



**AN INTEGRATED SCIENCE PROJECT AT CISA**

**Project overlook:**

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**Project evaluation:**

Physics: 10% course.

Pre-project & Synthesis questions  
Entry, exit and in class questions

Chemistry: 10% of course.

Lab. sheets completed with observations and questions 5%

**Absence policy:**

Zero on work done during that class.

## **PRE-PROJECT RESEARCH QUESTIONS**

Keep track of your references

- 1- Explain what is a renewable energy.
- 2- Explain/define what a biofuel is.
- 3- From what plants can we produce oil? Name at least 7 plants.
- 4- What are the main differences between diesel and regular car fuel?
- 5- Do you use cooking oil or frying oil at home? Estimate the volume of vegetable oil used per month in your household. Estimate the volume of oil per year.
- 6- Cooking and frying oils should not be discarded down the sink after usage. List and explain at least 3 environmental impacts when vegetable oils are released in the environment.
- 7- Briefly research the *Jatropha curcas* nut and its usage in Africa. Summarize your findings in 5-10 lines.
- 8- Several years ago, alcohol from corn plants began to be mass produced and added to regular fuels. At first, lobbyists and some governments were promising a bright future to this type of fuel, that could eventually replace “traditional fuels”. Time has shown several negative sides (impacts) of using alcohol as a fuel. Research and clearly explain three negative effects.
- 9- *Vulgaris* algae are grown in our laboratory by exposing them at 16 hours of light per day under light fluorescent tubes glowing mostly in the red and blue spectrum.
  - a) Research scientific papers online (<http://scholar.google.ca/>) and provide parameters that could be added in order to INCREASE algae and/or oil growth. Keep track of your references.
  - b) Explain why glycerol and glucose were chosen as good medium growth.

## Part 1A: Inoculation of algae

Teacher: Ms. Baddour

Time: 90 or 120 minutes

### Purpose:

To inoculate two species of algae for the biodiesel project.

### Evaluation:

Pre-Lab Assignment	Laboratory Work	Post Lab Assignment
none	none	none

### Material:

- Gloves
- Graduated cylinder 100 ml
- Concentrate Bold's Basal medium (BBM) (2 Eppendorfs of 1 ml)
- 2 Autoclave Erlenmeyer flasks + stirrer
- 3 Serological pipettes (5 ml, 25 ml)
- Micropipettes (P1000) + Tips
- Ethyl Alcohol (70 %)
- Distilled water
- Plastic beaker (waste)
- **Algae strain** : *Chlorella vulgaris* culture

### Procedure:

#### 1 - Preparation of Bold's Basal medium (BBM) in an aseptic environment

1.1 – Before preparing the BBM solution, you need to clean a portion of your bench area. This will be your 'aseptic manipulation zone'.

1.1.1 – Clean your 'aseptic manipulation zone' with 70 % Ethyl Alcohol.

1.1.2 – Gently clean everything that you need to manipulate in your 'aseptic zone' (micropipette, graduate cylinder, Erlenmeyer) with 70% Ethyl Alcohol.

1.2 – Preparing Bold's Basal medium (*Final volume = 100 mL*) from the concentrate BBM (all manipulations below are done in the 'aseptic zone')

- 1.2.1 – A **1/50** dilution is needed to obtain the final BBM solution. Calculate and record the amount of concentrated BBM & distilled water needed. **Ask Ms Baddour or Ms Otis to validate your results.**
- 1.2.2 – Watch these tutorials 'How to use a micropipette':  
[ow.ly/urBlu](http://ow.ly/urBlu) (English)  
[ow.ly/urBYq](http://ow.ly/urBYq) (French)
- 1.2.3 – Make the 100 mL BBM solution. Add the required volume of distilled water in the cylinder to 70% of the final required volume.
- 1.2.4 – Pipet the amount of concentrated BBM with P1000 micropipette from the Eppendorf. Transfer the concentrated BBM into the distilled water in your cylinder.
- 1.2.5 – Add the remaining necessary distilled water to top off the volume to 100 mL.

## **2 – Inoculation of the algae (all manipulations below are done in the 'aseptic zone')**

- 2.1 – Using a 25 ml sterile pipette, transfer 45 mL BBM solution from your cylinder to a 250 mL Erlenmeyer flask (which has already been sterilized in autoclave).
- 2.2 – Using the same pipette, transfer another 45 mL BBM solution to a second 250 mL Erlenmeyer flask (which has already been sterilized in autoclave).
- 2.3 – Identify the Erlenmeyer flask with a tape:  
First flask: **C. Vulgaris, date, your initials**
- 2.4 - Using a 5 mL sterile pipette, transfer 5 mL of Algae *Chorella Vulgaris* strain into the appropriate identified Erlenmeyer flask. (Note: Ask the technician for the C. Vulgaris culture).
- 2.5 – Cover the mouth each of your Erlenmeyer flasks with aluminium paper. Make sure the aluminium paper is well pressed down.
- 2.6 – Place your Erlenmeyer flasks on a stirring plate under the fluorescent lights in the Physics lab. (Ask Ms Baddour or Ms Otis to show you the right table).

2.8 – Clean your bench, materials & store everything in your baskets except for the pipets (garbage).

**To sign up for absorption measurements at lunch time, sign up here:**

[https://docs.google.com/document/d/1k\\_IKrFbh4z51Qz275u44z3UKTK-DXzd\\_fachxVCB8Zo/edit?usp=sharing](https://docs.google.com/document/d/1k_IKrFbh4z51Qz275u44z3UKTK-DXzd_fachxVCB8Zo/edit?usp=sharing)

## Part 1B: Take one spectrometry measurement

Teacher: Ms. Otis

Time: 25 minutes at lunch time

### Purpose:

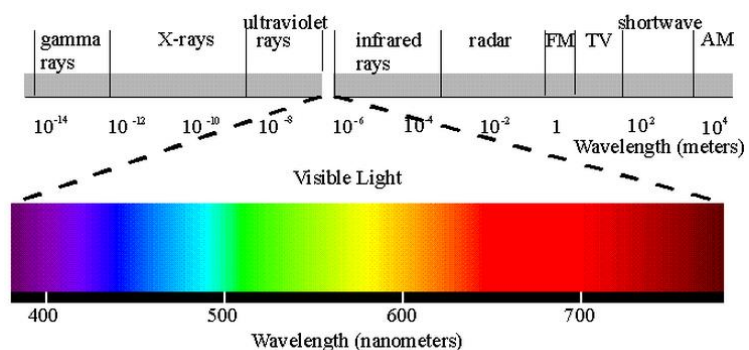
- To learn how to use a spectrophotometer and provide absorbance results of a solution.
- To develop micropipettes skills.

### Evaluation:

Points will be awarded to the graph created at the end of the project, only if measurements were taken. Absent = zero

Insert measurement in google form here:

[https://docs.google.com/spreadsheets/ccc?key=0Arue\\_5GKXYUQdElwTXNvOVB3aV9SQU5XMWNLAVhsN2c&usp=sharing](https://docs.google.com/spreadsheets/ccc?key=0Arue_5GKXYUQdElwTXNvOVB3aV9SQU5XMWNLAVhsN2c&usp=sharing)



**Figure 1 : Electromagnetic Spectrum**

## Part 2: Microscope observations

Teacher: Mr. Wikarski

Time: 90 minutes

Purpose: To observe algae cells and locate oil production sites.

