

BioFuel Production through Yeast Fermentation

Objectives

The objectives of this lab exercise are for you to:

- learn about the use of ethanol as an alternative energy source.
- learn about the process of fermentation by yeast cells.
- develop your ability to properly design an experiment and interpret the results.
- improve your writing skills and use of literature sources.

Exercise Synopsis

Week 1: have read assigned materials; will discuss reading of scientific literature; and groups will select a variable to study

Week 2: have read assigned materials; perform initial experiment

Week 3: have read assigned materials; we will discuss experimental design; groups will plan out experiment; and we will also have an Excel graphing session.

*** Lab report Introduction and Literature Cited due. ***

Week 4: have read assigned materials; groups perform their experiment

*** Completed lab reports due two weeks later. ***

I. Reading for Week 1

Before coming to lab for the first week of this lab exercise you should read the following introduction to biofuel production and review the basic experimental methodology.

Biofuel refers most commonly to **ethanol** which is produced through the **fermentation** of carbohydrates by **yeast** cells. Fermentation is an energy-yielding process that cells carry out in the absence of oxygen. Although fermentation does not provide much usable energy for the cell, it is sufficient for yeast cells. Yeast cells produce ethanol and CO₂ as byproducts, and the ethanol produced has become a valuable energy source. There is much interest in ethanol as an energy alternative to fossil fuels, which are both nonrenewable and contribute significantly to atmospheric pollution.

Ethanol, which is blended with gasoline (at about 5-10%) to yield gasohol, has several advantages over petroleum as an energy source. The presence of an oxygen atom in ethanol (Figure 1) allows gasohol to burn 'cleaner' than regular gasoline, with reduced emissions of carbon monoxide, nitrogen oxides and hydrocarbons. Ethanol also helps to address concerns about greenhouse gas emissions (mainly in the form of CO₂). Unlike petroleum, ethanol is 'carbon neutral', which means that the CO₂ released when it is burned is balanced by the uptake of CO₂ from the atmosphere by plants growing to produce more grain. In light of its advantages, world production of ethanol has increased dramatically in recent years (Figure 2).

Nevertheless, there are also reasons to be concerned about the increased reliance on ethanol.

Figure 1. The molecular structure of ethanol.

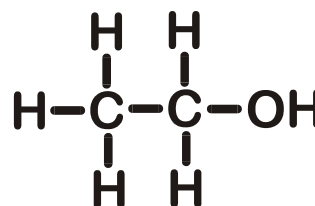
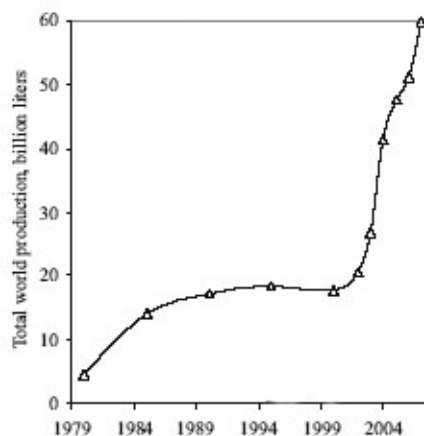


Figure 2. World ethanol production (Demirbas, 2009, 92).



From an energy balance perspective, more energy may be invested in the production of ethanol (harvesting of the crops, transportation, etc.) than it yields as a fuel (for differing views, compare Shapouri et al., 1995 and Pimentel, 2003). The need to divert food crops to fuel production also concerns many people. A bushel of corn (approx. 56 lbs) can yield approximately 2.5 gallons of ethanol, and at a corn yield of 125 bushels per acre (Shapouri, et al., 1995), an acre of farmland would yield enough corn for 310 gallons of ethanol. Thus, production of 8 billion gallons of ethanol requires about 2.5 million acres of land. The World Watch Institute (Gardner, 2000, 44) has reported that the amount of harvested (farmed) land in the world has declined over the last quarter century, while over the same time period world population has increased 50%. Thus, committing agricultural land to energy production will have consequences on available food in a world where the majority of people live in poverty.

