Biological Strategies Of Biodiesel Synthesis

By: David Samuelman

Submitted to:
Frank LaBanca, Ed.D.
Science Department Chair
Oxford High School

Abstract

Biodiesel is becoming more popular because of its environmental benefits, oil fueling high prices, and over use and unavailability. This project is aimed toward producing Biodiesel as a multiuse fuel; some of these uses include home heating systems and a variety of engines that power cars, boats, tractors, and other farm equipment. Methodology of producing this fuel includes transesterification of a fatty acid carbon source using genetically modified bacteria. The genetically modified bacteria produce the enzymes pyruvate decarboxylase, alcohol dehydrogenase, and an unspecific acyltransferase to catalyze the biodiesel formation. The project was sought to optimize a biofermentation protocol. For measuring the effectiveness of the produced fuel TLC, Think Layer Chromatography was used. Data indicated that a modified agitation growth fermentation method produced significantly higher quantity of biodiesel than the controlled fermentation method. For fuel separation a Rotovap is going to be used, a Rotovap uses evaporation to separate, using Diethyl Ether as a solvent.

Introduction

Biodiesel and alternative energy sources are becoming more and more problem since recent problems with oil. The problems are price inflation and environmental hazards, all these problems are very significant and need immediate change. This is where Biodiesel comes in; they are made from renewable resources such as vegetable oil. This is very dramatic because unlike oil, corn can always be produced. Oil is a non-renewable resource, once all of it is gone, it is gone forever. On an environmental stand point Biodiesel has many advantages. These advantages are low burning temperature, which means lower emissions released. Another advantage Biodiesel has is biodegradable, for example if ever spilled, unlike oil, biodiesel would not hurt the environment, because it was the environment. (Naymer,2008). These advantages are very important because green house gases are being emitted and it is having a negative effect on Earth. An example of a negative effect on Earth is the climate is changing, causing several animal species to be extinct and ice to break up in Antarctica. For another example, if a tenth of the people in the world used Biodiesel cars or other Biodiesel products, millions of gallons less of green house gases would be released, causing the climate not to change as dramatically; saving the environment and millions of animals. One problem with Biodiesel is the impracticality of the resources to power the world on this fuel source. Millions of acres of corn would have to be produced just to make Biodiesel. This is not practical because people are starving to death every day and to save the environment a possible food source is being used to burn as a car's fuel. (Carhed, 2009).

Biodiesel is typically manufactured using a transesterfication procedure with the use of methanol and the gas methoxide. The problem with this is that methanol is a harmful chemical and a great deal of energy is required for heat to catalyze the reaction. The traditional way of transesterfication of Biodiesel consists of Triglyceride bonded to 3 methanol's this gives the product of 3 Biodiesel's plus one glycerol. The glycerol in this reaction is a waste product; one use of this is makeup. This is an advantage because a waste product is being for other products, saving other materials. This is the basic "timeline" of producing biodiesel. In the process the triglyceride and the methanol break leaving the glycerol, 3 fatty acids, the OH-(hydroxyl group of the methanol.) A carbon bonded with three hydrogen's (1 extra bonding spot), and 3 carbons bonded to 4 hydrogen's (3 extra bonding spots). After break has taken place bonding must occur, the 3 carbons bonded to 4 hydrogen's bonds to the 3 OH- hydroxyl group of the methanol making Glycerol. 3 fatty acids bonded to the one carbon bonded to the 3 hydrogen's making 3 Biodiesel.