### Evaluation:

Pre-Lab. assignment	Laboratory work	Post. Lab assignment
None	Take pictures of specimens & answer questions	Questions to be answered and submitted on moodle

### Background information:

Algae are like plants in the sense that they photosynthesize and are multicellular organisms. These eukaryotic cells vary in size, but samples are in the order of 2-5 micrometers ( $2 \times 10^{-6} \text{m}$  to  $5 \times 10^{-6} \text{m}$ )

Microalgae (*Chlorella Vulgaris*) and yeasts are unicellular organisms. Each individual has only one cell. Yeasts and microalgae (like plants and algae) are eukaryotic cells. The nuclei of the cells are small dark round spot inside each cell. You must be able to see vacuoles, transparent white spots inside the cell.

### Procedure:

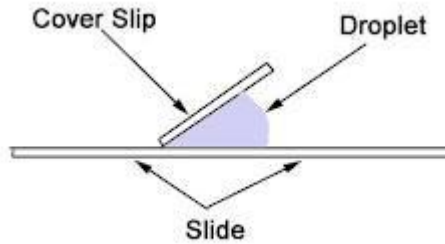
Today, you will observe fresh and dry algae cells under the microscope and try to locate oil within the cells.

Slides available for 4 observations:

- **Algae** : *Ulva* or *Fucus*
- **Yeast** : *Budding yeast, w.m.*
- **Plant (corn)** : Corn leaf, c.s.
- **Fresh Microalgae** : YOUR own *Chlorella Vulgaris*

- 1- Choose 3 slides amongst the ones available (1 Algae, 1 Yeast, 1 Plant) and record data in chart below.
- 2- Prepare a wet mount of your algae **C. Vulgaris** :
  - a. Clean the slide with alcohol 95% and a Kimwipe to remove dust.  
Take, with a transfer pipet, a small volume of your algae culture from your Erlenmeyer and **put 1 drop over the methylene blue** on your slide.  
**Note** : Methylene blue stains on the skin.

- b. Clean the cover slip with a Kimwipe to take remove dust.
- c. Add **gently** the cover slip on the slide with 45° angle. (See picture below).
- d. Remove excess liquid with Kimwipe.
- e. Observe under the microscope with a 1000X magnification using oil.



- f. When all the observations are done, clean each objective with **lens paper** & alcohol 95 %. \*\*\* Please, pay attention to clean well the 100X objective that had oil immersion. \*\*\*
- g. Take a picture of each observation to include in your chart.
- h. Complete chart (question 1) below.



**Part 2 Questions:**Keep track of your references

1- Complete the chart below:

	<b>YEAST</b>	<b>ALGAE</b>	<b>PLANT</b>	<b>MICROALGAE</b>
<b>Name</b>				
<b>Picture or diagram</b>				
<b>Magnification</b>				
<b>Brief description (color, size, etc.)</b>				
<b>Organelle(s) seen</b>				

- 2- Plants are already used in the production of biofuels. Research some examples and list two plants used in North America to produce fuel.
- 3- Briefly explain why algae cells are green. Be sure to explain which colors from the electromagnetic spectrum are essential for algae growth.
- 4- According to your observations, which cell organelle contains lipids? Verify your answer by researching information online.
- 5- Briefly research how a fluorescent microscope works. Explain how this type of microscope would be of great interest for identifying oil in cells.

### Part 3: Algae Explosion!

Teachers: Ms. Baddour

Time: 120 minutes

Purpose:

- To measure light absorbance of algae cells with the use of a spectrophometer.
- To extract oil from algae cells.

### Evaluation:

Pre-Lab. assignment	Laboratory work	Post. Lab assignment
Read background information and procedure	none	Questions to be answered and submitted on moodle

### Background information:

Production of biodiesel from algae cells has a promising future. The total yield of oil per hectare from algae cells is more than 10 times the yield production of traditional plants like soya or canola.

### Procedure:

- 1- Today, you will measure light absorbance of your own algae cells using a spectrophometer. Light absorbance is done at **685nm**.
  - a) Diluted your algae culture *Vulgaris* in a **ratio 1:5** in order to obtain a FINAL volume of 1ml.
  - b) Measure absorbance at **685nm** and record results.
  - c) Using the graph, below, calculate how many cells per ml you have in your diluted sample.
  - d) Multiply your answer in d) **by 5 (dilution factor)**. Record answer below.
- 2- The second part of today's laboratory is to remove algae cells from their nutritive culture, wash them properly to avoid contamination and break the cell wall of the cells to extract oil (faire une lyse cellulaire). Oil extraction is done by "cooking" the cells in a vapor sterilized oven.
  - a) Extract **50ml of algae culture** transfer into 50 ml centrifugation test tube (Rely on centrifugation tube graduation).
  - b) Centrifuge **4800rpm for 5 minutes**.
  - c) Remove surfactant and clean with **10 ml distilled water**.

- d) Centrifuge again at **4800rpm for 5 minutes**.  
 e) Remove surfactant and add **~2.5 ml (maximum) of distilled water** and dispose in official container.

*Détermination de la concentration cellulaire de P. subcapitata, C. vulgaris et Scenedesmus sp. à 685nm*

