

# **Supplemental Materials**

for

# Student-Scientist Curriculum: Integrating Inquiry-Based Research Experiences and Professional Development Activities into an Introductory Biology Laboratory Course

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# Sample Syllabus: BIOL 1202 - Principles of Biology: Molecules & Cells

Student-Scientist Lab Curriculum

### LABORATORY INSTRUCTOR

Name: Email Phone: Office Hours:

### **TEACHING ASSISTANTS**

Name: Email Phone: Office Hours:

### **BIG IDEAS:** *Students will understand:*

- 1. **ENERGY**: All living organisms need a continual supply of energy for growth and reproduction.
- 2. **NATURE OF SCIENCE**: The application of the scientific process enhances the interpretation of the biological concepts.
- 3. **BIOLOGICAL SYSTEMS**: All life on earth shares the basic characteristics of life and is interconnected.

**LEARNING OBJECTIVES**: Upon completion the student-scientist curricula, students will be able to:

- use Microsoft Excel to analyze data and create graphs
- properly operate a micropipette
- properly operate a compound microscope
- interpret data
- document inquiry-based research in a laboratory journal
- prepare research proposals for hypothesis-driven inquiry-based research experiments
- create posters to communicate their scientific findings
- exhibit and collaboratively present student-scientist poster to peers and STEM faculty
- design and conduct inquiry-based experiments using the scientific process

### **SUPPLEMENATARY RESOURCES:** These books are recommended for additional reading.

- How to Write and Illustrate a Scientific Paper by Bjorn Gustavii, 2008, 2<sup>nd</sup> ed.
- Statistics Explained: An Introductory Guide for Life Sciences by Steve McKillup, 2005.

**LAB GRADE EVALUATION:** As indicated in your BIOL \_\_\_\_\_ course syllabus, your lab participation grade is \_\_\_\_% of your overall BIOL \_\_\_\_ grade. Your lab grade will be determined based on the table below:

LABORATORY PARTICIPATION GRADE	
Lab Attendance:%	
Pre-Lab Assignments: <u>%</u>	
Lab Journal:%	%
Mini-Posters:%	
Student-Scientist Project:%	

	STUDENT-SCIENTIST CURRICULUM LABORATORY SCHEDULE				
DATE	LAB SKILLS	TOPIC	LABORATORY EXCERISE		
Jan 12	Lab Safety	Introduction to CURE Lab Lab Safety Rules Assessments & Waivers	Laboratory Safety & Rules Assessments Waivers		
Jan 19	Statistics Data Analysis	Data Analyses Microsoft Excel Interpreting Figures	In Lab Assignments: <i>Short Course Tutorials</i> : HHMI Microsoft Excel Data Analysis – Blackboard Assignment & <i>Worksheet</i> : Intro to Statistics and Data Analysis		
Jan 26	Scientific Method Research Proposal	Scientific Method Research Proposals	In Lab Assignment: Research Proposal Intro to Statistics and Data Analysis Worksheet DUE Short Course Tutorials : HHMI Microsoft Excel Data Analysis DUE (Blackboard)		
Feb 2	Micropipettes Lab Journals	Enzyme Activity Micropipette Lab Journals	<b>Pre-lab Assignment</b> : Micropipette Video and Questions Lactose Intolerance Research Proposal DUE <b>In Lab Assignment</b> : Lactase Activity Assay <i>Class Data for Analysis</i>		
Feb 9	Mini-Posters	Mini-Posters Results and Discussion	<b>Pre-Lab:</b> Complete Lab Journal <b>In Lab Assignment:</b> Revisit data analysis & Create Mini-Poster		
Feb 16	Microscopy Micropipettes Wet Mount Slides	Microscopy Micropipettes Yeast Enzyme Inhibition	<b>Pre-lab Assignment:</b> Microscope Video and Questions <b>In Lab Assignment:</b> Microscopy, Enzyme Inhibition and Cell Viability Assay Lactose Intolerance – Collaborative Mini-Poster DUE Individual Lab Journal DUE Peer-Self Reflection Worksheet <b>Due</b>		
Feb 23	Micropipette pH Strips Statistics Data Analysis Mini-Poster Lab Journals Research Proposal	Enzyme Inhibition Yeast Fermentation	<b>Pre lab Assignment:</b> Alcohol Fermentation and Enzyme Inhibition Research Proposal "Which Beer is Best" <b>In Lab Assignment</b> : Cell viability assay followed by Fermentation Assay - Class Data for Analysis Microscopy and Cell Viability Assignment <b>DUE</b>		
Mar 2	Scientific Method Microscope	Student-Scientist Project (SSP) Research Proposal	<b>In Lab Assignment</b> : Prepare Research Proposal for SSP Alcohol Fermentation and Enzyme Inhibition Group Mini- Posters <b>DUE</b> Alcohol Fermentation and Enzyme Inhibition Lab Journal <b>Due</b> Peer-Self Reflection Worksheet <b>Due</b>		
Mar 9	Research Proposal	SSP Research Proposal Review	<b>Pre-lab Assignment:</b> Draft of Research Proposal for SSP <b>In Lab Assignment:</b> Mock Review Panel of SSP Proposals		
Mar 23	Micropipette	Student-Scientist Projects	Lab Assignment: Conduct Student-Scientist Project		
Mar 30	Microscope	Cell Viability Assay	Revised SSP Research Proposal - DUE		
Apr 6	Lab Journals	Fermentation Assay	The data collected will be used for data analysis		
Apr 13	Final Posters	Create SSP (Final) Poster	Lab Assignment: Create Student-Scientist Posters		
Apr 20	Final Posters	Post-Assessments Revise SSP (Final) Posters	Lab Assignment: Revise Student-Scientist Posters Construct Final Posters – Due Mon April 14 by 12 pm		
Apr 27	Final Poster Presentations	SSP Poster Presentation Competition	<b>Simulate a Poster Competition at a Scientific Conference</b> Student-Scientist Project Lab Journals DUE Peer-Self Reflection Worksheet <b>Due</b>		

