, followed by slower (rate limiting) exchange of the remaining two glyceryl esters. However, further studies would have to be done to nullify other possibilities completely. Mono-/di-/tri-substituted acylglycerides would have to be used as starting reagents to clearly observe loss of either C1/3 or C2 signals.

The equilibrium concentration of biodiesel produced at 25°C is approximately 70% of theoretical yield, and underlies the importance for using an excess of methanol and removing the glycerol product to “force” the reaction to completion. Equilibrium greater than 50% also implies favorability of the products over reactants.

⁵⁸ Li, Kaixin; Yang, Zhihong; Jun Zhao et al. Mechanistic and kinetic studies on biodiesel production catalyzed by an efficient pyridinium based ionic liquid. *Green Chem.* Royal Society of Chemistry. **2015**, 17, 4271-4280.

Proper regulation of temperature and stirring was completed in a water bath using a variac, temperature controller and heat tape. Effective water bath temperature control resulted in increased rates suggesting that air cooling/conditioning may have been a factor in the observed slower rates during initial experiments. Signal to noise ratios were adjusted by increasing dilution of aliquot, and signal enhancement was done by adjusting fourier transform parameters of Delta program.

A simple, efficient and reproducible qNMR analytical method to monitor the transesterification of fatty acyl triglycerides was developed. This method can be used to further understand the kinetics and mechanism of small-scale biodiesel synthesis, and facilitate the optimization of reaction parameters and screening of new catalysts. Along with that, qNMR methodology can be modified to other kinetic studies by simply adjusting ratios between an internal standard and analyte signal(s).

Our results also suggest that room temperature (25°C) is preferred for rate studies on fatty acyl transesterification reactions, but more must be done in order to finalize rate analysis and determining reaction order. Our ongoing work is focused on determining the product distribution over the reaction time course. The complex mixture may contain tri-, di-, and mono-glycerides, free glycerol, biodiesel (methyl ester) and unreacted methanol.

For 2D NMR, scans were successful on aliquots selected and mono-/diesters, allowing for retrieval of reference spectra for specific C1/3 or C2 signals and characteristic peaks. Notable changes (although not quantitated) in the C1/3 peaks and not the C2 signal location, both in 2D and 1D spectra, imply that C1/3 chains are removed during the burst phase. However, due the high cost of even micro-scale volumes of certain esters (most importantly, the C2), micro-scale batch reactions were improbable with available equipment, so full analysis of each ester was not

completed with 2D NMR. Application of a controlled ampule environment with sonication instead of physical stirring may allow for the above micro-scale reaction to be performed.

Although full kinetic analysis of mono-, di-, and tri-esters were unable to be performed, preliminary ChemBio3D calculations (MM2) were executed to determine most favored structures and energy state of the ester (Appendix). Online calculations of pKa for target carbons was also performed, but no significant observations were made (Appendix).

5. Application

5.1 Catalyst Research

5.1.1 Catalysts

Catalysts are compounds or elements that lower the activation energy required for a reaction to proceed. Catalysts can be either homogeneous or heterogeneous to the reaction (same state or different state than the reagents). Homogeneous catalysts, such as potassium methoxide in the traditional biodiesel synthesis, have a greater contact or surface area to interact with the reaction mixture, but are sometimes difficult to remove from the products. Heterogeneous may have less surface area, but can easily be filtered out after the reaction (most metal catalysts). Biodiesel transesterification catalysis attempts have been made using a wide range of compounds, but more are still yet to be explored in depth (such as metal-organic frameworks or enzymes, which may also be less hazardous).

5.2 Mechanism

Results indicate that pathway B in Figure 22 is still the most likely candidate. Pathways A and C were not observed, but would require further examination as stated previously to validate removal of them as possible options. Also, if the catalyst is adjusted (modified or changed entirely) the reaction mechanism may or may not also be modified.

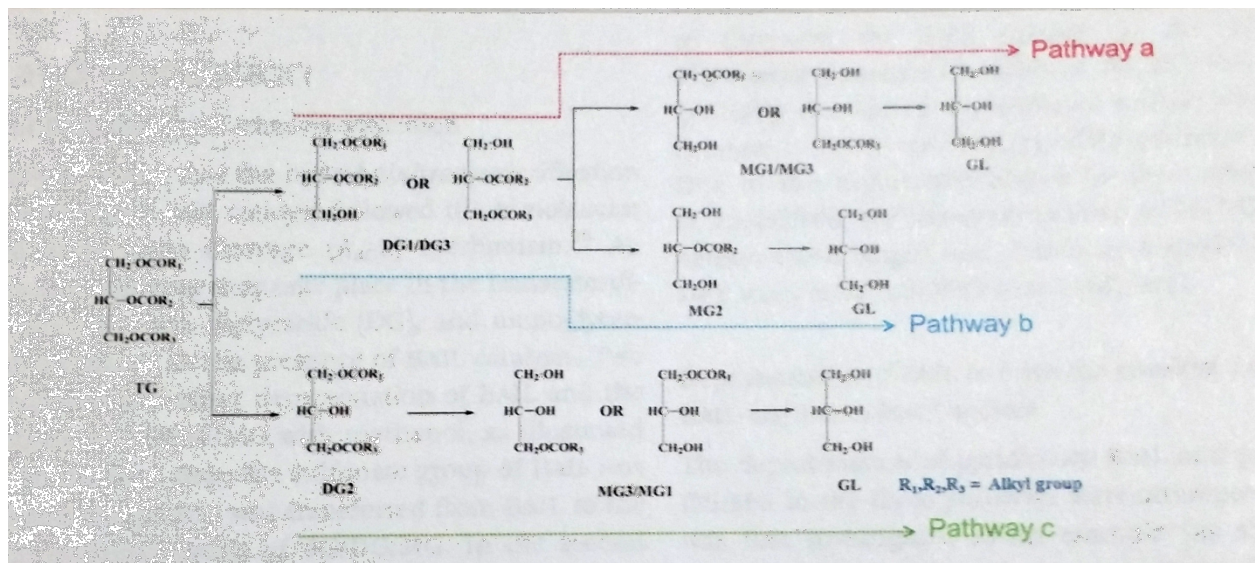


Figure 22 – three different reaction pathways by which vegetable oil transesterification may occur.