Variables that affect the rate of fermentation in yeast cells

A scientist wishing to increase biofuel ethanol production might evaluate the effects of many different factors (or **variables**) on the rate of yeast fermentation. There are many variables that could potentially affect the rate of yeast fermentation. Indeed, an infinite number of factors (e.g., the alignment of the planets) might *potentially* affect the outcome of an experiment; however, a scientist seeking to optimize biofuel fermentation must use judgment to decide which variables are *most likely* to significantly affect the process. Some of the variables known to affect fermentation in yeast cells are described below.

Type of carbohydrate. What are the rates of CO₂ production when fermentation is allowed to proceed in the presence of glucose, fructose, lactose or sucrose?

Concentration of carbohydrate. What is the effect of different concentrations of glucose on the rate of yeast fermentation?

Osmolarity. Osmolarity refers to the total concentration of sugars or salts in the fermentation solution, which affects water flow into or out of the cells. What is the affect of adding sorbitol (which cannot be utilized by yeast for fermentation) along with glucose on the rate of fermentation?

Concentration of salt. How does varying the salt (NaCl) concentration affect the rate of yeast fermentation?

Ethanol concentration. What are the effects of varying the initial ethanol concentration in the fermentation mixture on yeast fermentation?

pH. What is the effect of varying pH on yeast fermentation?

Temperature. What are the effects of different temperatures on yeast fermentation?

***** On the Biofuel Web Resource Page at <http://marietta.edu/~biol/introlab/index105.html> you will find further information about fermentation and these factors, as well as a list of pertinent literature resources on reserve in the library. *****

Name: _____

Prelab Questions for Biofuel Exercise – Week 1

1. What is biofuel and how is it produced?

2. What are three reasons why there is much interest in expanding production of biofuel in the US?

3. What are two reasons why there is concern about increasing the use of ethanol as an energy source?

4. What are the two products of fermentation by yeast cells?

Production of which one will you be measuring in this lab exercise?

II. Reading for Week 2

How can we measure yeast fermentation?

As grain will continue to be used for biofuel production, it is appropriate that we should seek to maximize the yield of ethanol. One way to do this is to study the effects of various factors on the rate of fermentation. Ethanol and CO₂ are produced by yeasts during fermentation (the CO₂ is what leavens bread and gives beer its fizz). Although measuring ethanol would be the most direct and useful, measuring ethanol released by the yeast cells is too involved for an intro biology lab. However, CO₂ production can be measured quickly and accurately, and it affords an acceptable means of studying the effects of different variables on yeast fermentation.

The experiments you will perform will be carried out using the respirometer apparatus shown in Figure 3. The principles of its operation are very simple: the yeast suspension is placed into a syringe and the CO₂ released by the cells pushes a small water droplet up a pipet, which allows the volume of CO₂ to be measured at different times over a period of 15-20 minutes.

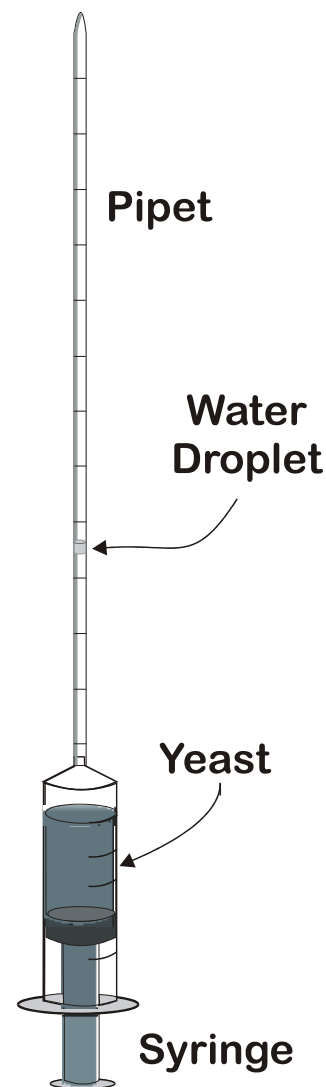
Experimental Procedure

In this week's lab you will study the rate of cellular respiration by yeast cells in the presence of a 5% (w/v) glucose solution. The procedure you will use is described below.