Appendix 2: Introduction to statistics and data analysis worksheet and answer key

Name\_\_\_\_\_

Lab Section

# **INTRODUTION to STATISTICS and DATA ANALYSIS**

**Instructions**: Use the raw data below to complete the data tables provided below. Use the background information to explain your results.

**Background Information:** *Lactose intolerance* is a human digestive system disorder that occurs when the cells in the small intestine produce low amounts of the enzyme lactase. Lactase is needed in order to hydrolyze the milk sugar lactose; it breaks lactose sugar down into glucose and galactose. These monosaccharides (glucose and galactose) are absorbed from the intestine into the bloodstream. When a *lactose intolerant* individual eats or drinks dairy products containing a large amount of lactose, there is not enough lactase in their small intestine to hydrolyze the lactose. Therefore, the lactose passes into their colon without being broken down and the bacteria living in the colon begin to ferment the lactose. Unfortunately, when bacteria metabolize the lactose it produces a lot of gas, which can cause cramping, pain, gassiness, and diarrhea. While most human infants produce lactase, not all adults do. Lactose intolerance between different racial groups is highly variable, but unusually rare in Northern European and some African countries. Populations that produce lactase into adulthood tend to be those with a long history of drinking fresh milk. However, the United States is a multicultural population with ancestries from all over the world. Even Though the percentage of lactose intolerance adults in the United States ranges between 11 to 20%, this number reflects an average across people of many different ethnic backgrounds (Jarvis and Miller, 2002 and Sahi 1994).

### **References:**

2.

1. Jarvis, JK. and GD Miller. 2002. Overcoming the Barrier of Lactose Intolerance to Reduce Health Disparities. Journal of the National Medical Association, 94:55-66 of

Sahi, T. 1994. Hypolactasia and Lactase Persistence: Historical Review and Terminology. Scandinavian Journal of
Gastroenterology. 29(202): 1-6.

Patient Number	Race	Glucose Conc. After 7mins (mg/dL)
1	Hispanic-American	500
2	Hispanic-American	500
3	Hispanic-American	1000
4	Hispanic-American	500
5	Hispanic-American	1000
1	African-American	250
2	African-American	250
3	African-American	500
4	African-American	100
5	African-American	1000
1	European-American	1000
2 European-American		1000
3 European-American		1000
4	European-American	500
5	European-American	1000
1	Asian-American	0
2	Asian-American	0
3	Asian-American	250
4	Asian-American	500
5	Asian-American	0

1. Using Excel, calculate the sample size, mean, standard deviation, and standard error for each racial group. Input the data in the table below. (16pts)

Sample	Sample Size (n)	<b>Mean</b> (mg/dL)	Standard Deviation	Standard Error
Hispanic American				
African American				
European American				
Asian American				

 We don't know what results to expect for any of these groups, so we don't have a control group for comparison. Instead, you should compare the different groups against each other using the online ttest calculator (<u>http://www.graphpad.com/quickcalcs/ttest1/?Format=SEM</u>). Input the p-values in data table below. (12 pts)

t-test	Hispanic-	African-	European-	Asian-
p-values	American	American	American	American
Hispanic-				
American				
African-				
American				
European-				
American				
Asian-				
American				

3. Using excel, create a bar graph with the standard error bars. The graph can be attached to this assignment on a separate sheet. The bar graph should include the x and y axes with titles and units, a legend if needed, graph title. (20 pts)

# **Explanation of Data**

- 1. A brief overall summary of your data. Discussion the descriptive statistics provided in table and the graph created using excel. (12 pts)
- 2. What does your p-value tell you about the different ethnic groups? Discuss if these groups are significantly different or not? (10 pts)

Name\_

# **INTRODUTION to STATISTICS and DATA ANALYSIS - Key**

**Instructions:** Use the raw data below to complete the data tables provided below. Use the background information to explain your results.