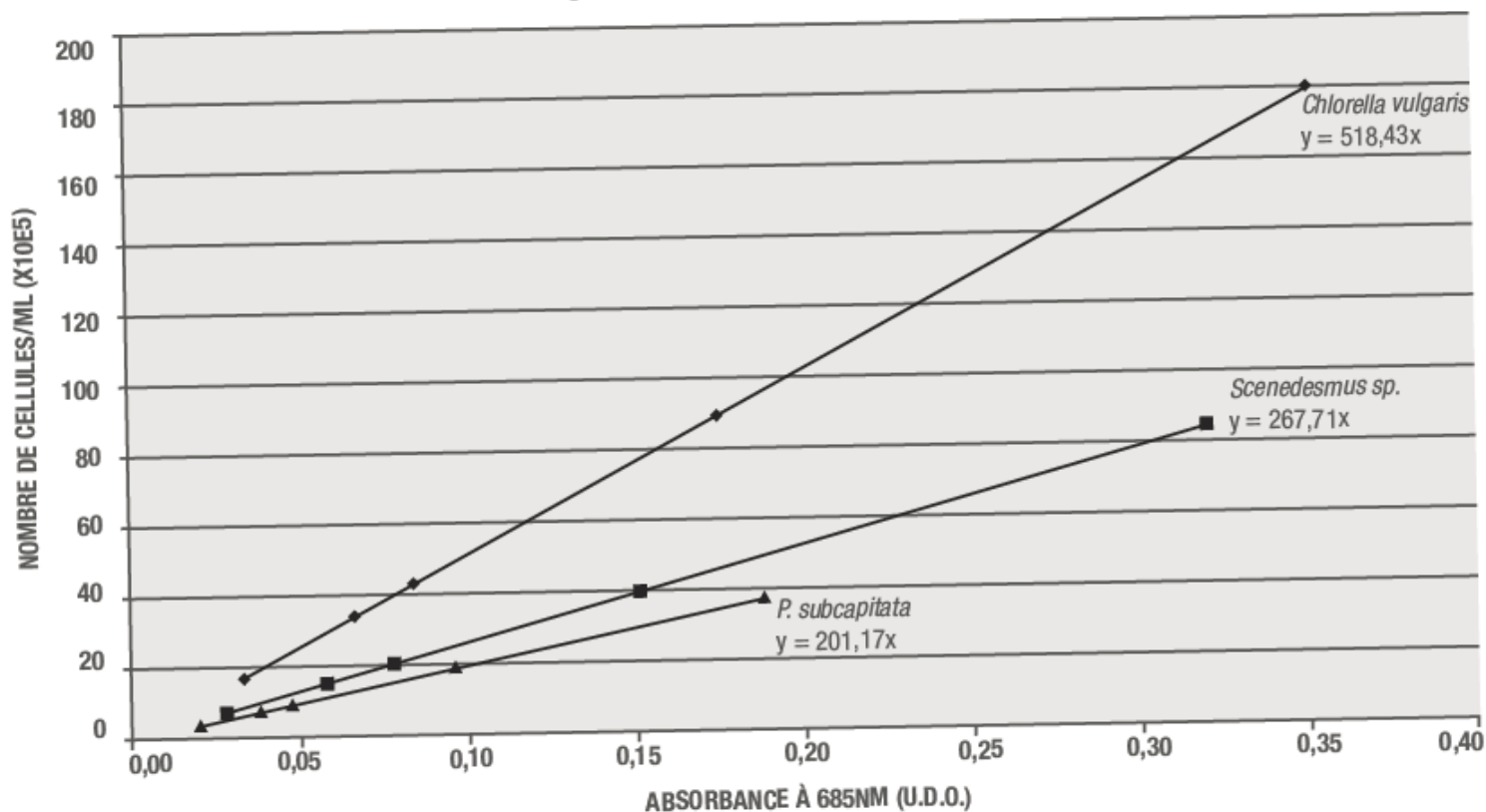


Figure 1: Growing trends for three types of unicellular algae cells.

### Part 3 Questions:

Keep track of your references

- 1- How many microliters of solution did you dilute in water to obtain a final volume of 1ml? Show calculations below.
- 2- State absorbance value at 685nm.
- 3- Use the figure above to calculate how many cells you have per milliliter of nutritive culture. Show calculations below. (Do not forget to multiply by 5).
- 4- Why is it necessary to zero the spectrophotometer before measurements?

- 5- The absorbance is done at 685nm. What color is this?
- 6- Why is the absorbance done at 685nm? Explain.
- 7- Provide a free body diagram of forces acting on an algae cell when inside centrifuge.
- 8- What is the centripetal acceleration on an algae cell located at the bottom of a test tube when inside the centrifuge? radius= 10.3cm
- 9- Our centrifuge at CiSA rotates up to 6000rpm. State this frequency in Hertz.
- 10-The word centrifuge comes from the centrifugal force. What is the centrifugal force? Compare it to centripetal force studied in class.
- 11-Modern ultracentrifuges have a frequency of about 60000Hz. If a red blood cell (mass 0.01g) is at the bottom of a test tube inside a centrifuge with a radius of 15 cm, find the centripetal force exerted on the red blood cell.

## Part 4: Titration of soybean oil and used oil

**Teachers: Ms. Baddour**

**Time: 90 minutes (if 120 minutes, better)**

### Purpose:

The purpose of this experiment is determine the quantity of free fatty acids (FFA) in two samples of oil (virgin soybean oil and used oil) by titration with KOH. This information is needed in order to calculate how much catalyst is needed for the conversion of these oils into biodiesel.

### Evaluation:

Pre-Lab. assignment	Laboratory work	Post. Lab assignment
Entry quiz on procedures that will be done today	Google doc. entry	Questions to be answered and submitted on moodle

### Introduction

What is a standard solution?

A solution prepared using a substance of known high purity, dissolved in a known volume of solvent<sup>1</sup>. Thus, the concentration of a standard solution is accurately known and is usually expressed as a molarity (mol of solute/L of solution). It is important that the mass of solute and the final volume of the solution are measured **precisely** (mass of solute on an electronic balance and volume of solution with a volumetric flask).

**Note:** Usually chemists prepare a *stock* solution when using standard solutions in the lab. A stock solution is a concentrated solution, which is then diluted to a lower concentration for actual use.

What is a titration?

Titration is a technique commonly used by chemists to determine the concentration of a solution. It involves the *slow* addition of one solution of known concentration (e.g. a standard solution) to a known volume of another solution of unknown concentration. The solution of known concentration is called the titrant. The addition of the titrant to the

<sup>1</sup> <http://goldbook.iupac.org/S05924.html>

solution of unknown concentration continues until the reaction reaches neutralization (typically indicated by a change in colour)<sup>2</sup>. Figure 1 illustrates a typical titration setup.