Concise Guide for the New NMR User (and Error Solutions for others)

As a new researcher and NMR instrument user, many new experiences are about to unfold in front of you. Good luck. :D

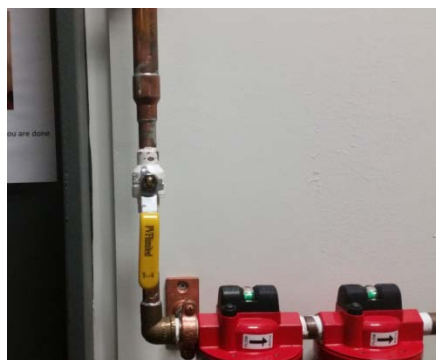
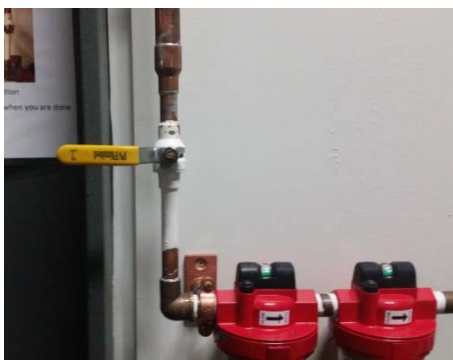
The Basics: Running a ^1H or ^{13}C NMR (with advanced side notes)

Note: Delta software is an old program. All typing must be done with mouse cursor hovering over text box. If cursor is moved, information in the box is submitted and typing cannot be continued until the cursor is brought back.

Note 2: Be nice to the NMR, and it will be nice to you.

Note 3: Please follow these directions carefully, and refer to explanations for further understanding of steps.

- 1) First and foremost, turn the air compression valve to the “Open” position



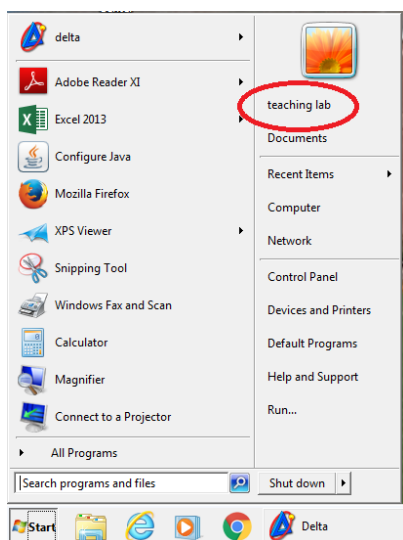
You should hear the air begin moving through the lines, and any samples loaded in the load slot will begin floating. If no sound is heard, refer to Error #09 in the Error Correction Section (ECS).

- 2) Login to the computer.

Username: **organic**

Password: =01teaching

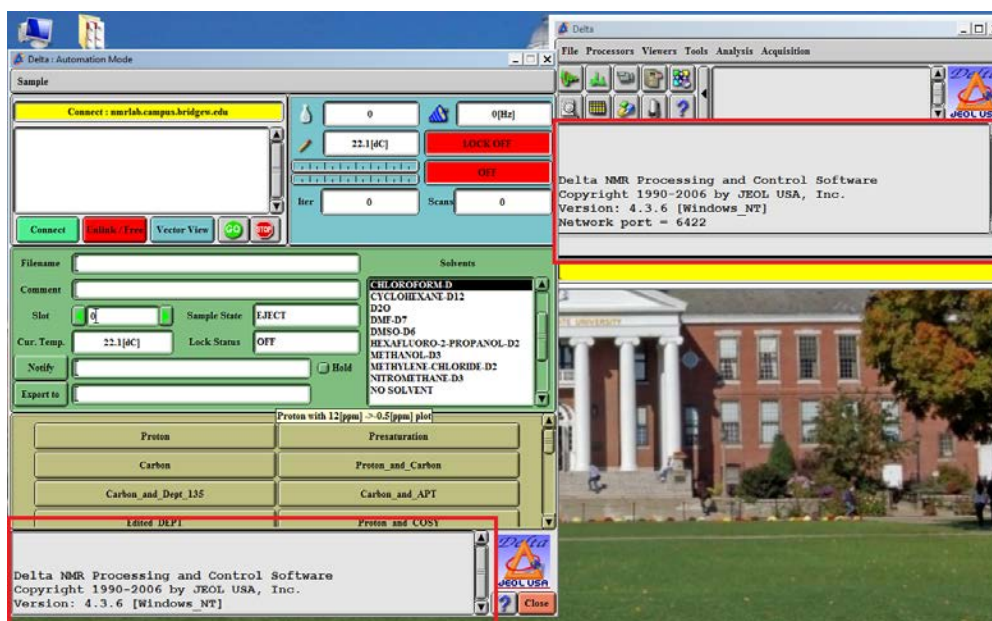
- 3) If computer was already logged in, check under windows icon to make sure the proper account is in use.



- 4) Open Delta program at bottom of screen.
- 5)



Check running task section (below). If there are experiments currently running, DO NOT interrupt them.



- 6) Two windows will open, the automation window and the advanced tools window.

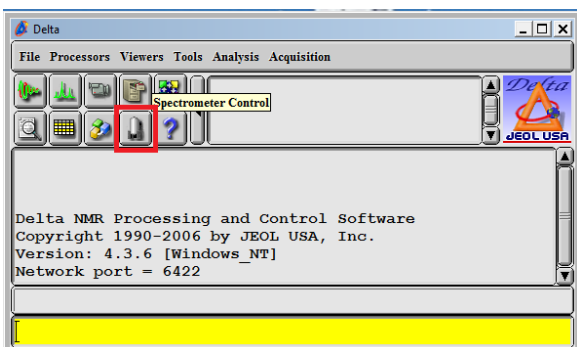
(Shown in step #5, left and right windows, respectively.)