**Background Information:** *Lactose intolerance* is a human digestive system disorder that occurs when the cells in the small intestine produce low amounts of the enzyme lactase. Lactase is needed in order to hydrolyze the milk sugar lactose; it breaks lactose sugar down into glucose and galactose. These monosaccharides (glucose and galactose) are absorbed from the intestine into the bloodstream. When a *lactose intolerant* individual eats or drinks dairy products containing a large amount of lactose, there is not enough lactase in their small intestine to hydrolyze the lactose. Therefore, the lactose passes into their colon without being broken down and the bacteria living in the colon begin to ferment the lactose. Unfortunately, when bacteria metabolize the lactose it produces a lot of gas, which can cause cramping, pain, gassiness, and diarrhea. While most human infants produce lactase, not all adults do. Lactose intolerance between different racial groups is highly variable, but unusually rare in Northern European and some African countries. Populations that produce lactase into adulthood tend to be those with a long history of drinking fresh milk. However, the United States is a multicultural population with ancestries from all over the world. Even Though the percentage of lactose intolerance adults in the United States ranges between 11 to 20%, this number reflects an average across people of many different ethnic backgrounds (Jarvis and Miller, 2002 and Sahi 1994).

### **References**:

3. Jarvis, JK. and GD Miller. 2002. Overcoming the Barrier of Lactose Intolerance to Reduce Health Disparities. Journal of the National Medical Association. 94:55-66

Patient Number	Race	Glucose Conc. After 7mins (mg/dL)
1	Hispanic-American	500
2	Hispanic-American	500
3	Hispanic-American	1000
4	Hispanic-American	500
5	Hispanic-American	1000
1	African-American	250
2	African-American	250
3	African-American	500
4	African-American	100
5	African-American	1000
1	European-American	1000
2	European-American	1000
3	European-American	1000
4	European-American	500
5	European-American	1000
1	Asian-American	0
2	Asian-American	0
3	Asian-American	250
4	Asian-American	500
5	Asian-American	0

4. Sahi, T. 1994. Hypolactasia and Lactase Persistence: Historical Review and Terminology. Scandinavian Journal of Gastroenterology. 29(202): 1-6.

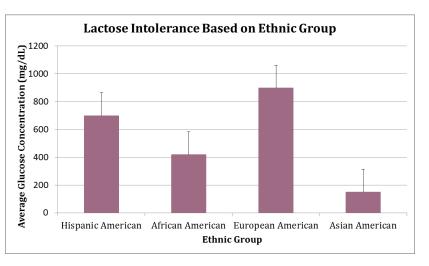
4. Using Excel, calculate the sample size, mean, standard deviation, and standard error for each racial group. Input the data in the table below. (16pts)

Sample	Sample Size (n)	<b>Mean</b> (mg/dL)	Standard Deviation	Standard Error
Hispanic American	5	700	273.86	122.47
African American	5	420	354.61	158.59
European American	5	900	223.61	100
Asian American	5	150	223.61	100

 We don't know what results to expect for any of these groups, so we don't have a control group for comparison. Instead, you should compare the different groups against each other using the online ttest calculator (<u>http://www.graphpad.com/quickcalcs/ttest1/?Format=SEM</u>). Input the p-values in data table below. (12 pts)

t-test	Hispanic-	African-	European-	Asian-
p-values	American	American	American	American
Hispanic-		0.1998	0.2415	0.0083
American		0.1998	0.2415	0.0085
African-			0.0336	0.1878
American			0.0550	0.1078
European-				0.0007
American				0.0007
Asian-				
American				

6. Using excel, create a bar graph with the standard error bars. The graph can be attached to this assignment on a separate sheet. The bar graph should include the x and y axes with titles and units, a legend if needed, graph title. (20 pts)



# **Explanation of Data**

- 3. A brief overall summary of your data. Discussion the descriptive statistics provided in table and the graph created using excel. (12 pts)
- 4. What does your p-value tell you about the different ethnic groups? Discuss if these groups are significantly different or not? (10 pts)

# Lactose Intolerance Lab Preparation Information

- Make 100mL solutions by dissolving lactose powder into water. Autoclave and store in fridge.
   White Milk
- 2. Make a solution of **100% Lactaid** according to instructions on the box (2 tablets in 100 mL of water).
- 3. Use **100% Lactaid** solution to prepare **"single race"** solutions as shown in chart below. *Save the rest in fridge or freezer*.
- 4. Use the **"single race"** solutions to prepare **Multi-racial patient samples** that students will use in lab. See chart below. *Save the rest in fridge or freezer*

"Single-race" Solution	Racial group	<b>C2</b> Final lactaid concentration	<b>V1</b> Volume of "100%" lactaid" solution (mL)	<b>Water</b> (mL)	<b>V2</b> Total Volume Needed (mL)
1	European American	100%	50	0	50mL
2	African American	50%	25	25	50 mL
3	Hispanic American	20%	10	40	50 mL
4	Asian American	10%	5	45	50 mL
5	Native American	5%	2.5	47.5	50 mL

Multiracial Patient Samples	Racial Combinations	Mix together as follows, total of 10mL
Patient A	European-American	5 mL Solution 1
Fatient A	African American	5 mL Solution 2
Patient B	African American	5 mL solution 2
	Hispanic	5 mL solution 3
Patient C	Asian American	5 mL solution 1
Patient C	European American	5 mL solution 4
Dationt D	African American	5 mL solution 2
Patient D	Native American	5 mL solution 5

# Supplies per Lab Bench

- 10 mL Milk per bench
- 1 Waste bucket labeled "WASTE" per bench
- 2 Micropipettes p1000 (pre-set to 500ul)and p200 (pre-set to 100ul) per bench
- Micropipette tips
- I Microfuge rack per table
- 5 mL each of Patient samples A, B, C, D per table (4 of each patient per group bench)
- 5 mL of water labeled "negative control solution" (4 per group table)
- 5 mL of 10% glucose labeled "positive control solution" (4 per group table)
- 2 Containers of Glucose test strips per table
- 2 Timers per table
- 2 Permanent Markers per table