*** Each group member should perform his or her own experiment. ***

1. Add 10 ml of dH₂O to a 50 ml flask.
2. Weigh out 1.0 g of glucose, place it in the flask and gently swirl until the carbohydrate is completely dissolved.
3. Using a 10 ml pipet, transfer 10 ml of the yeast suspension into the flask.
4. Allow the yeast suspension to incubate for 5 minutes, with occasional swirling.
5. Take up exactly 3 ml of the yeast suspension into a syringe. Invert the syringe and draw 1 ml of air above the liquid.
6. Complete the assembly of the respirometer (don't forget the water droplet) and place it into your test tube rack.
7. As soon as the water droplet reaches the 0 ml mark, begin taking measurements at 1 minute intervals and record the data in the tables provided at the end of this lab exercise. You can mark measurements using either the top *or* the bottom of the droplet, but be consistent. You may need to decrease the time interval between measurements as the rate of CO₂ production increases.

Figure 3. Respirometer



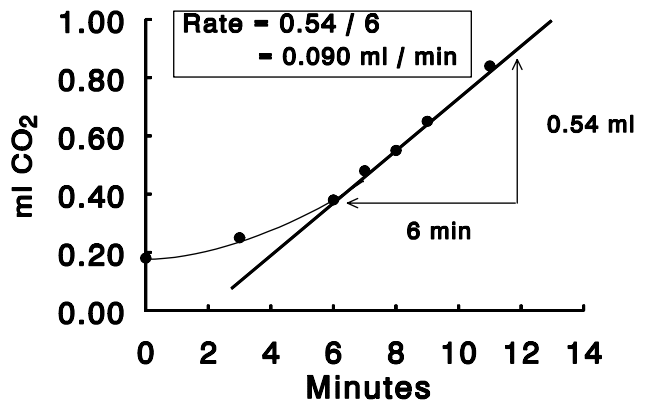
8. Plot the raw data on graph paper and draw a line through the linear part of the curve (Figure 4). Using only the **linear part of the data**, calculate the slope of the line as change in volume change in time. This yields the rate of respiration in units of ml CO₂/minute.

Table 1. Sample raw data

<u>Time</u>	<u>Reading</u>
0 min	0.18 ml
3	0.25
6	0.38
7	0.48
8	0.55
9	0.65
11	0.84

rate: 0.09 ml/ min
(calculated from graph)

Figure 4. Graphing fermentation raw data. The raw data (Table 1, the actual CO₂ volume measurements) is graphed as shown below. Note that the rate of fermentation is calculated from the slope of the line drawn through the **linear part of the curve**.



Graphing Results

The results of your experiment will show the effect of some factor over a range of conditions (e.g., temperatures) on the rate of yeast fermentation. The data shown in Table 1 and Figure 4 are examples of “**raw data**,” which are the original values collected in the lab. Raw data are used to calculate “**processed data**,” which in this case are the “rates” of fermentation calculated from the raw data. It is not sufficient in a lab report to include only graphs of the raw data; you must also include a **summary graph** to show the relationship between the variable you studied and the rate of fermentation. Sample summary graphs are shown in Figures 5 and 6.

How to arrange the axes of a graph

The “**independent variable**” must be plotted on the X-axis (abscissa) and the “**dependent variable**” must be plotted on the y-axis. The independent variable is the one that you controlled during the experiment (e.g., time, pH, temperature, or type of carbohydrate). The dependent variable is the value that was measured or calculated during the experiment. In this case, the dependent variable was either CO₂ volume (ml) or the calculated respiration rate (ml CO₂/min). In a properly designed experiment, the measured value is dependent upon the value of the independently controlled variable.

The two types of graphs you are most likely to use are a “bar graph” and a “line graph”. A **bar graph is appropriate when there is not a mathematical relationship between the values of the independent variable.** For example, the results of a study of fermentation in the presence of different types of carbohydrates should be presented in a bar graph (see Figure 5), because there is no “mathematical” relationship between ‘types of sugars’.

