Lab 6. Alcoholic Fermentation in Yeast—an Independent Project

Prelab Assignment

Before coming to lab, read carefully the introduction and the procedures, below, and then complete the prelab questions at the end of this lab handout. Hand in the prelab assignment just *before* the start of your scheduled lab period.

Goals of this Lab

After completing this lab exercise you should be able to cooperate with group members to carry out the steps of the scientific method while carrying out an independent laboratory investigation:

- Select a problem to study
- Propose one or more testable hypotheses
- Make predictions from the hypotheses
- Design and perform experimental procedures to test the hypotheses
- Analyze/interpret the results
- Present your team's research findings by making an oral presentation to the class.

Introduction

Over the next few weeks you will work collaboratively with your group to adapt the procedure used in Lab 5 to answer a scientific question concerning alcoholic fermentation by yeast. This investigation will allow you to be creative while getting practice applying the steps of the scientific method. This experience is designed to further your understanding and appreciation of the scientific process in a semi-structured situation similar to that used by researchers in their work.

The success of your group depends entirely on the careful and conscientious effort of each person. The division of labor is the responsibility of the team. This dependence on others is also characteristic of research and many other aspects of life (as you may already know!!). Upon completion of the lab work *your group* will present before your peers in class an oral presentation of your findings, an activity done daily by scientists around the world.

As mentioned above, the lab work required for this project will span over several weeks. The primary factors that will influence the amount of time spent in the lab include how well you design your lab experiment(s), your lab skills, and how efficiently and effectively you work as a group. Moreover, thorough preparation by all group members *before* performing experiments in lab will minimize the amount of time required in the lab.

One two hour lab period will be provided for this experiment, the first hour of which should allow you to complete Lab 5, while the second half of the lab period will allow you to get started on Lab 6, your independent investigation***. You are expected to utilize the biology lab outside of class time to complete the bulk of your project.*** The laboratory rooms are open Monday through Friday for activities involved in this lab—check with the Biology lab technician, Kathleen, the for the exact times the biology lab will be open.

Prelab Tasks

1. **Before coming to lab complete the Prelab Questions on the last two pages of this handout.** Use the information below as a guide:
   a. Select a question that you would like to investigate from the list below. If you have an idea for a question that is not listed below check it out with your instructor. Your instructor must approve the question your group selects. Things to consider when selecting a question to investigate:
      - You should understand the underlying concepts behind question selected. For example, if question #7 was selected, then you should have a thorough understanding of how and why changes in pH affect biological reactions. Or if question #8 was selected, you should have some knowledge about how and why ethanol affects cells and their plasma membrane.
      - You will need to consult your textbook, the introduction section of handout, and other resources to obtain supporting/background information about the question you select.
   b. Propose one or more testable hypotheses.
   c. Design an experiment using the materials available to test your hypothesis using the *KISS!* philosophy (Keep it simple stupid!). Consult with the Biology Lab Technician and/or your instructor concerning the availability of materials not listed in the materials section below.
   d. Be sure that all group members understand the techniques and procedures that you will use.
   e. Form a research team with two or three other students. The maximum group size is four.
   f. Assign tasks to be performed by each team member.

Possible Questions to Investigate

Background: At least three quarters of all women will experience a "yeast" infection at some point in their lives. A yeast infection is simply an imbalance of yeast in the vagina caused primarily by an overgrowth of the yeast fungus, *Candida albicans*. *Candida* live in small numbers and is always present in a woman's body (and in a man's too!). It's also the same fungus that causes diaper rash. Usually yeast is kept in check by "helpful" bacteria which are naturally present. Sometimes the normal balance of the vagina is disturbed. When that happens, *Candida albicans* can multiply, resulting in a vaginal yeast infection known as candidiasis. Yeast infections are the second most common cause of vaginal irritation, and can also occur on the male penis, particularly in uncircumcised men. Symptoms include severe itching, burning, and soreness, and irritation of the vagina and/or vulva, and a whitish or whitish-gray discharge that may have a "yeasty" smell like beer or baking bread—the odors of alcoholic fermentation!