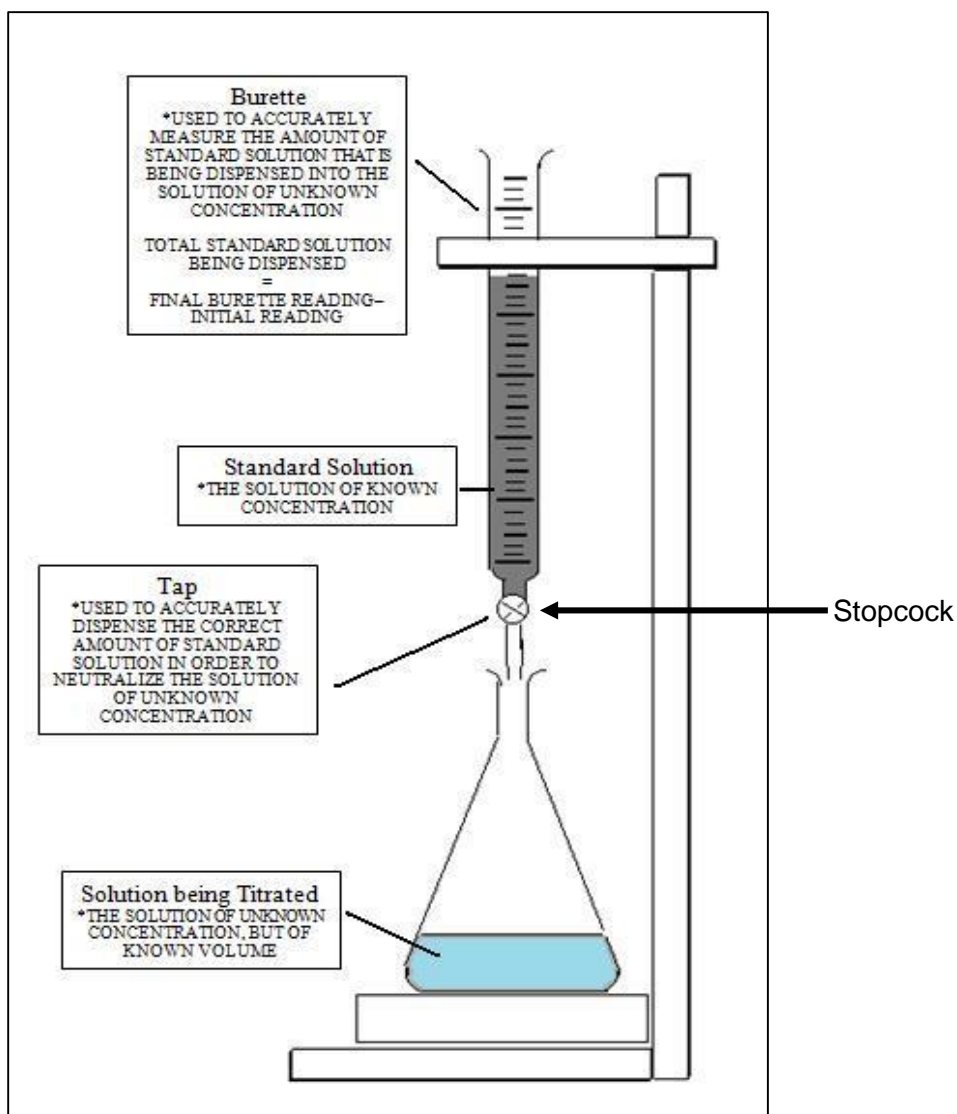


Figure 1 - Typical Titration Setup<sup>3</sup>

**Remember:** *Burette readings always have 2 decimal places.*

<sup>2</sup> [http://chemwiki.ucdavis.edu/Analytical\\_Chemistry/Quantitative\\_Analysis/Titration](http://chemwiki.ucdavis.edu/Analytical_Chemistry/Quantitative_Analysis/Titration)

<sup>3</sup> [http://chemwiki.ucdavis.edu/@api/deki/files/5184/=new\\_burette.jpg](http://chemwiki.ucdavis.edu/@api/deki/files/5184/=new_burette.jpg)

## Background information:

Used oils (ones that have been used once already for cooking) contain free fatty acids (FFA). Recall that all oils are composed of triglycerides and that triglycerides are made up of 3 fatty acid chains connected to a glycerin backbone. During heating, the triglycerides break down into diglycerides and FFA, as shown in Figure 1. The more an oil is used, the more the concentration of FFA increases. Used oils can contain up to 15 % (m/v<sup>4</sup>) FFA. The darker the used oil, the higher the FFA content.

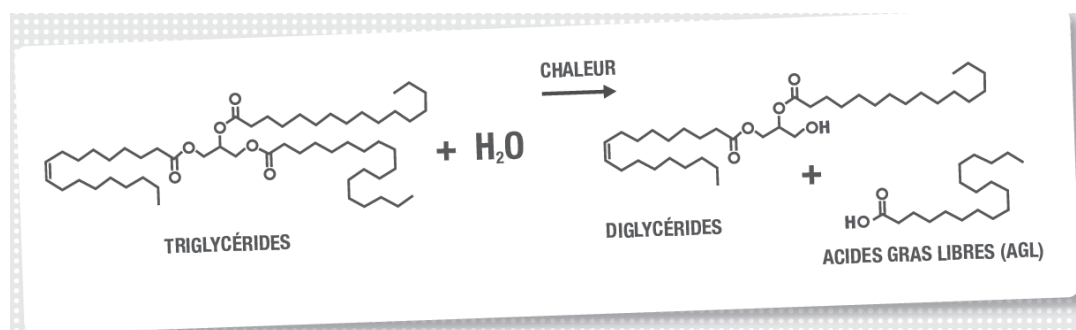


Figure 1: Formation of free fatty acids (FFA)

Why are we concerned about FFA? Because during the transesterification reaction to produce biodiesel, the FFA react with the catalyst (a strong base: NaOH or KOH) and form...soap! (Figure 2). The presence of this soap causes problems during biodiesel purification and it hinders the separation of the biodiesel phase from the glycerin phase.

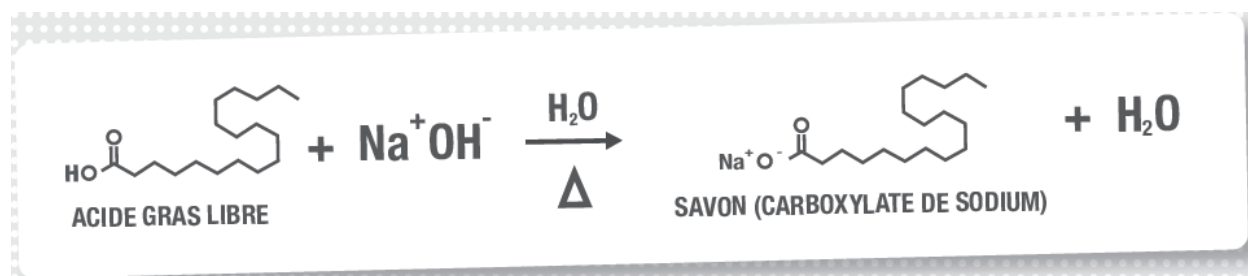


Figure 2: The reaction of FFA with a strong base to form soap

Thus, the amount of FFA must be determined by titration. Then, the extra amount of catalyst (KOH) that will be needed for the conversion of used oil to biodiesel can be calculated. In this way, we will ensure that there is enough available catalyst for the transesterification reaction.

<sup>4</sup> 15 % m/v = 15 grams of FFA per 100 mL of used oil

## Material

- Isopropanol
- KOH stock solution (1 % m/v<sup>5</sup>)
- Phenolphthalein
- Soybean oil
- Used oil
- Universal support
- Burette support
- 50 mL burette
- 125 mL Erlenmeyer flask x 4
- 5 mL transfer pipettes
- 10 ml graduated cylinder
- 100 ml volumetric flask
- Funnel
- Gloves

## Procedure

**A)** Prepare your titrant Standard KOH solution [0,1% m/v] ( $V_f = 100\text{ml}$ ) from a KOH stock solution [1% m/v]

1. Dilute the KOH stock solution **by a factor<sup>6</sup> of 10** by transferring \_\_\_\_\_ mL of your stock solution to a clean volumetric flask using a graduate cylinder. Add distilled water to the appropriate final volume. This new solution is your titrant, label it.

**B)** Titration of a Used & Soybean oil

2. **Fill the burette** with the titrant solution (**0.1 % m/v KOH**). **Record** the exact initial volume of the solution in the burette (*Remember: Burette readings always have 2 decimal places*).

**Note :** *Remove the air bubble, if present, from the bottom of the stopcock by gently tapping on the burette.*

3. Add **10 mL isopropanol** (use a graduated cylinder), **1 mL of used oil** (use transfer pipette), and **2-3 drops of Phenolphthalein** to a **125 mL Erlenmeyer flask**.

**Note:** *Make some up & down with isopropanol in order to dissolve and transfer all of the oil from the pipette to the Erlenmeyer.*

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<sup>5</sup> 0.1 % m/v = 0.1 grams of KOH per 100 mL of water

<sup>6</sup> Dilution factor =  $V_{\text{new}}/V_{\text{old}}$



4. Titrate **drop by drop** and agitate **continuously** the Erlenmeyer flask until the end point is reached.

**Note :** *Titrate by adding the titrant from the burette to the titrated solution in the flask. Do this by opening the tap(stopcock) to deliver a small stream of titrant to your flask. The indicator will change colour when the titrant hits the solution in the flask, however this colour change will disappear once the solution is mixed. As you get close to the end point, slowly add the titrant **drop by drop!** The endpoint occurs when a permanent **dark pink colour** is obtained.*

5. Once the endpoint is reached, record the exact final volume of KOH in the burette.



Ref : Image by Valerie Otis

6. **Repeat steps 1 to 4** for a second trial with the **used oil** and calculate the **average volume of KOH** used. The results should not vary by more than 0.10 mL. **Remember to add more titrant solution to the burette before starting the next titration and don't forget to record the initial volume.**

**Note:** *After your first titration – keep your Erlenmeyer flask nearby to compare with the remaining titrations.*

7. Repeat **steps 1 to 5** with the **soybean oil**.
8. Completely remove the KOH titrant solution from the burette and put it in the appropriate waste container.