# LACTOSE INTOLERANCE RESEARCH PROPOSAL GRADING RUBRIC

USE YOUR OWN HANDWRITING FOR EVERYTHING (exception = printed charts and graphs)

EXPERIEMENTAL DESIGN ITEMS	Points Possible	Points Earned
<ul> <li>Background Information (4 pts each)</li> <li>Background information should be written in clear and complete sentences.</li> <li>Explain what it means to be lactose intolerance.</li> <li>Explain the relationship between lactase and lactose intolerance.</li> <li>How do enzymes affect lactose intolerance?</li> <li>What is the importance of your experimental variables on lactose intolerance?</li> <li>What is the purpose of the experiment?</li> <li>List of the references you used to complete the background information section.</li> </ul>	28	
<ul> <li>Hypothesis: that matches your experimental design. (3 pts each)</li> <li>"if then "statement that clearly maps out the experiment</li> <li>Explain the scientific reasoning behind your prediction</li> </ul>	6	
<ul> <li>Materials &amp; Methods (6 pts each)</li> <li>Include all a list of materials need to conduct the experiment</li> <li>Using complete sentence in future tense, summarize how you will carry out the experiment</li> </ul>	12	
<ul> <li>Variables: Clearly describe, in complete sentences (2 pts each)</li> <li>Does the experiment design match your hypothesis?</li> <li>Control and Experimental (test) groups</li> <li>Independent variable(s) and Dependent variable(s)</li> <li>Standardized Variable(s)</li> </ul>	12	
<ul> <li>Data Analysis: Explain how you will record your data. (3 pts each)</li> <li>What will you measure?</li> <li>What equipment will you use to measure it?</li> <li>Which statistical methods will you use to analyze your data?</li> <li>Which websites or computer programs did you use to analyze your data?</li> </ul>	12	
<ul> <li>Conclusions (5 pts each)</li> <li>In order to reject your hypothesis, describe the data you would need to see. Explain your reasoning.</li> <li>In order to accept your hypothesis, describe the data you would need to see. Explain your reasoning.</li> </ul>	10	
Total Points	80	

### **Additional Comments:**

# Lab Journal Grading Rubric

USE YOUR OWN HANDWRITING FOR EVERYTHING (exception = printed charts and graphs)

LAB JOURNAL ITEMS	Points Possible	Points Earned
<ul> <li>Appearance (2 pts each)</li> <li>Table of contents is present and accurate</li> <li>Neat look, legible and pleasant to behold</li> <li>Pages are numbered</li> <li>Any charts, graphs, etc. are affixed inside the notebook (not sticking out)</li> <li>Permanent Ink Only</li> </ul>	10	
Date for each entry	4	
Goal: What you plan to accomplish today during lab?	4	
<ul> <li>Notes (2 pts each)</li> <li>Record any notes you took during lecture</li> <li>Record any plans you made for designing experiments</li> <li>State your hypothesis</li> <li>Record data collected during the experiment</li> <li>Describe methods and procedures used during the experiment</li> <li>Make note of any problems or limitations during the experiment</li> <li>Make note of any problems or limitations during the experiment</li> <li>Results, Discussion and Reflection (2 pts each)</li> <li>Was the goal accomplished?</li> <li>Explain your results.</li> <li>Provide data tables with descriptive statistics (<i>mean, std. dev, std. error, n</i>)</li> <li>Provide an overall summary of your study.</li> <li>Do you accept or reject your hypothesis?</li> <li>What did you learn?</li> </ul>	12	
Total Points	44	
<ul> <li>***Bonus Points: (1 pt. each)</li> <li>Pictures</li> <li>Figures</li> <li>Diagrams</li> <li>Signature on each page detailing the experiment</li> </ul> Total Score		

### Appendix 6: Mini-poster evaluations and mock review panels

Names of Group Members (Evaluators) \_\_\_\_\_

Date\_\_\_\_\_

Section\_\_\_\_\_

### MINI-POSTER EVALUATIONS Mock Review Panels of Final Posters from Previous Semesters

Write number assigned to the mini- poster that your team will be evaluating: \_\_\_\_\_\_

Evaluate the Mini-Poster. For each component of the Mini-Poster, circle a number 1-5.