It may take a few moments for them to fully load, so give it a few seconds. The automation window is all it takes if you wish to run a quick scan, although I recommend following the next steps in the advanced window instead. More control and variation is available through that method than via the automated version, and troubleshooting can be done from there (not from the automation window).

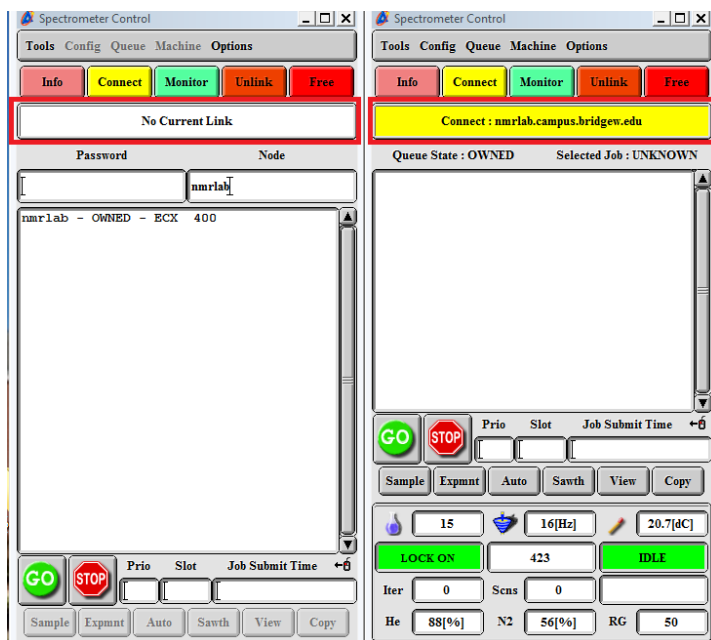
- 7) Insert NMR tube into sample holder. USE GAUGE. Should be set to 20mm. Push only until bottom touches the stopper inside the gauge, **do not** push past.*
- 8) Slowly insert sample holder into empty slot in carousel.

Note: sample holder can be inserted into active slot (on front screen of carousel), but if sample does not float up and down ($\pm \frac{1}{2}$ inch), place in a different (non-active) slot and follow Error #09 in ECS.

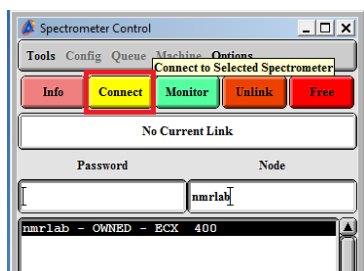
- 9) Click on the NMR spectrometer control in the advanced window.



- 10) Check to see that the JEOL NMR is connected (a yellow bar should say “connected to”).

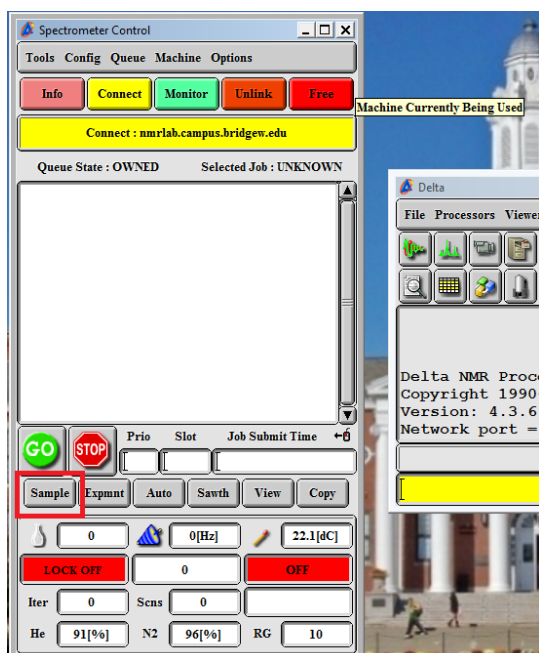


11) If not connected, click appropriate instrument (nmrlab – OWNED – ECX 400) and click Connect.