Anyone that experiences the symptoms of a yeast infection should consult their medical doctor A.S.A.P. That said, below are listed ***five "home remedies"*** used to treat yeast infections. Select *one of the five* home remedies and design an experiment to determine if it decreases the rate of alcoholic fermentation in baker's yeast. If it does, then what is the minimum concentration required to *stop* alcoholic fermentation? Develop a quantitative relationship that will allow you to predict the rate of fermentation at varying concentrations of the home remedy.

1. **Hydrogen peroxide.** Hydrogen peroxide is produced naturally by *acidophilus* bacteria in the vagina, and kills yeast. One home remedy uses 1 teaspoon of 3% hydrogen peroxide from your market or pharmacy with 1 cup of water.
2. **Garlic.** Garlic is believed to contain natural antifungal substances that kill off yeast.
3. **Boric acid** is a great remedy for a cockroach-ridden apartment, and is believed to kill yeast, too.

4. **Yogurt** contains *Lactobacillus acidophilus*, a bacterium that also happens to be found in a healthy vagina. *Acidophilus* is thought to kill yeast by producing hydrogen peroxide. One home remedy involves the application of yoghurt directly to the vulva and gently applied to the vagina. Another remedy involves the insertion of an ***acidophilus*** **pill** obtained from the local supermarket, pharmacy or health food store.
5. **Potassium sorbate** is a potent fungicide that is widely used in preserving foods, and is often used in brewing beer to stop the growth of the yeast at the correct time. Potassium sorbate is therefore available at places that sell home-brewing supplies, and at some natural foods stores. One home remedy uses a 3% solution by adding 8 g potassium sorbate to a cup of water.

6. **Sodium fluoride** is found in most brands of toothpaste since it helps to decrease the activity of bacteria involved in tooth decay by inhibiting one of the enzymes involved in glycolysis. Does sodium fluoride, NaF, inhibit alcoholic fermentation in yeast? If it does, what is the minimum concentration of sodium fluoride needed to inhibit fermentation? If NaF doesn't affect the rate of fermentation, explain why. Note: Aqueous NaF reacts with glass, thereby decreasing its concentration over time. Hence, *NaF solutions must be stored in plastic containers*.

7. Is the rate of alcoholic fermentation sensitive to **pH**? If so, what is the optimum pH for alcoholic fermentation? Develop a quantitative relationship that will allow you to predict the rate of fermentation at any pH between 1 and 14.

8. Does **ethanol** affect the rate of alcoholic fermentation? How is the rate of alcoholic fermentation affected by increasing concentrations of ethanol? What is the maximum concentration of ethanol tolerated by yeast? Develop a quantitative relationship that will allow you to predict the rate of fermentation at varying concentrations of ethanol.

9. The addition of a solute such as **sodium chloride** to a solution alters the tonicity of a solution. Does sodium chloride affect the rate of alcoholic fermentation? How is the rate of alcoholic fermentation affected by increasing concentrations of sodium chloride? What is the maximum concentration of sodium chloride tolerated by yeast? Develop a quantitative relationship that will allow you to predict the rate of fermentation at varying concentrations of sodium chloride.

10. Most, but not all, of the **ultraviolet light** coming to us from the sun is absorbed by the ozone layer in the upper atmosphere and thereby prevented from reaching the earth. Since UV light causes mutations in cellular DNA that could ultimately result in cancer, and because DNA controls the production of all proteins in a cell, exposure to UV light may result in the production of enzymes and other proteins with reduced activity. Consequently, the world-wide thinning of the ozone layer is a potential threat to much of the life on earth. Does exposure to UV light affect the rate of alcoholic fermentation in yeast? Is there a minimum level of exposure to UV light that results in a depression of fermentation or a halt in fermentation?

11. **Soaps and detergents** kill germs (e.g. bacterial) by lysing the critter's plasma membrane. How does the addition of a soap or detergent (e.g. Sodium Lauryl Sulfate or Dish washing detergent) affect the rate of fermentation? If a soap or detergent decreases the rate of fermentation, then what is the minimum concentration need to stop alcoholic fermentation? Develop a quantitative relationship that will allow you to predict the rate of fermentation at varying concentrations of detergent.