Observations are clearly written and thoroughly explained	1	2	3	4	5
Hypothesis is clearly worded.		2	3	4	5
Hypothesis is detailed; explains the scientific reasoning behind the prediction.	1	2	3	4	5
Procedure is clearly worded and easy to follow.	1	2	3	4	5
Experimental approach is described accurately, including techniques	1	2	3	4	5
Experimental design match the hypothesis	1	2	3	4	5
Control and test groups match the hypothesis	1	2	3	4	5
Independent (IV), Dependent variable (DV), and Constants described accurately	1	2	3	4	5
IV and DV match the hypothesis	1	2	3	4	5
Statistical methods described accurately		2	3	4	5
Provide the websites and statistical software used to conduct the analysis			3	4	5
Tables organized, neat and easy to follow		2	3	4	5
Data tables contain the statistics (mean, n, std. dev, SEM and p-values)		2	3	4	5
Data tables includes units of measurement	1	2	3	4	5
Appropriate graph is used	1	2	3	4	5
Graphs contain a title, labeled X-axis and Y-axis with accurate units	1	2	3	4	5
Provides a detailed description of the results that matches the data	1	2	3	4	5
Provides a brief overall summary of the study that matches the data	1	2	3	4	5
Accurately accepted or rejected the hypothesis or found data to be inclusive	1	2	3	4	5
Future directions provided and explained	1	2	3	4	5

**1**= Strongly Disagree, **2**= Disagree, **3**= Neutral, **4**= Agree, **5**= Strongly Agree

MANDATORY: Write a few sentences of constructive feedback.

Add up the TOTAL POINTS: \_\_\_\_\_

### Appendix 7: Mock poster review panel follow-up self-reflection worksheet

## MOCK POSTER REVIEW PANEL REVIEW FOLLOW UP SELF-REFECTION WORKSHEET

1. Did you find the mock review panel of final posters from previous semester's activity useful? Explain your answer.

2. After completing the mock review panel of final posters from previous semesters, would you say it was **EASY** or **CHALLENGING** for you to work together to agree on a final score for the mini-posters? Explain your answer.

3. After completing the mock review panel of final posters from previous semesters, how did you feel about providing constructive feedback to another student scientist? Explain your answer.

4. After completing the mock review panel of final posters from previous semesters, how confident are you in constructing a mini-poster worthy of receiving an A for your grade? Explain your answer. (Scale of 1 = Not Confident; 2 = Somewhat Confident; 3 = Neutral; 4 = Confident; 5 Very Confident)

POSTER ITEMS AND GRADING CRITERIA	Points Possible	Points Earned
<ul> <li>Observations (2 pts each)</li> <li>Observations should be written in clear and complete sentences.</li> <li>Explain the importance of investigating lactose intolerance.</li> <li>Explain what it means to be lactose intolerance.</li> <li>What is the research objective for the experiment?</li> <li>Provide in-text citations and References in APA format</li> </ul>	10	
<ul> <li>Hypothesis: that matches your experimental design. (2 pts each)</li> <li><i>"if then</i> "statement that clearly maps out the experiment</li> <li>Explain the scientific reasoning behind your prediction</li> </ul>	4	
<ul> <li>Materials &amp; Methods (6 pts each)</li> <li>Using complete sentence in past tense, summarize how you conducted the experiment</li> <li>Include all of the materials needed to conduct the experiment</li> </ul>	12	
<ul> <li>Experimental Design: Clearly describe, in complete sentences (2 pts each)</li> <li>Does the experiment design match your hypothesis?</li> <li>Control and Experimental (test) groups</li> <li>Independent variable(s) and Dependent variable(s)</li> <li>Standardized Variable(s)</li> </ul>	12	
<ul> <li>Data Analysis (3 pts each)</li> <li>What did you measure?</li> <li>What equipment did you use to measure it?</li> <li>Which statistical methods did you use to analyze your data?</li> <li>Which websites or computer programs did you use to analyze your data?</li> </ul>	12	
<ul> <li>Data Tables</li> <li>Complete, organized and neat tables (2pts each)</li> <li>Appropriate labels and units (2 pts each)</li> <li>Descriptive Statistics Data Tables (10 pts each) - Sample size, mean (average), Standard Deviation, and p-values</li> </ul>	30	
<ul> <li>Graphs</li> <li>Title and legend (1 pt. each)</li> <li>Labeled X-axis with units and Y axis with units (1 pt. each)</li> <li>Appropriate graph used of class data (with standard error bars) (5 pts each)</li> </ul>	10	
<ul> <li>Results</li> <li>Provides a detailed description of the results. (10 pts)</li> <li>Which group(s) were significantly different at a p-value &lt;0.05? (3 pts)</li> <li>Which group(s) showed no significant differences? (3 pts)</li> <li>Did you experience any problems during the experiment? If so, how did you troubleshoot them? (4 pts)</li> </ul>	20	
<ul> <li>Conclusions</li> <li>Provide a brief overall summary of the study. 10 pts</li> <li>Should you accept or reject the hypothesis? Or is your data inconclusive? 5 pts</li> <li>Explain your reasoning. 5 pts</li> </ul>	20	
<ul> <li>Future Directions</li> <li>To extend your knowledge, what experiment would you like to do next? Briefly explain what else you'd like to discover, based on your results.</li> </ul>	5	
Total Points	135	

# Preparation Sheet for Enzyme Inhibition and Yeast Microscopy Lab Activity

### YEAST:

- 1. Pick a large 3mm colony (clone 9 or clone 10) into 5 mL SD-Ura or SD-Leu or SD-Trp.
- 2. Shake  $\sim$  20hr at 30 degrees.
- 3. Measure OD600, it should be at least 1.0.
- 4. Make aliquots, 90 microliters per tube, 2 tubes per bench.

### **SOLUTIONS**

- 1. HCl 0.01M make 5-10mL
- 2. Aliquot into tubes 50 microliters each, 2 tubes per bench.