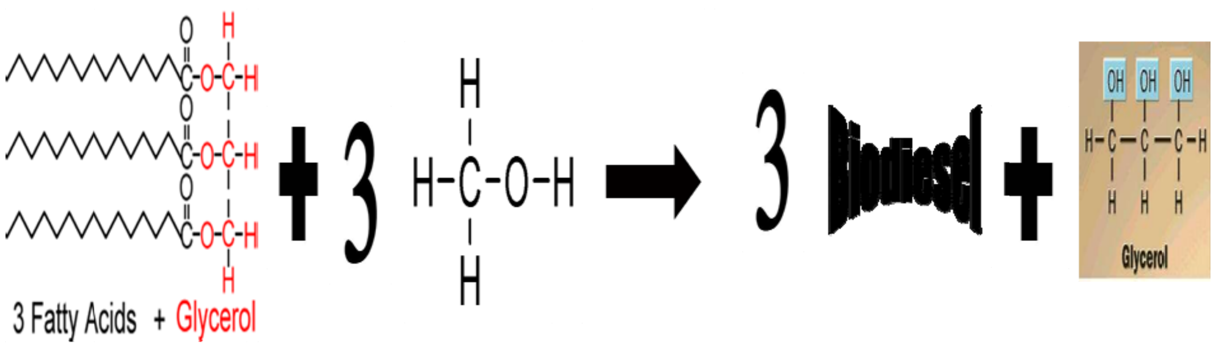

Figure 1- This figure shows the regular production of Biodiesel in picture form.

A new better way of transesterfication consists of one Triglyceride bonded to bacteria (pyruvate decarboxylsase, alcohol dehydrogenase, and unspecific acyltransferase) the product of this reaction equals biodiesel and the waste product Gylcerol. This eliminates the use of harmful methanol and the harmful gas methoxide. Replacing this chemical is bacteria that do the work for you. These genes listed above are responsible for transesterfication, without the use of methanol. The bacteria cell goes through cell respiration which it produces ethanol naturally. Than 3 enzymes work together to bond the ethanol and a fat source, which in this case is Oleic Acid, this produces Biodiesel.

The bacterium used in this Biodiesel production is an E. coli cell, but it is not a normal E. coli cell. Inside the cell is a Microdiesel plasmid, it gives the cell certain abilities it would not normally have. The genes in the plasmid consist of rep, which is the ability for the cell to replicate. Ampr is the gene that gives the cell ampicillin resistance, flori is also the ability to replicate. Afta, is alcohol dehygrogenase, Pdc and adhB , pyruvate decarboxylase and an unspecific acyltransferase which are the genes responsible for bonding ethanol and Oleic Acid. (Klaushernak, 2008).Using E. coli the use of methanol can be removed because the E. coli does the work for you.