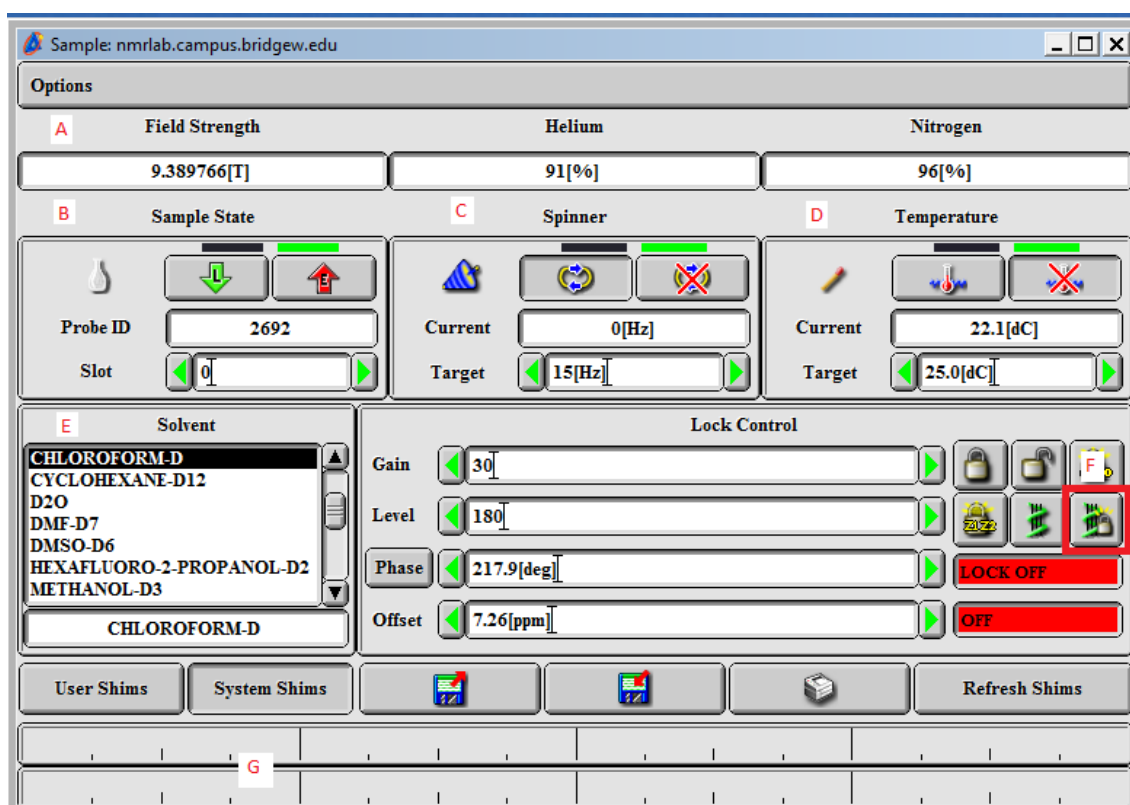


Note: if Delta program still does not connect to the NMR, refer to Error #03 in ECS.

12) Click Sample.




Work from left to right, top to bottom in order to properly set your sample:






- a) Check Helium and Nitrogen levels.* If either are below 50%, be sure to inform Lab Technician or Faculty.



Field Strength	Helium	Nitrogen
9.389766[T]	91[%]	96[%]

- b) Check slot number on carousel. If sample is in active slot, either click Load  or type slot number in, then move mouse cursor away.

Sample State




Probe ID

Slot  



If carousel is stuck or will not load/eject, Error #07.

- c) Watch spin rise, and verify steady spin at desired rate (15 Hz).

Spinner




Current

Target  



If spin does not rise or instead drops, Error #06.

- d) Verify Temperature is below limit (25°C)

Temperature

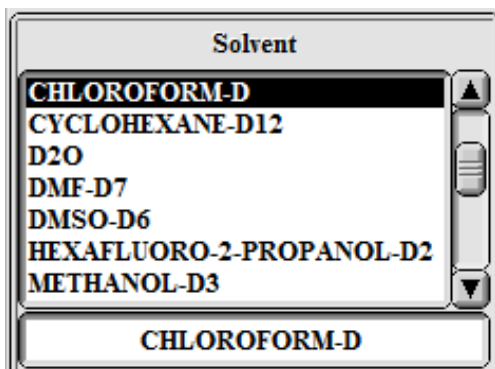
  

Current

Target  

If temperature is incorrect or above 25°C, **inform** faculty/lab tech.

- e) Select solvent. Observe adjustments to quick parameters (gain, offset, etc...)*



- f) Click Gradient Shim and Lock.* (first “lock on” will be a false positive. Wait until a steady “lock on” green signal is observed.)



If no lock (wait 5 to 10 minutes), Error #02.

- g) Verify after gradient shim and lock that both bars below are green (intensity is not as important)*



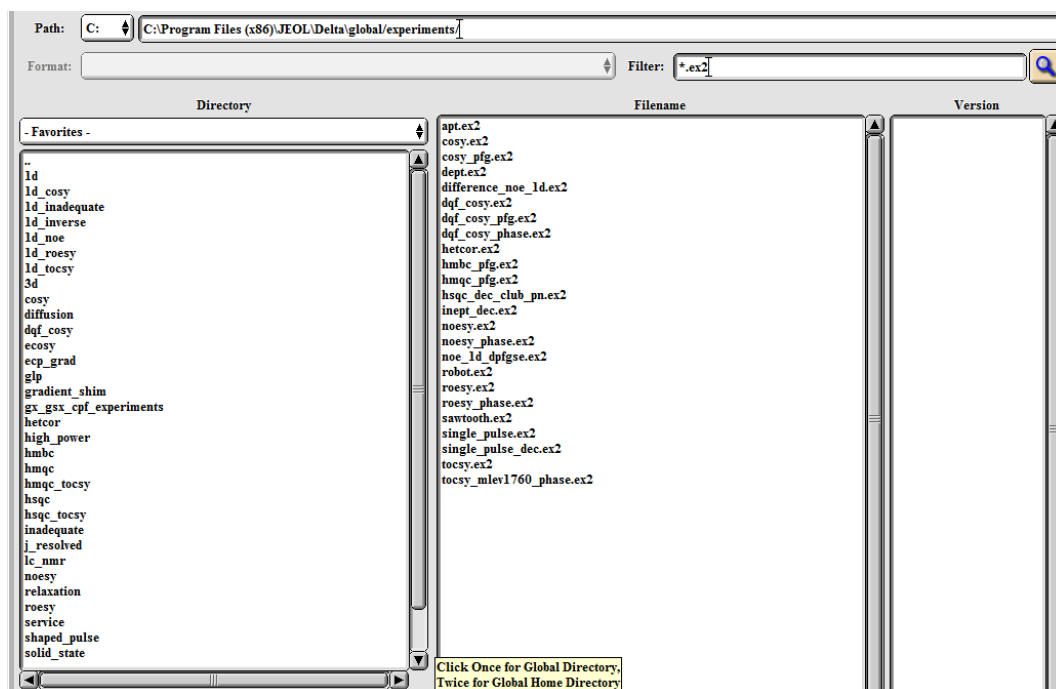
If both are not green, Error #10.

- 13) Click Expmt in Spectrometer Control window.



- 14) Open single_pulse.ex2 → If no files are visible, click Global Directory.





...leads to this.