**At the end only, cleaning:**

- *Dispose the solution titrated in the appropriate waste container.*
- *Rinse volumetric flask with water & let it dry.*
- *Wash the Erlenmeyer flasks with water & soap. Use a brush to help dissolve the oil.*
- *Place the glassware upside down in the drying rack.*

Name: \_\_\_\_\_

### **Part 4 Questions:**

1. Record your titration data in the following table.

	<b>Used oil</b>			<b>Soybean oil</b>		
	Initial KOH volume (mL)	Final KOH volume (mL)	Volume of KOH used (mL)	Initial KOH volume (mL)	Final KOH volume (mL)	Volume of KOH used (mL)
<b>Trial 1</b>						
<b>Trial 2</b>						
<b>Average</b>						

2. Calculate the % FFA in both oils.

	<b>Used oil</b>	<b>Soybean oil</b>
<b>% FFA</b>		

Calculations

3. Calculate the extra KOH that will be needed for the transesterification reaction tomorrow. Remember that 1 mL of oil was used

	<b>Used oil</b>
<b>Average volume of KOH 0.1 % used during titration</b>	
<b>Mass of extra KOH needed per L of oil</b>	
<b>Mass of extra KOH to add to 55 mL of oil</b>	

Calculations

## Part 5: Biodiesel from vegetable oil!

Teacher: Mr. Ian Wikarski

Time: 120 minutes

### Purpose:

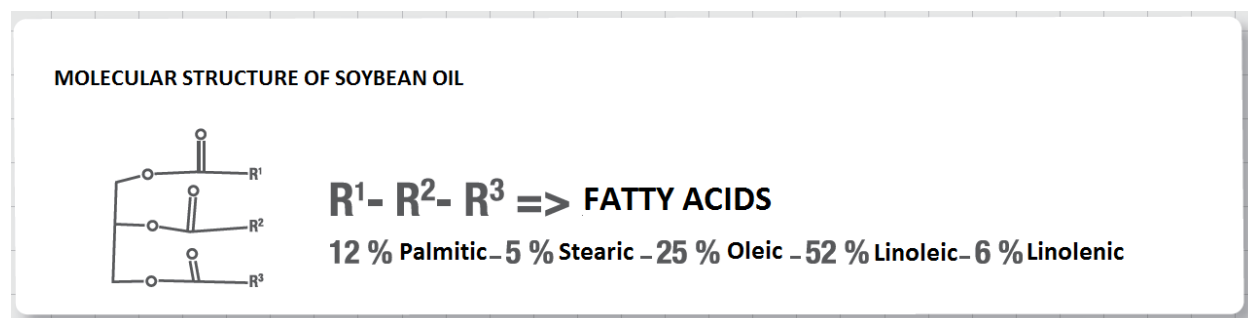
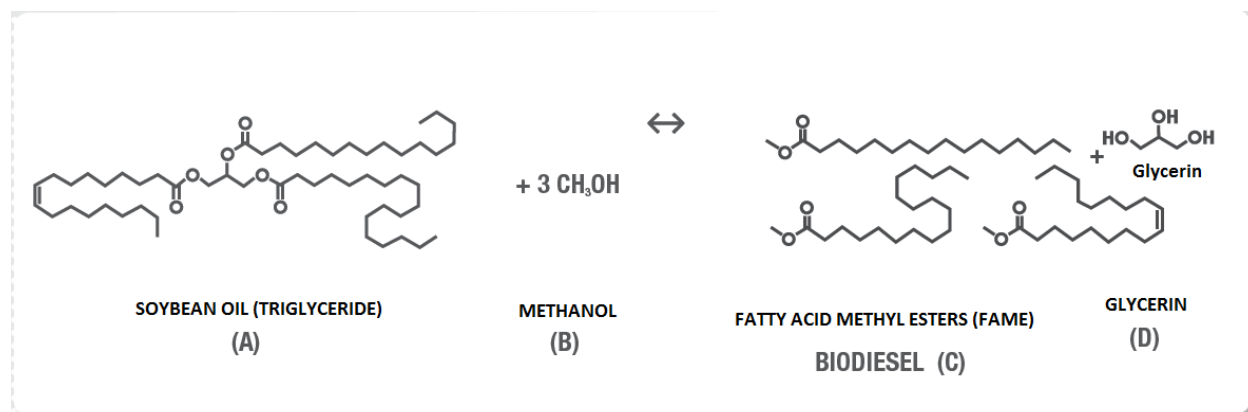
The purpose of this experiment is to produce biodiesel from soybean oil. You will perform the transesterification reaction and use stoichiometry to determine the percent yield of the reaction. The percent conversion of used oils and virgin oils will be compared.

### Evaluation:

Pre-Lab assignment	Laboratory work	Post. Lab assignment
Entry quiz on procedures that will be done today	none	Questions to be answered and submitted on moodle

### Background information:

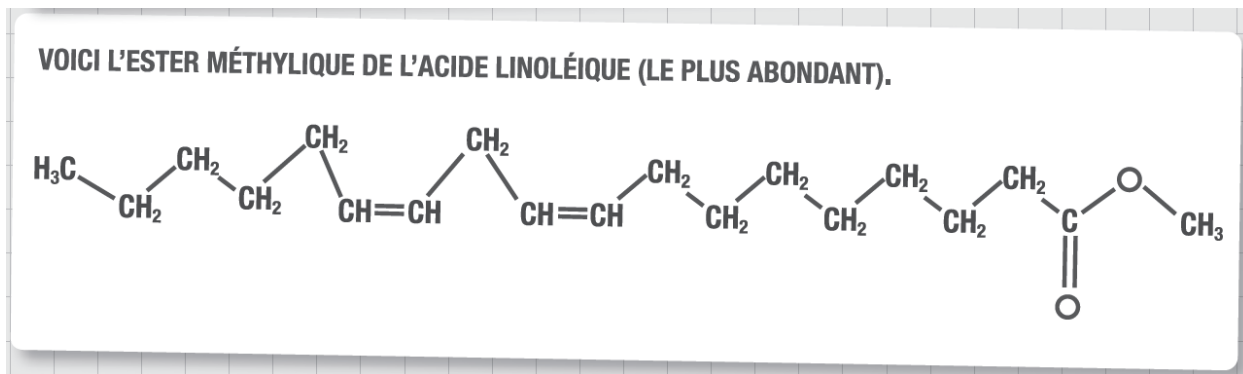
The transesterification reaction is as follows:



## Common Fatty Acids:

SATURÉ/ INSATURÉ	NOMBRE DE CARBONE		FORMULE STYLISÉE
Saturé	C <sub>12</sub>	Acide laurique	
	C <sub>14</sub>	Acide myristique	
	C <sub>16</sub>	Acide palmitique	
	C <sub>18</sub>	Acide stéarique	
	C <sub>20</sub>	Acide arachidique	
Insaturé	C <sub>18</sub>	Acide oléique	
	C <sub>18</sub>	Acide linoléique	
	C <sub>18</sub>	Acide linolénnique	