Figure 5. Fermentation rate in presence of different types of sugars.

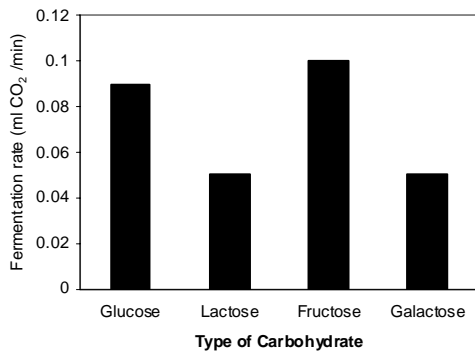
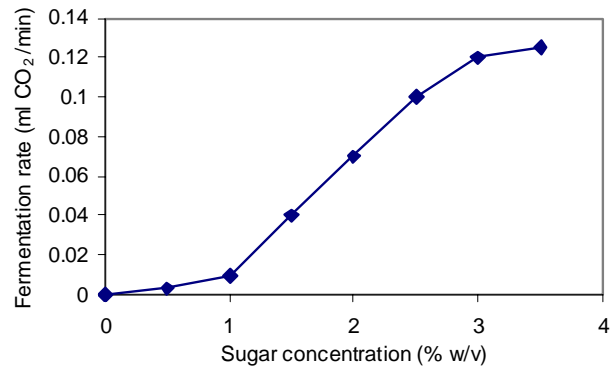


Figure 6. Rate of respiration in presence of different amounts of sugar.



However, a **line graph is only used when there is a mathematical or numerical relationship between the values of the independent variable.** Examples include amount of sugar added, temperature of incubation, incubation time, etc. (see Figure 6). Notice that the data points are clearly marked and connected by a line. This type of graph helps to reveal trends and relationships in the data that can be described and interpreted in the report.

III. Reading for Week 3

Some Considerations in Experimental Design

A well designed experiment tests the effect of only *ONE* variable at a time, the one described in the *hypothesis*. As you have already learned, a hypothesis is an educated guess of the outcome of the experiment. Often we have little knowledge by which to formulate a hypothesis, but there is always some knowledge that can be applied. Some examples of hypotheses would include:

- Yeast fermentation is fastest in the presence of glucose.
- Fermentation rates decrease as temperature is increased.
- The presence of calcium will increase the rate of fermentation.
- etc...

Often, a single experiment will test a range of **conditions of the variable**. For example, a study of the effect of temperature may test the effects of three or more different temperatures, and an investigation of sugar concentration may test the effect of a range of different values.

The results of such experimental tests are always compared to a **control**. You will recall that control tests are included to rule out other alternative explanations for the results. For example, someone might argue that the CO₂ released during this experiment is the result of chemical reactions that do not require living yeast cells (as was argued by some organic chemists in the early 1800s). What control would you suggest to 'control for' (rule out) this possible explanation?

Calculating Concentration

For all of these experiments you must calculate the amount of a material dissolved in the fermentation mixture. 'Concentration' is the most meaningful way to express this quantity. Concentration indicates the amount of a substance per unit volume of the liquid in which it is dissolved, and units of '% w/v' (percent weight/volume) or '% v/v' (percent volume/volume) are acceptable for this lab. When the substance being measured is added as a dry powder, '% w/v' should be used and is calculated by dividing the grams of the substance added by the final volume of the solution and multiplying by 100:

$$\% \text{ w/v} = \frac{\text{grams of substance added}}{\text{Final total ml of solution}} \times 100$$

For example, in the first week's exercise you measured fermentation by yeast cells provided with 1 gram (g) of carbohydrate dissolved in a total volume of 20 milliliters (ml), which represents a concentration of 5% w/v.