### Each bench needs:

- 2 markers
- 2 Waste buckets
- 2 p20 micropipettors
- Pipet tips
- 2 Microfuge tube rack
- 2 tubes of yeast, 90 microliters each
- 2 tubes of 0.1M HCl, 50 microliters each
- 2 tube of deionized water, 500 microliters each
- Slides and coverslips
- 2 tube of methylene blue
- glass waste beaker

# Altering Enzyme Activity on Yeast Cells, Cell Viability & Microscopy Lab Worksheet

### **OBJECTIVES**

- Identify the parts of a light compound microscope
- Properly operate a light compound microscope
- Create a wet mount
- Explain altering enzyme activity on yeast cells
- Perform a viability assay to determine the number of *living* cells in a sample.
- Define "enzyme inhibition" and explain how to inhibit enzyme activity using hydrochloric acid (HCl)

### **BACKGROUND**

**YEASTS** are unicellular Fungi, the simplest eukaryotic cells. They are easy to grow in a laboratory, since their nutritional requirements and environmental needs are few.

- They prefer to grow at 20-30°C, but can tolerate a range of growth temperatures between  $\sim$ 4  $\sim$ 45°C. Temperatures that are too high or too low may interfere with enzyme function.
- Yeast need a source of energy and can metabolize various carbohydrates including monosaccharides, disaccharides and some polysaccharides for this.
- Yeast need peptides or amino acids synthesize their own proteins. They need a supply of nitrogen and phosphorus to synthesize their nucleic acids.
- Yeasts require an appropriate concentration of salts and various other ions, to maintain an osmotically and chemically favorable environment.
- Having the proper pH is a major aspect of maintaining a chemically favorable environment. Inside cells, enzymes can become unfolded or denatured at the wrong pH, which reduces or eliminates their activity.

**YEAST VIABILITY** – Viability refers to the ability of yeast cells to stay alive. Yeast are easy to grow but, also easy to injure or kill by removing what they need. For instance, yeast cannot live if their enzymes cannot function. In this activity we will alter the pH of yeast liquid cultures, which inhibits enzyme activity. Then we will determine if the viability of the yeast was impacted by the change in pH.

**MEASURING YEAST VIABILITY** – The simplest way to measure yeast cell viability is using a light microscope and a simple stain called *methylene blue*, which stains DEAD yeast cells. When methylene blue is able to enter yeast cells it will make them appear blue. Yeast cells that are alive will appear clear or opaque because they are able to break down the methylene blue and so it disappears. Cells that are dead are unable to break methylene blue (or anything else) down – they have no metabolic activity. To measure yeast cell viability methylene blue is added to cells, then cells are observed using a light microscope and counted. Figure 1 shows what yeast cells would look like during a typical methylene blue viability assay, a viewed under conditions similar to what you will use. To calculate percent viability, simply divide the number of live (unstained) cells by the total number of cells seen and multiply by 100.

### Percent Viability = (number of live cells/total number of cells) X 100

### BASIC CHEMISTRY REVIEW! (http://www.chem.purdue.edu/gchelp/howtosolveit/Equilibrium/Calculating\_pHandpOH.htm)

**Calculating pH**. To calculate the pH of an aqueous solution you need to know the concentration of the hydronium ion in moles per liter (**molarity**). The pH is then calculated using the expression:

 $pH = -\log [H_3O^+].$ 

**Example:** Find the pH of a 0.0025 M HCl solution. The HCl is a strong acid and is 100% ionized in water. The hydronium ion concentration is 0.0025 M. Thus: pH =  $-\log(0.0025) = -(-2.60) = 2.60$ 

### PROCEDURES (Work in groups of 2.)

## Treat yeast with HCl to inhibit enzyme activity

- 1. Locate 2 microfuge tubes containing yeast.
- 2. Using the p20 micropipette, add 10 microliters of HCl to one tube and 10 microliters of water to the other tube.
- 3. Vortex tubes  $\sim 10$  sec to resuspend the yeast (disperse the cells evenly in the liquid).
- 4. Let tubes incubate on your bench for at least 20 minutes.

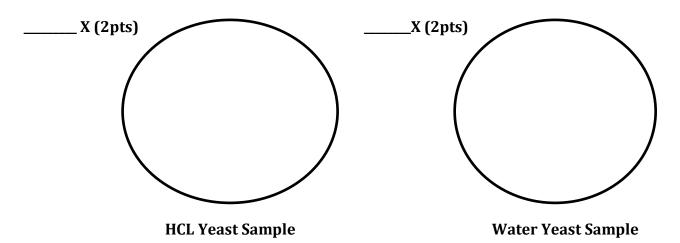
## Preparing and viewing wet mount (live) yeast slides

- 1. Label one empty glass slide for each tube of yeast.
- 2. Vortex the yeast tube.
- 3. Add 5 microliters of yeast to the slide then add 5 microliters of methylene blue on the slide. AVOID BUBBLES!
- 4. Following the procedure demonstrated in blackboard, carefully place a coverslip at a 45 degree angle at the edge of the liquid on the slide. Slowly and carefully, let the coverslip gently fall over the liquid to cover it.
  \*\*ANGLE and SLOW is important to reduce the chances of bubbles.
- 5. Examine slide in a microscope, moving from total magnification of  $40X \rightarrow 100X \rightarrow 400X$ .
- 6. Count a total of 50 cells per slide for the viability assay.
- 7. Enter data in Table 1.