Biodiesel is a fatty acid methyl ester (FAME) the most abundant FAME in biodiesel is linolenic acid methyl ester:



## Material

- 55 mL of soybean oil and 55 mL of used oil
- 40 mL methanol x 2
- KOH pellets
- 100 mL beaker x 2
- 125 mL Erlenmeyer flask x 2
- 100 mL graduated cylinder x 2
- 25 ml graduated cylinder x 1
- thermometer
- universal stand
- thermometer clamp
- heating plate
- transfer pipettes
- magnetic stir bars
- Timer

## Setup

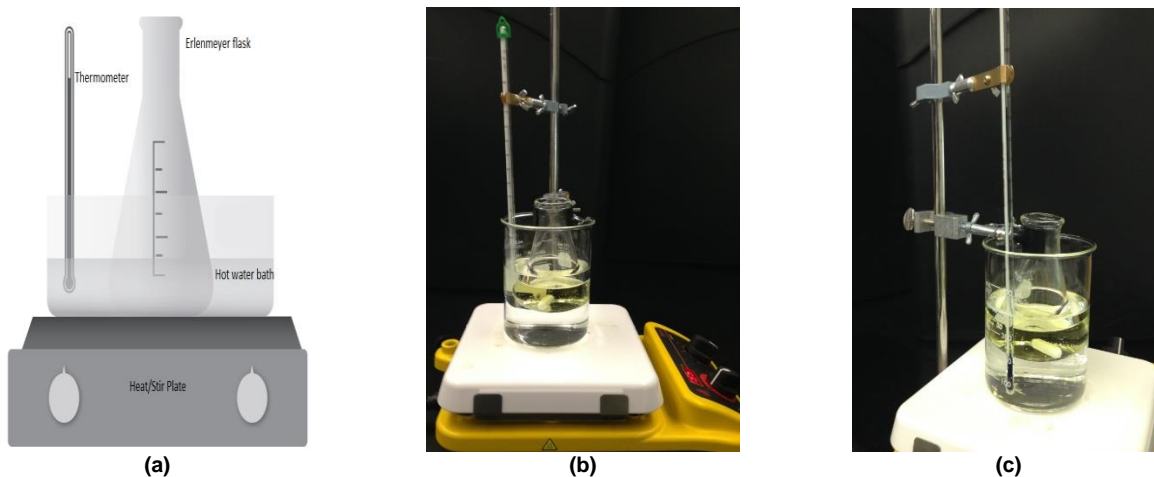


Figure 1 (a)-(c): Setup for transesterification

## Procedure:

1 – Prepare your caustic methanol solutions (one to be used for soybean oil and one to be used for the used oil).

2.1 **Label two 100 mL beakers:** “Caustic methanol for soybean oil” and “Caustic methanol for used oil”.

2.2 **In the first beaker (for soybean oil):** Weigh **1.4 g of KOH**. Dissolve the KOH in **40 mL of methanol (measure with 50 ml cylinder)** in a 100 mL beaker using a magnetic stir bar. Wait until the KOH dissolves completely (this may take up 10 minutes).

2.3 **In the second beaker (for used oil):** Weigh **1.4 g + 0.0605 g = 1.4605 g of KOH**. (The extra KOH amount is determined from the titration results from

yesterday). Dissolve the KOH in **40 mL of methanol** in a 100 mL beaker using a magnetic stir bar. Wait until the KOH dissolves completely (this may take up 10 minutes).

2 - Prepare your setup as shown in Figure 1.

3 – The transesterification reaction:

3.1 Measure **55 mL of soybean oil** using a 50 ml graduated cylinder. Transfer the oil into the **Erlenmeyer flask** and add a magnetic stir bar. Make sure your flask is stabilized and well supported by clamping it to the universal stand (see setup image).

3.2 Add **hot water** from the tap to the water bath container and **gently heat** using the heating plate.

3.3 Using the **thermometer clamp**, fix the **thermometer** in order to be able to determine the water bath temperature.

3.4 **Start the agitation** on the heat/stir plate. Adjust it such that you form a little whirlpool in the oil, without causing any splashing.

3.5 **Heat** until the temperature of the water bath reaches **50 °C**, then turn **off** the heating.

3.6 **Add 14 mL of caustic methanol to the Erlenmeyer flask** using a 25 ml graduated cylinder.

3.7 Maintain the temperature of the water bath between **45 and 55 °C** for **20 minutes**. To do this, wait until the temperature **decreases to 45 °C** before turning on the heating again.

**Note:** The temperature must not exceed 55 °C.

3.8 After 20 minutes, the reaction is done. **Stop the agitation and turn off the heating plate.**

3.9 Remove the Erlenmeyer flask from the hot water bath and **leave it on the lab bench to cool down** for about **10 minutes**, so that it reaches room temperature.

3.10 Meanwhile, **repeat steps 3.1 to 3.9 with 55 mL of used oil**. Remember to use the **caustic methanol** that was prepared for the **used oil**.

3.11. Transfer in 150ml beakers. Cover them with aluminum foil. Label each with your initials.



Figure 2: Erlenmeyer flask with two phases (upper: biodiesel, lower: glycerin). Image by Ian Wikarski

**Waste Disposal Instructions:** Dispose of caustic methanol in the appropriate container under the fumehood.

**Cleaning:**

1. Graduated cylinders: Clean every cylinder. If it contains oil, use soap and wash thoroughly with the brush.
2. Beakers and Erlenmeyer flasks: Wash with water & soap.
3. Drying: Place the glassware upside down in the basket to let them dry.



Name: \_\_\_\_\_

### **Part 5 Questions:**

Keep track of your references

#### **Background Questions**

1. Fill in the following table to indicate the stoichiometric coefficients of the transesterification reaction performed today.

REACTANTS			PRODUCTS	
Soybean oil (triglyceride)	Methanol	↔	Biodiesel (fatty acid methyl esters or FAME)	Glycerin
A	B		C	D

2. In this lab, we produce a biodiesel from soybean oil at the experimental scale via the transesterification reaction. Would this reaction be doable with other types of oils? Briefly explain.

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3. What factors could influence the speed of the transesterification reaction performed today with soybean oil?

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**Observations**

4. Complete the table below

Data	Values
Volume of soybean oil used (mL)	
Volume of methanol used (mL)	
Reaction time (minutes)	
Temperature of the water bath (°C)	
Volume of biodiesel collected (mL)	

The following information will be useful to answer the next few questions.

	Soybean oil	Methanol	Biodiesel	Glycerin
<b>Molar mass (g/mol)</b>	872	32	292	92
<b>Density (g/mL)</b>	0.92	0.79	0.88	1.25
<b>Boiling point (°C)</b>	257	65	348	290

**Synthesis Questions**

5. Using the volumes and experimental information, determine the mass and number of moles of the two reactants. Show your calculations below and enter your values into the table provided.

Reactants	Volume (mL)	Mass (g)	Moles
<b>Soybean oil</b>			
<b>Methanol</b>			

Calculations:

6. Fill in the following table and show your calculations below. *The experimental ratio corresponds to the number of moles of methanol in the solution for every 1 mole of soybean oil present.*

Reactants	Stoichiometric coefficient from the equation	Experimental number of moles	Experimental ratio
Soybean oil			1
Methanol			

Calculations:

Does the quantity of reactants used agree with the stoichiometric coefficients from the balanced equation? Explain briefly.