- 15) Name your experiment (sample ID not required, but may be useful) in the window that pops up (Experiment Window). Use initials first, then shorthand of analyte (e.g. LS_Biodiesel).
- 16) Be sure to check off “auto-gain” in the first tab, beneath file naming.
- 17) Options for increased number of scans and other parameters are underneath each tab. Only adjust them if required by procedure or test. Main parameter adjustment would be the number of scans.
- 18) Click Submit and Go (“acknowledge” will queue but not run the experiment).
- 19) Click View (optional) to observe scans taking place.




- 20) Final spectra pops up. On right hand side, click FFT → Autophase → Base Correct
- 21) Use Toolbar to zoom, integrate, scale, etc...*
- 22) Print. CutePDF with selection of file type will give a picture file, while printing to LaserJet will just print a hard copy.

If file is not created (under shortcut on desktop), Error #04.

If printer doesn't print, Error #08.

23) Go to Sample window.

24) Eject sample .

25) Retrieve sample. Remove from holder.

26) Thank the NMR. Seriously.

Error Checking and Glitch Solutions

01. Tuning the NMR – How to reconnect the tuning probe when probe information is lost

- 1) Open Delta program and click NMR icon in non-automation window.
- 2) If no connection, click Connect to link to NMR. Yellow bar should replace grey bar.
- 3) Click Tools → Mode → Console
- 4) Type “console” in password box (default login/username)
 - a. Note: connection bar should turn blue.
- 5) Click Config → Pretune
- 6) Check probe numbers underneath physical NMR, and compare to numbers in Pretune menu. Change them ON COMPUTER to match those under the NMR.
- 7) Click Create Dial File, press [Ok].
- 8) Close menu.
- 9) Click Tools → Mode → Operator (i.e., revert out of Console mode)

02. No Lock

Stop gradient shim and lock in tasks running menu.

Check sawtooth, look for: (picture)

Move with cursor under menu to symmetric point

If above is not observed, and instead all observed is: (picture)

Check different Z_0 ranges (-1000, -1500, 500, etc...)

If still not found, eject sample using Sample window, and recheck gauge level*

Reload.

Attempt Gradient Shim and Lock again.

03. No Connection

Check username computer is logged into

If not the proper one, log out/log in

Try Connecting again.

If right username and still no connection, close Delta Program.

Reopen Delta, wait 15 seconds, try again.

If restarting Delta did not work, close Delta Program.

Restart Computer under windows taskbar.

Reopen Delta, wait 15 seconds, try again.

If still not connecting, press power off above main server box.

Keep off for 30 seconds.

Turn on, let NMR reboot. Carousel will move, numbers on screen will change.
After 15 minutes (about), reopen Delta program and try again

04. Cannot Print to PDF/JPEG/etc...

Printer preferences from Spectrometer Control Window.
Find section under Ghostscript
Check location of save and check off options that all it as a choice.
Save changes.

05. Excess Noise (poor S/N ratio)

Amount of sample/solvent
Checking sawtooth/alignment

06. No Spin

Clean holder
Check Air flow
Swap holder

07. Loading/Carousel Stuck

Restart Delta.
Restart Computer.
LAST RESORT... reboot nmr.
Lots of rebooting. xD

08. Printer refuses to print

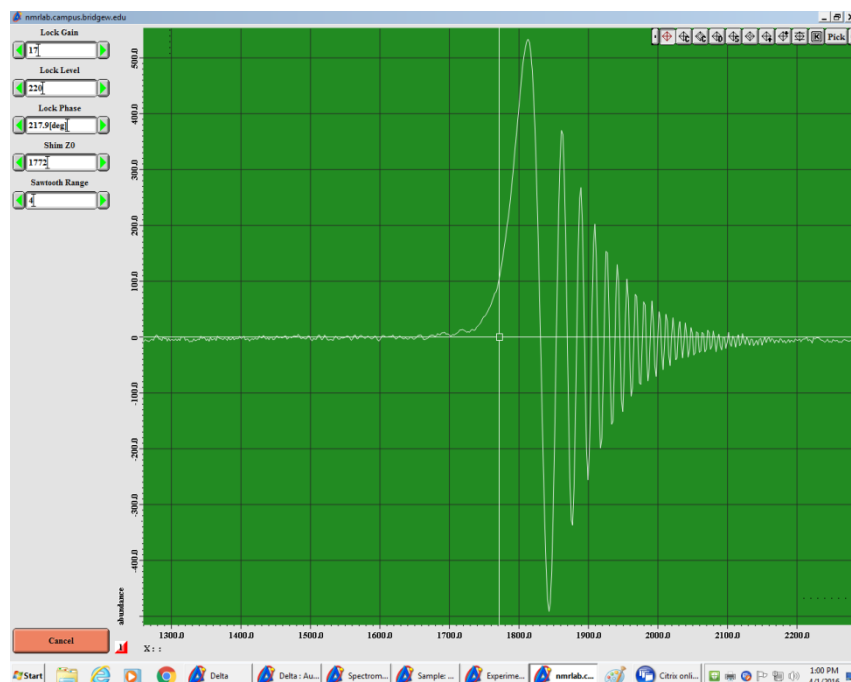
Equivalent to “paper jam” error.
Open paper tray, then close.
Hit green check mark.
If still will not print, make sure there is paper in the tray.
Add paper (ensure above 10 pages).
If still will not print,
...plug in the printer, or buy a new one.

09. No Float

Close compressor shut off valve against wall.
Wait 30 seconds.
Reopen and assess if sample is floating or not.
If not, Check gauges behind NMR.
Make sure they are reading at black mark when line is OPEN.
If gauges are empty and no sound is heard/no sample is floating.
Find a lab technician or faculty member and ask to check on the compressor upstairs.