Go to the next page for materials and methods

Materials Available

All materials from Lab 5

Additional Equipment
- Beakers
- Volumetric flasks
- Graduated pipettes
- Disposable gloves for handling the NaF
- Ultraviolet light source
- Safety glasses that filter out UV light

Chemicals
- pH Buffers: 1, 3, 5, 7, 9, 11, 13
- 95% Ethanol
- Sodium Fluoride powder
- Sodium Chloride powder
- Sodium Lauryl Sulfate or Dish Washing Detergent
- Labeled waste container for NaF
- 3% hydrogen peroxide (fresh)
- *Acidophilus* tablets (fresh)
- Fresh garlic cloves
- Boric acid powder
- Potassium sorbate powder
- Fresh *Acidophilus* yoghurt
- Ice

Cautions !! **Follow the cautions below if your experiment involves the use of any of the following!!**

Sodium Fluoride, NaF	– NaF is a poison. Clean up all spills immediately. – Dispose of unused NaF in the "NaF waste container." – Wear protective gloves and goggles. – Use a pipette pump or bulb when piping.
Ultraviolet light	– Ultraviolet light damages skin and eyes. – Do not expose any body parts to UV light. – Where safety goggles that filter out UV light. – Turn off the UV light before placing a sample under or removing it from the UV light.

Procedure

1. **Perform the experiment** designed by your research group. Your instructor will explain the methods of storage for your experimental set-ups.

2. **Record your procedures, data and observations** neatly and clearly in a *ruled* data table on separate paper, preferably in a lab notebook. Be sure to include units of measure an use significant figures.
   - Show clearly the meaning of all data. It is not always obvious whether data will be important later on. Therefore, record everything that is remotely relevant to the experiment since you will probably forget it if you rely on your cerebral neurons to keep track of data!!
   - To help illustrate possible relationships graph your data using graphing software such as *Excel*.

3. **Write a Discussion Section.**
   Explain your results, speculating on trends, possible causes, and conclusions. Try to present the principles, relationships, and generalizations shown by the results. Bear in mind, in a good discussion, you discuss—you do not recapitulate the Results. Your discussion should address each of the following....
   - What is the hypothesis you tested? Does your data support or falsify you your hypothesis? Explain using your experimental data to substantiate your response.
   - Summarize what can be concluded from the results of your investigation.
   - If the results do not support your hypothesis, attempt to explain why this is so.
   - Did you experience any problems or difficulties while performing the experiment? If so, what were they? How do they influence the validity of your results and conclusions?
   - How could your procedure be modified or improved to get better results?
   - What future experiments or research is in order?

4. **Communication of results.**

   - **Oral presentation of your research to the class.** For details, refer to Appendix A, *How to Make an Oral Presentation of a Scientific Experiment* (available at the lab section of the Biol 211 class website). Although you will not be writing a scientific paper, you will find Appendix B, *General Format for Writing a Scientific Paper* (also at the lab section of the class website), helpful when preparing for the oral presentation.


THEORIES
PROOFS


off the mark.com
by Mark Parisi
NOW, IF YOU'LL JUST HAND ME MY MUTATED VIRUSES...
O.J.
offthemark.com
OLIVER JOHNSON LOSES ANOTHER LAB PARTNER


off the mark.com
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...AND IN HERE WE'RE ENGINEERING THE ULTIMATE POLITICIAN. HE'LL BE ABLE TO SHAKE HANDS AND KISS BABIES WITH STUNNING EFFICIENCY...
offthemark.com

Lab 6 Prelab Questions	**Name** _____
Alcoholic Fermentation—Independent Project	**Group Number** _____ **Date** _____
Biol 211	**Date:**

Refer to "Prelab Tasks" on page 2 as you compete the following.

1. **The question you would like to investigate:**
   - Question Number: _____
   - Restate the Question:

2. **Hypothesis or Hypotheses to be Tested:**

3. **Overview of the Procedure:** (Summarize in a paragraph what you will do)

5. **Detailed Procedure:** On the back of this page make a numbered list of each step of your procedure. Include the amounts and concentrations of the substances used, time intervals involved, and appropriate controls.

6. **Names of all Members of You Team**

First and Last Names	Phone number and/or email address
1.	
2.	
3.	
4.	
5.	

Detailed Procedure