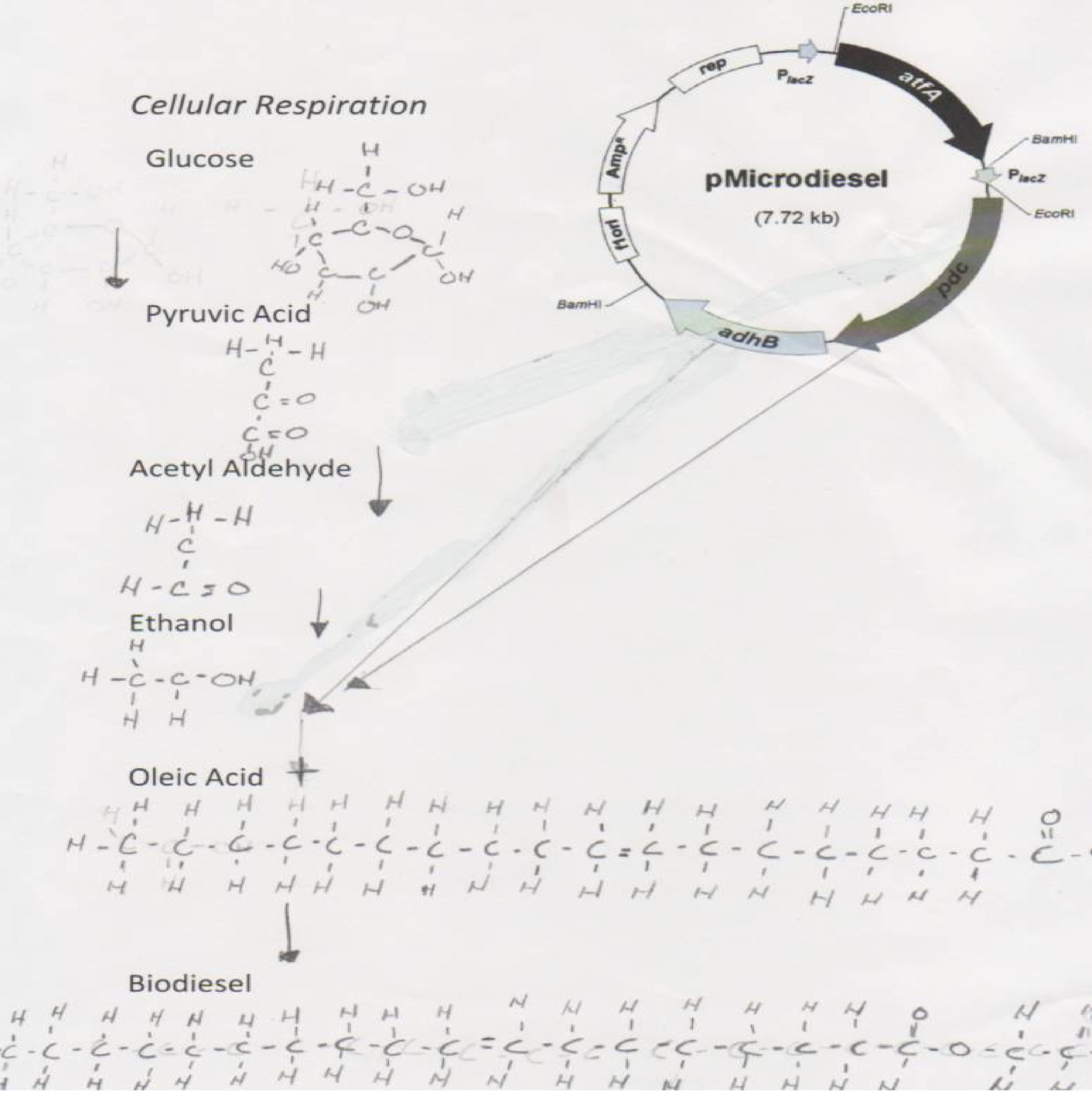

Figure 2- This image describes how the Microdiesel plasmid works to make the Biodiesel. It shows how the cell goes through cell respiration and produces Ethanol naturally. Than it shows

the 3 enzymes that bond together the Ethanol and the Oleic Acid, this bonding together produces the Biodiesel.

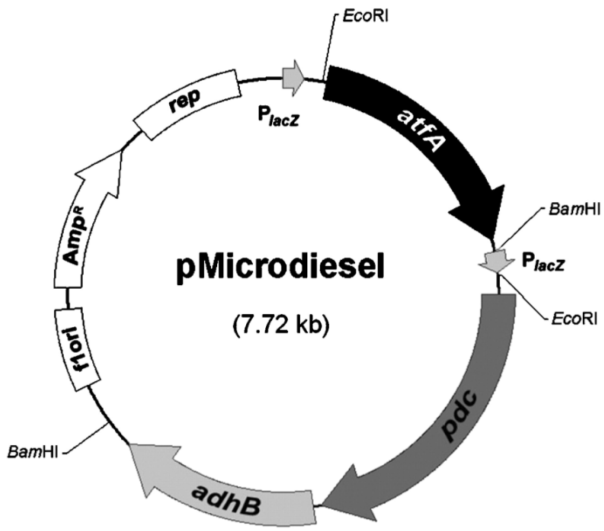

Figure 2- Shows the Microdiesel plasmid that is in the E. coli cell. (Kalscheuer, 2006)

History of Biodiesel

The history of Biodiesel is not as most people think; Biodiesel has been created since 1920s. On August 31, 1937, G. Chavanne of the University of Brussels (Belgium) was granted a patent for a “Procedure for the transformation of vegetable oil for the uses as fuels’. This would be later known as transesterfication. Rudolf Diesel used peanut oil in his first compression-ignition system; these engines were run on vegetable oils, the first Biodiesel. This vegetable oil engine was still in use until the 1920’s when petro-diesel took over. (Faryl,2001).

Figure 2- This shows a container full of vegetable oil that would be used for Biodiesel production (Patrick, 2005).