10. Shim and Lock, No Green Bars

If locks but bars are not green, click Sawtooth. (if not locking at all, refer to #02)
Look for large peak in wave, and the cursor should lie over the center of that wave (Example below)



If it looks sort of like that, but scaled differently, move the cursor slightly towards the left portion of the peak.

Retry gradient shim and lock.

If there is no shape resembling this, check solvent, and also check to make sure you properly gauged the NMR tube (if it is not far or too far in the holder, the NMR cannot see it)

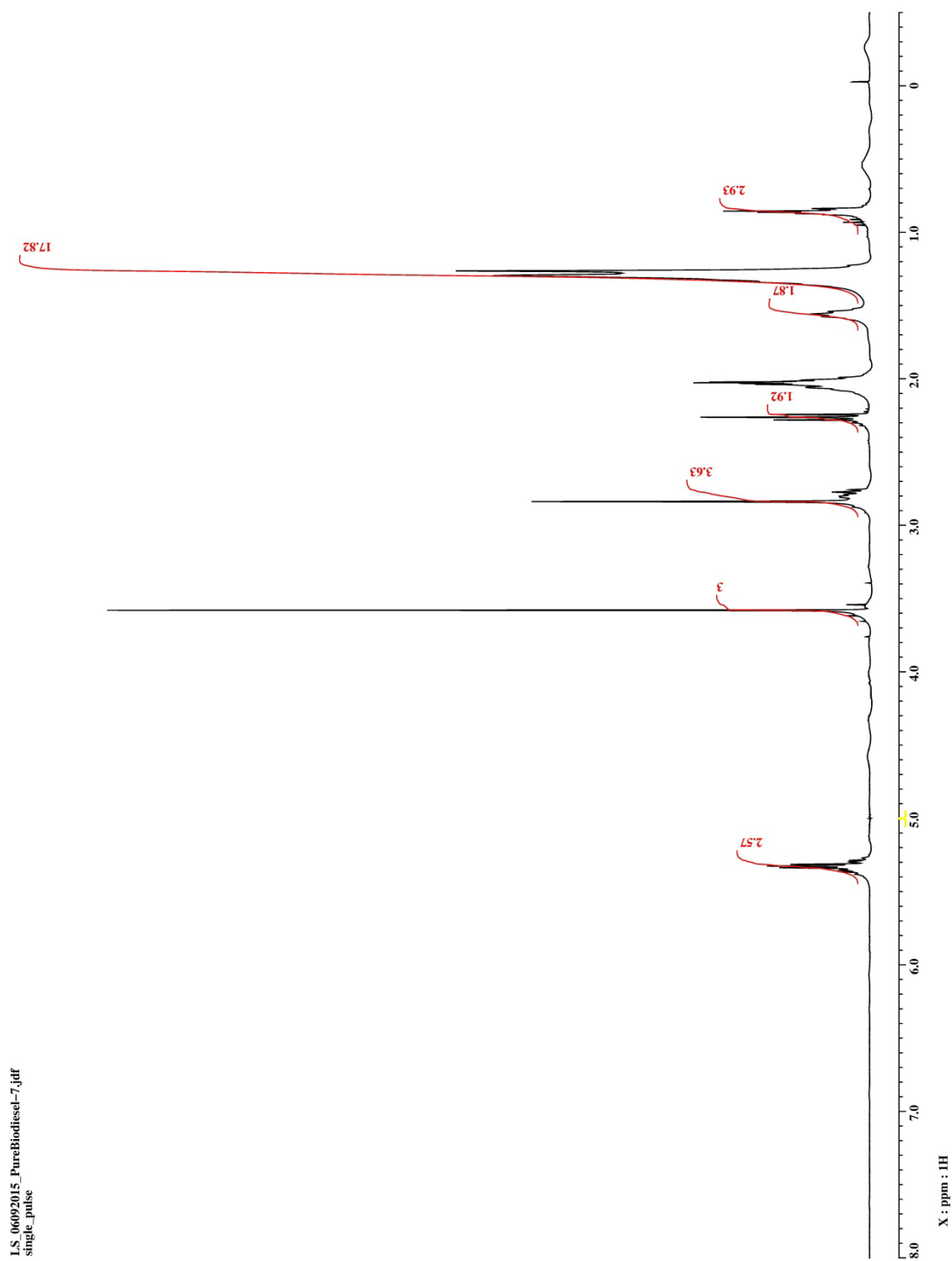
Explanations of *markings

*1) Checking Field strength, He, and N₂ levels = He and N₂ are used to keep the inner temperature surrounding the supermagnet at or below 4 Kelvin. Liquid nitrogen cools the magnet to under ~30 Kelvin (and lower), while expansion of the Helium gas cools it down further (a “neat” characteristic of expansion at those low temperatures).

*2) Gradient Shim and Lock = “Shim” refers to the NMR attempting to locate the requested solvent signal by pinging the sample from various locations and frequencies. “Lock” refers to having found the signal and the appropriate orientation, which is then held for the rest of the experiment.