When the substance being measured is added as a liquid, '% v/v' should be used and is calculated by dividing the volume (ml) of the substance added by the final volume (ml) of the solution and multiplying by 100:

$$\% \text{ v/v} = \frac{\text{ml of substance added}}{\text{Final total ml of solution}} \times 100$$

For example, to make a 5% solution of ethanol in a mixture with a final volume of 20 ml, you would need to combine 1 ml of ethanol + 19 ml of the other components.

Prelab Questions for Biofuel Exercise – Week 3

Name: _____

1. What is the experimental variable that your group will be investigating?

2. What is a hypothesis that you might test concerning this variable?

3. What would be a suitable control for your experiment?

4. Write full, properly formatted references for two sources of information identified on the Biofuel Web Resources Page that have information pertinent to your experimental variable.

1.

2.

5. For each of these sources, identify something you learned about your experimental variable. For each, cite the source using the appropriate citation format [“(author, 1999, 34)” or “(author and author, 1999, 34)” or “(author et al., 1999, 34)”] as described in the lab report guidelines.

1.

2.

Sample calculations

1. 4 g of salt in 20 ml =
 $(4 \div 20 \times 100) = 20\% \text{ w/v}$

2. 6 ml of ethanol in 20 ml =
 $(6 \div 20 \times 100) = 30\% \text{ v/v}$

Practice calculations

(complete these before lab)

1. 2 g of lactose in 20 ml:

2. 3 ml of ethanol on 20 ml:

3. 5 g of MgCl in 15 ml:

4. 6 ml of glycerol in 18 ml:

IV- Reading for Week 4

Looking for patterns and trends in results

The effect of quantitative variables (a factor that can be varied by a quantitative amount, e.g., temperature, amount of sugar, etc.) on a process generally show one of two patterns. When the rates from a series of experiments where the variable differs quantitatively are plotted, sometimes the rate of the reaction decreases at values above and below a particular **optimal value** (Figure 7). Other variables may show a **maximal value**, above which the rate remains constant (Figure 8). You should look for such patterns in your results. Why might the variable you studied show an optimal or maximal pattern? What processes in the cell or fermentation (properties of membranes, enzyme, pathways, etc) would account for the results you obtain?

Figure 7. Sample results showing an optimal value. These data show that the rate of fermentation is highest when the independent variable has a value of 5 (the optimal value).

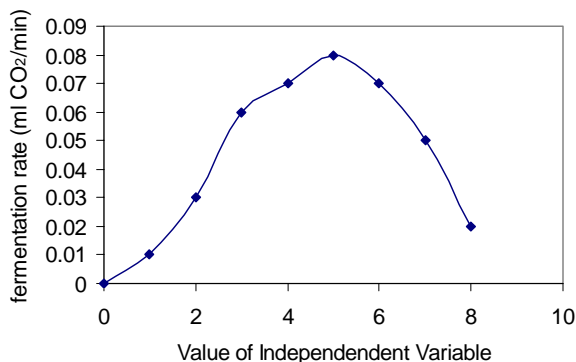
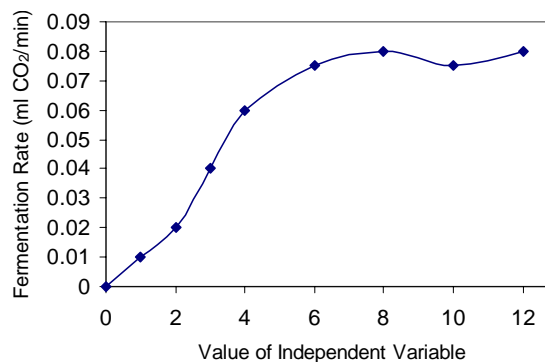


Figure 8. Sample results showing a maximal value. These data show that the rate of fermentation does not increase when the value of the independent variable exceeds 8 (the maximal value).



- Demirbas A. 2009. Biofuels, In: Demirbas A, editor. Biofuels: Securing the Planet's Future Energy Needs. London (GB): Springer. pp 87 – 101.
- Gardner G. 2000. Grain Area Shrinks. In: Brown LR, Renner M, Halweil B, editors. Vital Signs 2000. New York (NY): WW Norton and Co. p 44 - 45.
- Pimentel D. 2003. Ethanol Fuels: Energy Balance, Economics, and Environmental Impacts are Negative. *Natural Resources Research* 12:127-133.
- Shapouri H, Duffield JA and Graboski MS. 1995. Estimating the Net Energy Balance of Corn Ethanol. USDA Agricultural Economic Report Number 721.