# MICROSCOPE IMAGES OF YEAST SUSPENDED IN HYDROCHORLIC ACID AND WATER

Use the microscope to draw the yeast cells suspended in water and yeast cells suspended in HCl using the 40X objective lens (label each drawing with its TOTAL magnification).

Indicating which yeast cells are dead or alive by labelling them on your drawings (4pts).



**RAW DATA TABLE** 

Table 1:	Yeast Survival in Water and HCL after a 20 Minute Incubation Period (2	16 pts	)
----------	--	--------	---

Yeast Sample	# Live Cells	# Dead Cells	Total	% Live Cells
Water				
HCL				

## **POST-LAB QUESTIONS**

5. Explain your results. Compare and contrast the survival of yeast resuspended in HCL and water. Specifically, discuss the effect of HCl on yeast survival and enzyme activity? (**30 pts**)

6. Did you experience any problems while conducting this experiment? If so, how did you troubleshoot them? (5 pts)

# ALCOHOL FERMENTATION AND ENZYME INHIBITION RESEARCH PROPOSAL

USE YOUR OWN HANDWRITING FOR EVERYTHING (exception = printed charts and graphs)

EXPERIEMENTAL DESIGN ITEMS	Points Possible	Points Earned
<ul> <li>Background Information (4pts each)</li> <li>Background information should be written in clear and complete sentences.</li> <li>In your own words, summarize the alcoholic fermentation process.</li> <li>Describe the environmental conditions that will cause yeast to carry out alcohol fermentation.</li> <li>Discuss how an enzyme inhibitor that alters pH could stop enzymes from working.</li> <li>In your own words, explain the incident at the brewery (THE CASE)</li> <li>What is the purpose of the experiment?</li> <li>List of the references you used to complete the background information section.</li> </ul>	28	
<ul> <li>Hypothesis: that matches your experimental design. (5 pts each)</li> <li><i>"if then</i> "statement that clearly maps out the experiment</li> <li>Explain the scientific reasoning behind your prediction</li> </ul>	10	
<ul> <li>Materials &amp; Methods (6 pts each)</li> <li>Include all a list of materials need to conduct the experiment</li> <li>Using complete sentences in future tense, summarize how you will carry out the experiment</li> </ul>	12	
<ul> <li>Variables: Clearly describe, in complete sentences (2 pts each)</li> <li>Does the experiment design match your hypothesis?</li> <li>Control and Experimental (test) groups</li> <li>Independent variable(s) and Dependent variable(s)</li> <li>Standardized Variable(s)</li> </ul>	12	
<ul> <li>Data Analysis: Explain how you will record your data. (4 pts each)</li> <li>What will you measure during this experiment?</li> <li>What equipment will you use to measure it?</li> <li>Explain why you will use the equipment.</li> <li>Explain how you plan to use it.</li> <li>Which statistical methods will you use to analyze your data?</li> <li>Which websites or computer programs did you use to analyze your data?</li> <li>Explain why you will use them to analyze your data.</li> </ul>	28	
<ul> <li>Conclusions (5 pts each)</li> <li>In order to reject your hypothesis, describe the data you would need to see. Explain your reasoning.</li> <li>In order to accept your hypothesis, describe the data you would need to see. Explain your reasoning.</li> </ul>	10	
Total Points	100	

ALCOHOL FERMENTATION AND ENZYME INHIBITION MINI-POST	ER GRADI	NG
POSTER ITEMS AND GRADING CRITERIA	Points Possible	Points Earned
<ul> <li>Observations (2 pts each)</li> <li>Observations should be written in clear and complete sentences.</li> <li>What is fermentation?</li> <li>Explain the incident at the microbrewery. (IN YOUR OWN WORDS!)</li> <li>What is the purpose of the experiment?</li> <li>Provide in-text citations and references in APA format</li> </ul>	10	
<ul> <li>Hypothesis: that matches your experimental design. (2 pts each)</li> <li><i>"if then</i> "statement that clearly maps out the experiment</li> <li>Explain the scientific reasoning behind your prediction</li> </ul>	4	
<ul> <li>Materials &amp; Methods (6 pts each)</li> <li>Using complete sentence in future tense, summarize how you will carry out the experiment</li> </ul>	6	
<ul> <li>Experimental Design: Clearly describe, in complete sentences (2 pts each)</li> <li>Does the experiment design match your hypothesis?</li> <li>Control and Experimental (test) Groups</li> <li>Independent Variable(s) and Dependent Variable(s)</li> <li>Standardized Variable(s)</li> </ul>	12	
<ul> <li>Data Analysis (3 pts each)</li> <li>Which statistical methods did you use to analyze your data?</li> <li>Which websites or computer programs did you use to analyze your data?</li> </ul>	6	
<ul> <li>Data Tables (one for fermentation pH and cell viability)</li> <li>Complete, organized and neat tables (1pt each)</li> <li>Appropriate labels and units (1 pt. each)</li> <li>Descriptive Statistics Data Tables (10 pts each) - Sample size, Mean (average), Standard Deviation, Standard Error of the Mean and p-values</li> </ul>	45	
<ul> <li>Graphs (one for fermentation pH, and cell viability)</li> <li>Title and legend (1 pt. each)</li> <li>Labeled X-axis with units and Y axis with units (1 pt. each)</li> <li>Appropriate graph used of class data (with standard error bars) (5 pts each)</li> </ul>	27	
<ul> <li>Provides a detailed description of the results. (10 pts)</li> <li>Which group(s) were significantly different at a p-value &lt;0.05? (3 pts)</li> <li>Which group(s) showed no significant differences? (3 pts)</li> <li>Did you experience any problems during the experiment? If so, how did you troubleshoot them? (4 pts)</li> </ul>	20	
<ul> <li>Conclusions <ul> <li>Provide a brief overall summary of the study. (10 pts)</li> <li>Should you accept or reject the hypothesis? Or is your data inconclusive? (5 pts)</li> <li>Was an inhibitor added to the suspected medium? If so, what evidence do you have to support your findings? (5pts)</li> <li>Do the results support the claims of the brewery concerning the alleged contamination of the medium? (5 pts)</li> <li>Should the employee be compensated for loss of work and rehired? Why? (8 pts)</li> </ul> </li> </ul>	33	
Total Points	153	
<ul> <li>***Bonus Points: (1 pt. each)</li> <li>Pictures</li> <li>Diagrams</li> </ul>		