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7. Recall that we used a caustic methanol solution today (methanol containing a small amount of KOH, a strong base). The KOH acts as a catalyst. Explain the role of a catalyst and its impact on the reaction.

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8. Why is it important to maintain the temperature of the mixture between 45 and 55 °C during the reaction?

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9. What property of the two products (biodiesel and glycerin) lets us deduce that the top phase is the biodiesel?

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10. Calculate the **theoretical masses and volumes** of biodiesel and glycerin that should have been obtained based on stoichiometry. Use the number of moles of the limiting reactant (soybean oil) for your calculations and consider that methanol is in excess.

	Soybean Oil	Biodiesel	Glycerin
<b>Stoichiometry coefficients</b>			
<b>Number of moles</b>			
<b>Mass (g)</b>			
<b>Volume (mL)</b>			

Calculations:

11. Use your experimental volumes and theoretical volumes to help you answer the following questions:

a) Calculate the percent yield of biodiesel. *Remember: percent yield = experimental volume / theoretical volume x 100*

Data	Values
<b>Experimental volume of biodiesel produced (mL)</b> <i>Refer to Question 4</i>	
<b>Theoretical volume of biodiesel (mL)</b> <i>Refer to Question 10</i>	
<b>% yield</b>	

b) Knowing that the yield of this reaction should be around 98 %, how does your yield determined above compare? Discuss briefly.

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12. Calculate the percent conversion of the soybean oil and used oil and summarize in the table below.

	Soybean oil	Used oil
<b>Volume of oil used (mL)</b>	55	55
<b>Volume of biodiesel produced (mL)</b>		
<b>% conversion = <math>(V_{\text{biodiesel}}/V_{\text{oil}}) * 100</math></b>		

**13.** How does the percent conversion of the used oil compare to the virgin soybean oil? Explain.

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**14.** Explain briefly why converting used oils to biodiesel is problematic compared to virgin oils. How can you overcome this problematic?

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## Part 6: Biodiesel cleaning

Teacher: Mr. Wikarski

Time: 60 minutes

Purpose: To clean the produced biodiesel from free fatty acids and methanol and create a true acceptable commercial fuel.

### Evaluation:

Pre-Lab. assignment	Laboratory work	Post. Lab assignment
none	none	N/A

### Background information:

Biodiesels produced from used oils contain free fatty acids, glycerol, methanol residues and soap -which are byproducts of the titration process-. To clean your biodiesel from these impurities, water will be used. This process is widely used in the industry and washing accurately the biodiesel will make a truly acceptable commercial fuel.

### Procedure:

The following steps must be done for each biodiesel produced in step 5 (from soya and from used oils). You can do the steps at the same time, but use different containers.

- 1- Get your **2 erlenmeyers** containing biodiesel from Part 5 lab.
- 2- **Transfer the biodiesel** (upper phase – refer to Figure 2) by decanting to a 100 mL graduated cylinder. Complete the remaining transfer using a transfer pipette. Make sure **not to transfer any glycerol** to the graduated cylinder!
- 3- **Determine the exact volume** of the biodiesel collected. Record your volume in the observation sheet.
- 4- Transfer the biodiesel into a **cooking jar** (pot Masson) : 1 jar for soya oil; 1 jar for used oil (**Identified the jars** : type oil, initials, date).  
**Note** : You can combine your biodiesel with another team to reduce the amount of jars used.
- 5- Add **20% of volume of tap water** to the biodiesel.  
 For example, add 20ml of water to 100ml of biodiesel.
- 6- Add the lid to the jar and **shake vigorously for 1 minute**. Turn the jar upside down several times.

- 7- Wait **5 minutes** and note observations.
- 8- Repeat **steps 1 to 4** with the other biodiesel.
- 9- Wait at least **24 hours** to record final observations (done next class).



## Part 7: Combustion of biodiesels

Teacher: Mr. Wikarski

Time: A solid 120 minutes (65 minutes of theory + 55 minutes of laboratory)

**Purpose:** To verify the energy content of biodiesel fuels and compare it to regular fuels.

### Evaluation:

Pre-Lab. assignment	Laboratory work	Post. Lab assignment
None	Fill out google document	Questions to be answered and submitted on moodle

### Background information:

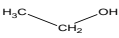
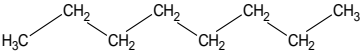
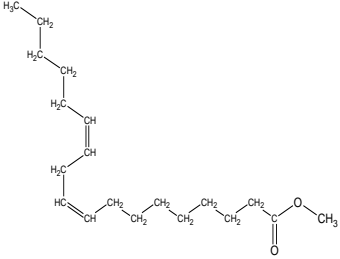
Renewables biofuels offer a promising hope in the reduction of production of global warming molecules (methane, carbon dioxide, etc.). In the past years, the federal government has imposed new regulations in fuel composition. Table 1 summarizes some regulations.

**Table 1 : New regulations on commercial fuels**

Type of fuel	Content required	Type of biofuel	Date of regulation
Fuel	5%	Ethanol	December 15th 2010
Diesel and heating crude oil	2%	Biodiesel	July 1st 2011 January 2013 (Québec et maritimes)

Adding the biofuels to regular fuels leads to the following questions: What are the environmental and economic benefits of doing so? To answer this question, some calculations are required.

Table 2: Molecular structure of some fuels

	Éthanol	Essence (Octane)	Biodiésel *Esther méthylique d'acide linoléique	Pétrodiesel
Structure				
Formule moléculaire	C <sub>2</sub> H <sub>6</sub> O	C <sub>8</sub> H <sub>18</sub>	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	
Masse molaire M (g/mole)	46	114	294	
Masse volumique ρ (g/mL)	0,79	0,70	0,88	0,85

**Results:****Table 3 : Theoretical heat combustion of various fuels.**

Fuel	Heat combustion $\left(\frac{kJ}{mole}\right)$	Molar mass (g/mole)	Heat combustion (theoretical) $\left(\frac{kJ}{g}\right)$
Ethanol			
Octane			
Biodiesel (from soya)			

## Procedure:



Figure 1 : Set-up

**Note :** Distance between burner and flame should be 0.5cm to 1cm max.

**WARNING :**

**Lab. coat and goggles needed.**

**Watch out for fumes.**

**HIGHLY FLAMMABLE!!!**

1. First, transfer **your biodiesel** from soya oil or used oil, as decided in class, using a funnel under the fume hood.  
**Note : Fill the burner approximately to three-quarter.**
2. Add **300ml of tap water** to soda can. Measure volume as accurately as possible using a graduated cylinder.
3. Take burner with **identified fuel**.
4. Measure the **mass of burner** and record result.
5. Measure **initial water temperature**. Make sure thermometer does not touch bottom of can.
6. Light up burner located at 0.5cm from bottom of can.
7. Wait **approximately 40-50 seconds OR** until water temperature has changed by **4-5 degrees Celsius**.
8. Measure the **mass of burner again** and record result.
9. Repeat **steps 1 to 7 with three other types of fuel**.