Writing the lab report

General instructions

- Each student must write an independent report and prepare his or her own graphs.
- The report will include an Introduction, Hypothesis, Procedures, Results, Discussion and Literature Cited sections.
- General information about writing lab reports is provided in the Document entitled “Guidelines for Writing Lab Reports”.

Introduction

You are expected to read the pertinent sections on the Biofuel Web Resource Page at <http://marietta.edu/~biol/introlab/index105.html> and use pertinent literature resources on reserve in the library.

- You must use at **least five of the literature sources** on reserve in the library for background information (see the web resource page for a list of these literature sources).
- You can use your textbook, lab manual and web resource page as literature sources.
- All information sources must be cited and referenced using the formats as specified in the lab report guidelines.
- Topics that should be discussed in Background Information section:
 - Why are people interested in biofuel production?
 - What is the nature of fermentation; when do cells carry out this process, what are the end products?
 - What are some of the characteristics of yeasts, and why are they used in biofuel production?
 - Why is it important to study factors that affect biofuel production; what is the variable that your group is studying; what do literature sources say about this variable?

Procedures

You will thoroughly describe the experiment that your group performed this week, including the procedure for measuring yeast respiration described above. Remember, the procedure must be written in essay format, in the third person passive voice, and in enough detail for some else to repeat the experiment and obtain the same results. You are encouraged to include a picture of the respiration apparatus (with a figure legend).

Results

Tables and Figures

- Graphs of all raw data, prepared on Excel, with trendlines drawn through the linear region of the data. To conserve paper, plot the raw data for all conditions of your variable for a given experimental replicate in a single figure; all lines must be distinguishable and clearly labeled.
- **Do not include tables of the raw data.**
- A table of calculated respiration rates for each replicate and the averages.
- A summary graph showing average respiration rates vs your independent variable. You should use the graph format appropriate to your variable.

Note: all tables and graphs must be formatted (Figure numbers, title, axes labels, etc.) as described in the Lab Report Guidelines.

Description of Data

- Describe the raw data for one typical replicate, but only describe any unusual aspects in the raw data from other replicates.
- Fully describe the summary graph that presents average fermentation rates, this represents the most important results of the experiment.

Discussion section

Conclusions

- How does the trend in the summary graph compare with your initial predictions?
- Do the results support your hypothesis or not?

Explanation of results. Questions that should be discussed include:

- If appropriate, explain how the results differed from your expectations or were ambiguous; is there a better alternative conclusion?
- Do the results for your variable shows an optimal or maximal value? Why might it show such a pattern?
- How do your results compare to related literature sources about your variable? (be sure to cite sources)
- Why does the variable you tested affect ethanol production as shown in the summary graph?
- What are the implications of your findings in context of biofuel production; how would you apply your findings if you were actually attempting to improve the process?

Future experiment

- What is a logical extension of the experiment that you performed involving the same variable?
- A poor future experiment will merely call for more replicas or a wide range of conditions of the variable.

Literature References (and Citations)

- Must follow correct formats
- Must included at least 5 sources on reserve
- No references of web sites (except Biofuel Web Resource Page)

Group member names: _____

Experimental Plan

Each group will turn in a plan describing the experiment you will perform during week 4 of the Biofuel fermentation lab. The group should work together to plan the experiment and consult with the lab instructor as necessary. Each member should prepare an individual copy.

Variable:

Conditions of variable to be tested:

	Quantity & type of test substance to be added	Volume of water	Volume of yeast	Final volume	Final conc'n of test substance
1					
2					
3					
4					
5					
6					

Number of replicas:

Control:

Predicted results. Draw the appearance of the summary graph showing the expected relationship between your variable and rate of fermentation. Label axes correctly.