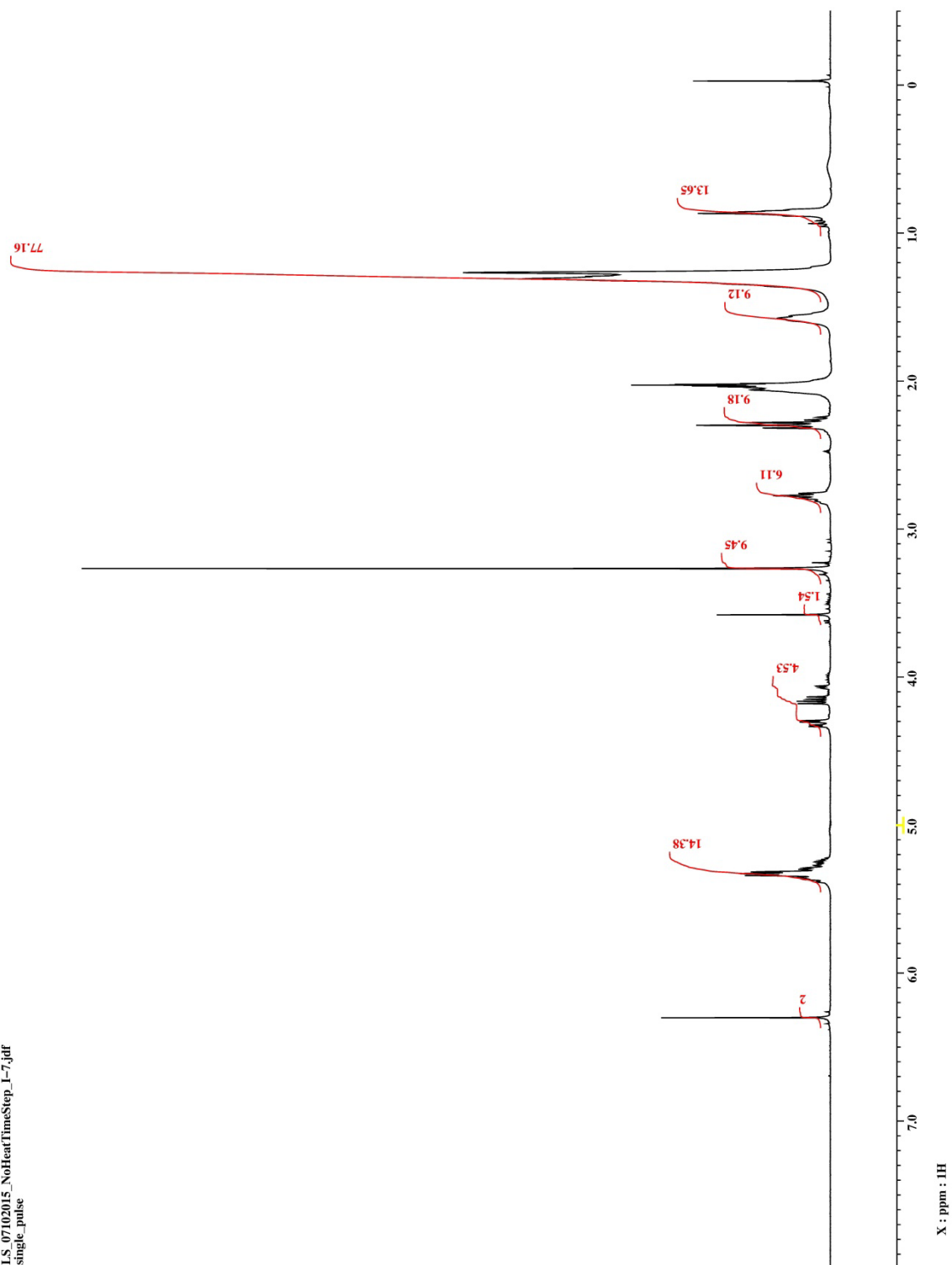
*3) Intensity refers to the strength or amplitude of the first wave/signal received by the instrument, not as to how well locked or shimmed it may be.