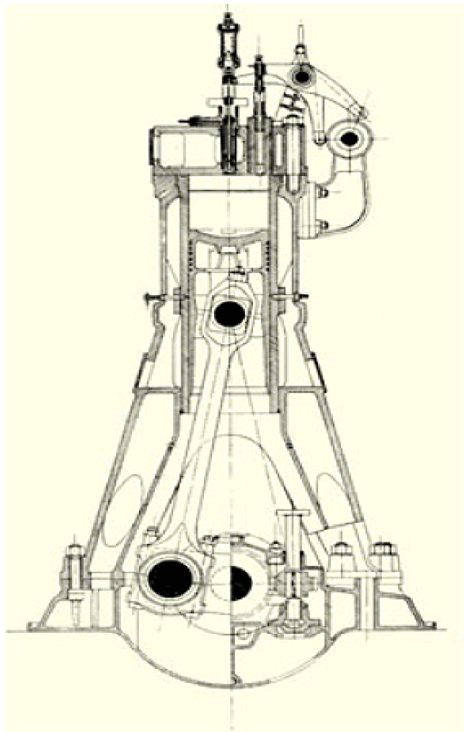

Figure 3 - A blueprint of Rudolf Diesels' Compression ignition system (one of the first known uses of Biodiesel.) (Dade, 2004.)

In the mid 70's fuel shortages re-energized the production of Biodiesel as an alternative to Petroleum diesel. As the petroleum based diesel soon grew superior, Biodiesel was pushed aside to as "alternative" again. Now, with increasing concerns about global warming, declining water and air quality, serious human health concerns are inspiring the thought of increasing usage of Biodiesel. (Chavaene, 2000.) This is because of the environmental benefits that make Biodiesel superior to oil based products. Much of the world is starting to slowly switch to Biodiesel, yet Biodiesel consumption accounts for less than 1% of the total diesel production in the United States.

Chemicals/Consumables	TLC plates*Supplies*	Incubator *Equipment*
E. coli Lennox Broth Sodium Oleate Acetic Acid Petroleum Ether Ethel Acetate Sulfuric Acid	Petri Dishes Inoculating Loops Beaker 2000ml containers 1000ml flasks	Centrifuge Micropipette Magnetic Stir Bar

Problems with Chemically Produced Biodiesel

Biodiesel is typically manufacturing using a transesterfication procedure with the use of methanol and the gas methoxide. The problems with this procedure are the very harmful chemical methanol, and the harmful gas methanol. Making this fuel this way is very difficult without the use of a lab because of these chemicals; they are very difficult to because they are harmful chemicals. (Lanster, 2007). Not to mention the danger involved with handling this harmful chemicals. Also extremely high temperatures of heat are required for this reaction to catalyze the reaction. This is not only dangerous to the person making the fuel, but very expensive for whoever has to pay for the heating. This way of producing Biodiesel is also not very practical because people are dying every day because of starvation, and the world is not going to allow corn or other products that are being used for food be destroyed for a fuel source. By doing this the world would have much more starvation deaths.

E. coli Transformation

This is the first step in the process of making Biodiesel. An E. coli cell was transformed, putting a Microdiesel plasmid inside the E. coli cell, now giving the whole E.coli cell an ampicillin resistance and other genes. This is a resistance that the cell would not normally have. Now when this E. coli cell replicates the plasmid will be inside the E. coli cell. This ampicillin resistance provides a “marker” to ensure the correct product was made. The transformed E. coli cells were placed on Ampicillin plates; show that these products have the Microdiesel plasmid inside them. If the E. coli cell had died you know it was not the correct because it did not have the ampicillin resistance meaning it did not have the Microdiesel plasmid.

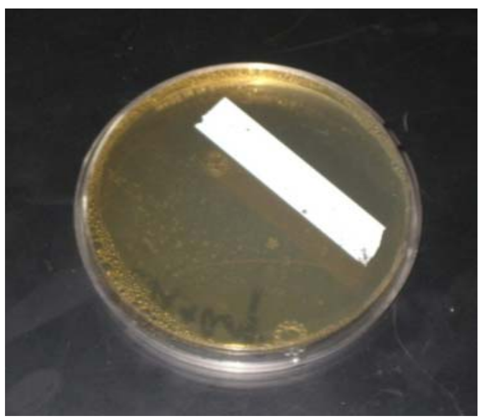

Figure 4- This plate shows the transformed E. coli cells containing the Microdiesel plasmid.

Biodiesel Fermentation

Lennox Broth, Sodium Oleate, and the transformed E. coli cells were mixed in a 1000mL flask, placed in an incubator for growth. Fermentation is the reaction of Transesterfication, where the production of Biodiesel occurs. By placing the flask inside the incubator you allow the bacteria to be exposed to warm temperatures; this is where bacteria thrive to grow. By adding the Sodium Oleate this provides the carbons for the fat source; which in this case is Oleic Acid. The E. coli cells used in this fermentation are the cells that have the Microdiesel plasmid. The plasmid has the three enzymes that bond the naturally produced Ethanol to the Oleic Acid; producing the Biodiesel.