Insert all your results in the **google doc**. \* Enter values in google form :

Group 1: <https://docs.google.com/spreadsheets/d/1aRpKDTewiRhI-rkLuz7GI4mwZwkVVI32an0sPLz7pqA/edit?usp=sharing>

Group 2 and 3: [https://docs.google.com/spreadsheets/d/1A26mDOZryhotx-X\\_HQbfngnjS8qfucZcCp1rBNtmYcQ/edit?usp=sharing](https://docs.google.com/spreadsheets/d/1A26mDOZryhotx-X_HQbfngnjS8qfucZcCp1rBNtmYcQ/edit?usp=sharing)

Name: \_\_\_\_\_

**Table 4 : Burned mass**

	Initial mass (g)	Final mass (g)	Burned mass (g)
Ethanol			
Octane			
Petrodiesel			
Biodiesel (soya) <b>OR</b>			
Biodiesel (used oil)			

**Table 5 : Water temperature variations**

	Initial temperature (°C)	Final temperature (°C)	Temperature difference ( $\Delta T$ ) (°C)
Ethanol			
Octane			
Petrodiesel			
Biodiesel (soya)			
Biodiesel (used oil)			

**Table 6\* : Heat combustion of various fuels (kJ/g)****Use  $c = 4.186$  joule/gram °C for water.**

	$\Delta T$ (°C)	Energy absorbed by water (kJ)	Burned mass (g)	Experimental heat of combustion* $\left(\frac{kJ}{g}\right)$
Ethanol				
Octane				
Petrodiesel				
Biodiesel (soya)				
Biodiesel (used oil)				

\* Enter values in google form :

Group 1: <https://docs.google.com/spreadsheets/d/1aRpKDTewiRhI-rkLuz7GI4mwZwkVVI32an0sPLz7pqA/edit?usp=sharing>Group 2 and 3: [https://docs.google.com/spreadsheets/d/1A26mDOZryhotx-X\\_HQbfngniS8qfucZcCp1rBNtmYcQ/edit?usp=sharing](https://docs.google.com/spreadsheets/d/1A26mDOZryhotx-X_HQbfngniS8qfucZcCp1rBNtmYcQ/edit?usp=sharing)**Table 7\* : Heat combustion and % difference between theoretical and experimental values.****\*USE CLASS AVERAGES (GOOGLE FORM) TO FILL THIS CHART**

	Heat of combustion (theoretical) $\left(\frac{kJ}{g}\right)$	Average heat of combustion (experimental) $\left(\frac{kJ}{g}\right)$	% différence
Ethanol	27.4		
Octane	44.7		
Petrodiesel	N/A		N/A
Biodiesel (soya)	37.8		
Biodiesel (used oil)	37.8		

**Part 7 Questions:**

Keep track of your references

1. Describe your observations between biodiesel and petrodiesel (smell of fumes, color of fuel, color of flames, size of flames, etc.)
2. According to your experimental results, which fuel has the greatest heat combustion per gram? Is your answer identical to class averages? Explain.
3. State at least three sources of error in this laboratory explaining significant differences between theoretical and experimental values

## Part 8: Automation of biodiesel production

Teacher: Mr. Wikarski

Time: 120 minutes

**Purpose:** To visualize and analyze a semi-automatic biodiesel machine.

### Evaluation:

Pre-Lab. assignment	Laboratory work	Post. Lab assignment
None	none	Questions to be answered and submitted on moodle

### Background information:

The following semi-automatic machine is controlled by a programmable logic controller and can be controlled manually. The machine is used to convert soya oil (or any other clean vegetable oil) into biodiesel.

### Procedure

Analyze carefully the machine and press various switches (manually) to verify which valve they control.

### Questions:

1. Carefully identify container A,B,C,D by outlining what reactant(s) (or liquid(s)) you would add to each respective container. Record answers in the chart below.

Container	Reactant(s) or Liquid(s)
A	
B	
C	
D	

2. Identify a possible valve number that would be opened at the following times:

<b>Time (minutes)</b>	<b>VALVE NUMBER(S)</b>
0	
90	
91	
720 (12 hrs)	

3. Calculate and state all quantities needed (including concentrations and times) that would produce a FINAL volume of 250ml of biodiesel. Leave calculations below.



## Part 9: Visit of Rothsay Biodiesel Company

Teachers: Mr. Wikarski & Valérie Ottis

Time: 60 minutes

Purpose: To visit a real industrial biodiesel production plant.

### Evaluation:

Pre-Lab. assignment	Laboratory work	Post. Lab assignment
none	none	none

### Background information:

Briefly visit the company website to learn more about biodiesel:

<http://www.rothsay.ca/francais/produits/biodiesel/>

Direction to the company: (a 15 minute drive from school)

Rothsay Sainte-Catherine

605 1ere Ave.

Sainte-Catherine, QC J5C 1C5



## SYNTHESIS QUESTIONS

1. We saw in this project how to produce biodiesel by transesterification as a batch operation. Say you wanted to perform the same transesterification from clean vegetable oil as we did in the lab, but with larger quantities AND as a continuous automatic process. Draw, label and explain a diagram of what your continuous set-up would look like. Be sure to include quantities (e.g. volumes, times, concentrations, etc). Use information from the fieldtrip.
2. Briefly explain why ethanol is now added to regular fuel and why biodiesel is added to regular petro diesel.
3. Converting used oils into biodiesel is not as easy as it sounds. Explain why a titration of used oils is necessary and explain the steps needed in order to obtain clean biodiesel from used oils.
4. Theoretical values for oil content for the algae *Vulgaris* is approximately 15% (mass oil/mass algae) under good growing conditions. You are part of a company that would like to produce 20000 liters of biodiesel from algae *Vulgaris* per month.
  - a) How much raw oil from algae would you need in order to produce the desired quantity? (look at your results from the soya experiment)
  - b) How much mass of algae would you required? (Hint: density of oil is approximately 0.92g/ml).
5. In your own words, explain the difference between a first generation, second generation and third generation biofuel, give examples and state the advantages and disadvantages of each.
6. In plain and simple terms (such that a 10 year old child can understand), explain what is a biodiesel and how it is produced by transesterification.
7. Based on your knowledge of the various biofuel generations, describe a possible  $n^{\text{th}}$  generation biofuel.

8. Create an absorption / time graph for the growth of algae over a 3 week period. Use class data created at lunch time using the google document.  
[https://docs.google.com/spreadsheets/ccc?key=0Arue\\_5GKXYUQdElwTXNvOV3aV9SQU5XMWNLaVhsN2c&usp=sharing](https://docs.google.com/spreadsheets/ccc?key=0Arue_5GKXYUQdElwTXNvOV3aV9SQU5XMWNLaVhsN2c&usp=sharing)
9. What are your thoughts and opinions about this project? Please fill de survey online at: [https://docs.google.com/forms/d/1awhVswEtrwEZn8cT6Z4LPAE\\_YpwF9bNyXGUKjFbHBk4/viewform](https://docs.google.com/forms/d/1awhVswEtrwEZn8cT6Z4LPAE_YpwF9bNyXGUKjFbHBk4/viewform)

### **References:**

Camden Council, Camden. "Biodiesel Project." *Biodiesel Project*. N.p., n.d. Web. 25 Mar. 2014.

<[http://www.camden.nsw.gov.au/page/biodiesel\\_project.html](http://www.camden.nsw.gov.au/page/biodiesel_project.html)>.

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[http://teachers.yale.edu/curriculum/viewer/initiative\\_13.05.03\\_u](http://teachers.yale.edu/curriculum/viewer/initiative_13.05.03_u)