Appendix

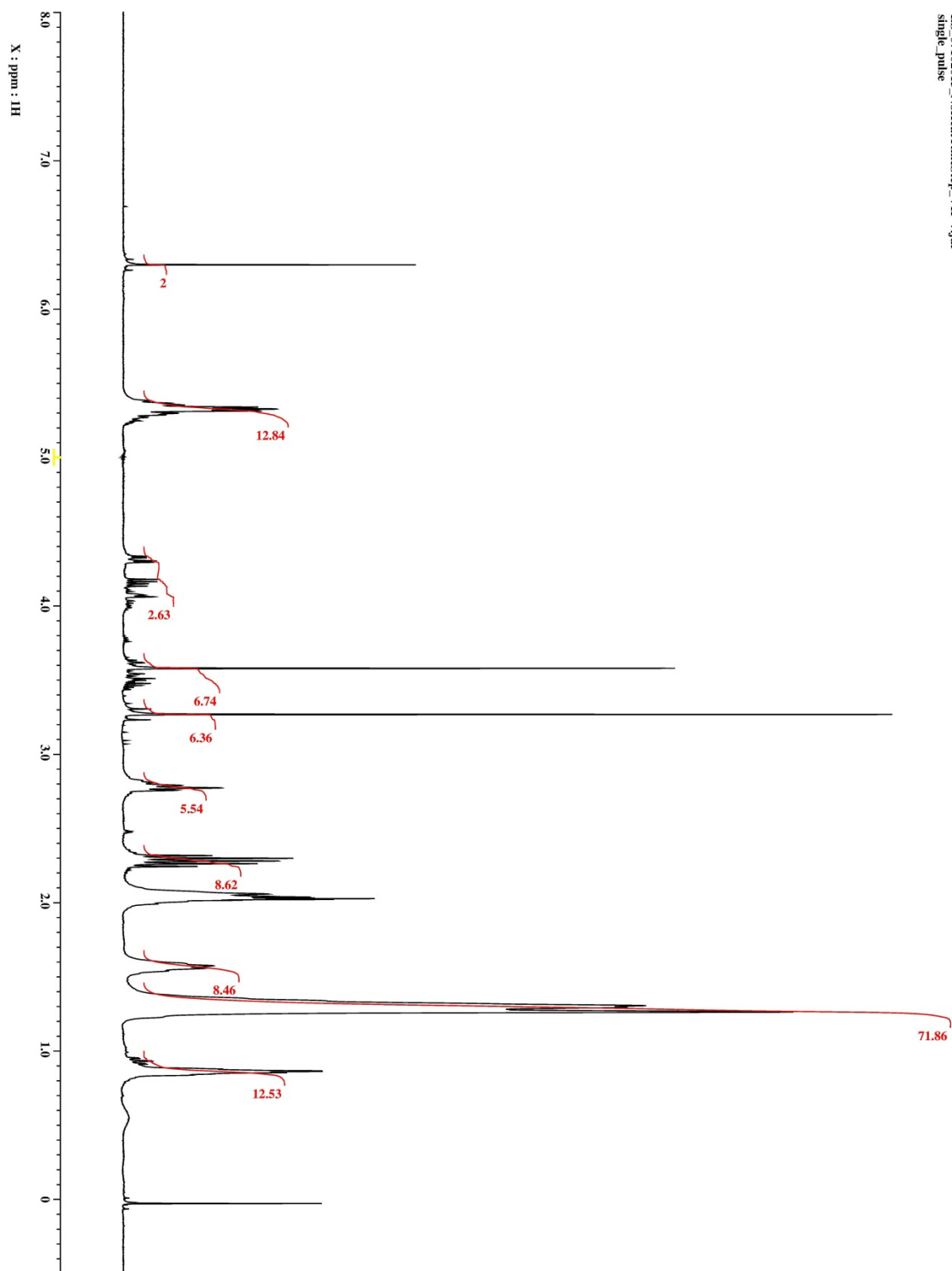


NMR Spectra of Pure Biodiesel.

LS_07102015_NoHeatTimeStep_1-7.jdf
single_pulse



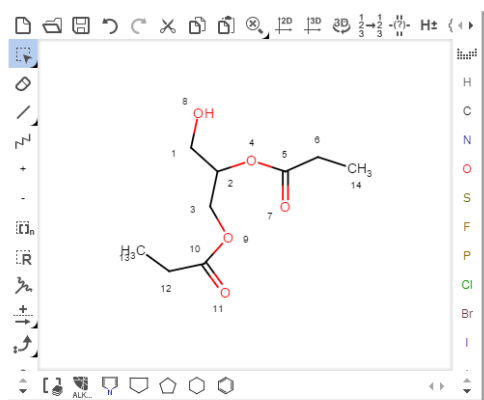
NMR Spectra of first aliquot taken, <5 minutes.



NMR Spectra of final aliquot taken, ~50 minutes.

The following are computer images calculating pKa values:

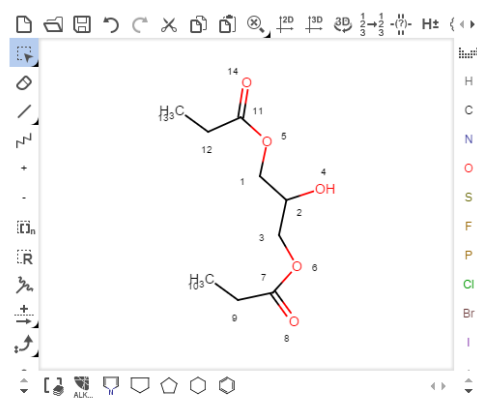
(Here, pK_b is defined as the pK_a of the conjugate acid.)



View pKas Switch to Java applet

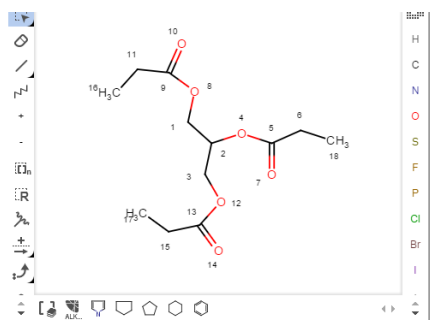
Atom	pK_a	pK_b
C1	31.4	
C2	29.7	
C3	32.0	
O4		
C5		
C6	25.5	
O7	-7.0	
O8	14.6	-3.0
O9		
C10		
O11	-7.0	
C12	25.5	
C13	50.0	
C14	50.2	

(Here, pK_b is defined as the pK_a of the conjugate acid.)



View pKas Switch to Java applet

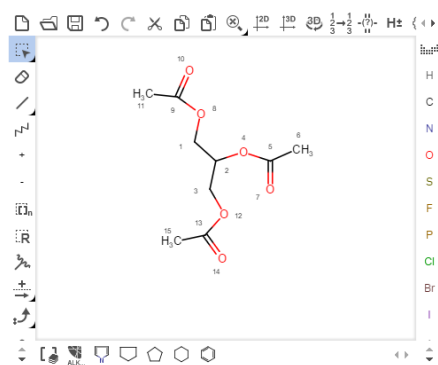
Atom	pK_a	pK_b
C1	32.4	
C2	28.8	
C3	32.4	
O4	13.6	-3.4
O5		
O6		
C7		
O8		-7.0
C9	25.5	
C10	49.8	
C11		
C12	25.5	
C13	49.8	
O14		-7.0



View pKas Switch to Java applet

Atom	pK_a	pK_b
C1	32.0	
C2	29.3	
C3	32.0	
O4		
C5		
C6	25.5	
O7	-7.0	
O8		
C9		
O10	-7.0	
C11	25.5	
O12		
C13		
O14	-7.0	
C15	25.5	
C16	49.6	
C17	49.6	
C18	49.9	

(Here, pK_b is defined as the pK_a of the conjugate acid.)



View pKas Switch to Java applet

Atom	pK_a	pK_b
C1	32.0	
C2	29.3	
C3	32.0	
O4		
C5		
C6	25.0	
O7	-7.0	
O8		
C9		
O10	-7.0	
C11	25.0	
O12		
C13		
O14	-7.0	

pKa Calculator:

<https://epoch.uky.edu/ace/public/pKa.jsp>

Table consisting of the MM2 energies calculated via ChemBio3D. Energies were calculated for the diesters possible after the burst phase would have ended. Energy difference corresponds to the energy it would have released to get to that diester from the triester.

Type of Ester	MM2 Energy (kcal/mol)	Energy Difference from Triester
1,2 di-ester	16.0695	21.3151
1,3 di-ester	21.8922	15.4924
Triester	37.3846	0

“That’s all, folks!”