Figure 5- This shows the flask containing Lennox Broth, Sodium Oleate, and the transformed E. coli cells in an incubator for growth.

Results of Non Aerated Biodiesel Fermentation

Normal Fermentation	Area (cm^2)
Day 0	1.1
Day 1	1.6
Day 2	1.97
Day 4	2.13

Measuring Effectiveness

For measuring the results TLC (Thin Layer Chromatography) was used. It shows the separation of products to more closely see the true components of it. Using Petroleum Ether, Acetic Acid, and Ethyl Acetate as a mobile phase shows the separation of each product. After the separation occurred, a solution of 40% Sulfuric Acid was sprayed on the plate. Holding the plate periodically over a Bunsen burner the separation became clearer and more easily quantified. On the TLC plates a circle of growth occurred, copies of the plates were taken. The circle was cut out and weighed. Using proportions to find the area in $cm^{2.}$ By using a unison measurement different tests could be closely compared. This image of TLC plates shows the separation of the fuel over different times in incubator.

Figure 7- This image of TLC plates shows the separation of the fuel over different times in incubator. The image on the right shows the separation of the fuel at mixture. The middle one shows separation overnight, and the one on the left shows the separation after 2 days. As you can see the longer in the incubator the bigger the circle of growth, after two days the growth was very similar. Showing two days is the optimal time for growth.

Biodiesel Aeration Fermentation

Lennox Broth, Sodium Oleate, and transformed E. coli cells were placed in a 1000mL flask, placed in an incubator for growth. This time the flask was not placed in the incubator, it was placed on a magnetic stir bar in an incubator. This provided agitation and aeration, hopefully optimizing results. This fermentation was done to optimize the results of the original fermentation. The results are being optimized by adding the magnetic stir bar; this spins the substrate. By doing this it adds Oxygen and Aeration to the reaction and catalyzes it.

Figure 8- shows the flask placed on a magnetic stir bar in an incubator.

Results of Aerated Biodiesel Production

Day 0	1.15
Day 1	2.85
Day 2	4.12
Day 4	4.16

These results show that Aeration Biodiesel is 127% more effective than non-aeration Biodiesel, when produced for the same time.

Rotary Evaporator

After Biodiesel was created it is still in the presence of sodium Oleate and other materials other than Biodiesel. For the Biodiesel to be a useable product, it needs to be separated out so that it is pure Biodiesel.

A rotary Evaporator is a separation technique that uses evaporation and condensation. To start the starting liquid and a solvent is put into a circular class sphere that is dropped into a hot water bath. The circular sphere spins so the liquid can evaporate in the hot water. When it evaporates it travels up this thin glass tube with glass coiling inside the tube, this glass coiling is attached to a refrigerator that pumps cold water through the system cooling down the evaporated liquid allowing it to condense. Also attached to this glass tube is a vacuum that sucks out all the air from the tube, to ensure that nothing else is inside the tube.

Figure 9- shows the Rotary Evaporator without the refrigerating unit and the vacuum pump.

Results of Rotary Evaporator Separation

Rotary Evaporator Separation	Area (cm^2)
Pre-Separation	1.48
Separated Once	2.74
Separated Twice	4.99
Separated Three Times	5.03

Conclusion

Using a Rotary Evaporator was an effective way to separate biologically produced Biodiesel. When the Biodiesel was produced there were still waste products, such as E.coli and glycerol. For this Biodiesel to become a useable product there needed to be a separation technique. For this technique a rotary evaporator was used. Using evaporation and condensation and Diethyl Ether as a solvent separated out into Biodiesel.

After several tests through the Rotary Evaporator it becomes conclusion that running the sample through the Rotary Evaporator was the most successful after the third time. Running the sample through the Rotary Evaporator one time was nearly 3.37 times less successful than three times through the Evaporator. Running through the second time was less than 1.85 times successful than the third time. Running through the fourth time was only .03 times more successful than running through the third time. This shows that the fourth test is irrelevant and could be cut out. This is very convenient because the solution does not have to be run through ten-plus times to get the results desired.

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