Appendix 13: SSP research proposal preparation follow-up self-reflection worksheet

Name \_\_\_\_\_

Section\_\_\_\_\_

# STUDENT SCIENTIST RESEARCH PROPOSAL PREPARATION FOLLOW UP SELF-REFLECTION WORKSHEET

- How beneficial did you find preparing your research proposal before starting your student scientist project (SSP)? Explain your answer. (Scale of 1 = Not Beneficial; 2 = Somewhat Beneficial; 3 = Neutral 4 = Beneficial; 5 Very Beneficial)
- 2. After preparing a research proposal, would you say it was **EASY** or **CHALLENGING** to work together to design a detailed experiment? Explain your answer.
- 3. How useful was the constructive feedback provided the committee that reviewed your proposal? Explain your answer. (*Scale of* **1** = *Not Useful*; **2** = *Somewhat Useful*; **3** = *Neutral*; **4** = *Useful*; **5** *Very Useful*)

4. After preparing a research proposal, how confident are you in carrying out your experiment? Explain your answer. (Scale of 1 = Not Confident; 2 = Somewhat Confident; 3 = Neutral; 4 = Confident; 5 Very Confident)

5. Did you feel like a student scientist while completing your research proposal? Explain your answer.

6. Discuss additional knowledge you gained from preparing the SSP research proposal.

RESEARCH PROPOSAL GRADING RUBRIC CRITERIA TYPED ONLY	Points Possible	Points Earned
<ul> <li>Background Information (4pts each)</li> <li>Background information should be written in clear and complete sentences.</li> <li>What is yeast fermentation?</li> <li>Explain the effects of your independent variable on yeast fermentation.</li> <li>Explain how the independent variable in your study potentially impact the enzymatic activity during yeast fermentation?</li> <li>What is the importance of your experimental variables on yeast fermentation?</li> <li>What is the purpose of the experiment?</li> <li>Provide in-text citations and references in APA format</li> </ul>	28	
<ul> <li>Hypothesis: that matches your experimental design. (5 pts each)</li> <li><i>"if then</i> "statement that clearly maps out the experiment</li> <li>Explain the scientific reasoning behind your prediction</li> </ul>	10	
<ul> <li>Materials &amp; Methods (6 pts each)</li> <li>Include a list of all the materials need to conduct the experiment</li> <li>Using complete sentence in future tense, summarize how you will carry out the experiment</li> </ul>	12	
<ul> <li>Variables: Clearly describe, in complete sentences (2 pts each)</li> <li>Does the experiment design match your hypothesis?</li> <li>Control and Experimental (test) groups</li> <li>Independent variable(s) and Dependent variable(s)</li> <li>Standardized Variable(s)</li> </ul>	12	
<ul> <li>Data Analysis: Explain how you will record your data. (4 pts each)</li> <li>What will you measure during this experiment?</li> <li>What equipment will you use to measure it?</li> <li>Explain why you will use the equipment.</li> <li>Explain how you plan to use it.</li> <li>Which statistical methods will you use to analyze your data?</li> <li>Which websites or computer programs did you use to analyze your data?</li> <li>Explain why you will use them to analyze your data.</li> </ul>	28	
<ul> <li>Conclusions (5 pts each)</li> <li>In order to reject your hypothesis, describe the data you would need to see. Explain your reasoning.</li> <li>In order to accept your hypothesis, describe the data you would need to see. Explain your reasoning.</li> </ul>	10	
Rough Draft Attached	10	
Reviewer Comments - Rough Draft	10	
Total Points	120	

ADDITIONAL COMMENTS WILL BE PROVIDED ON THE BACK SIDE OF THIS DOCUMENT

# **COMMENTS AND SUGGESTIONS FOR SCIENTISTS**

The reviewers need to provide a detailed explanation for their scores.

Does the title accurately describe the research study?

Introduction:

Hypothesis:

Methods and Materials:

Variables:

Data Analysis:

Conclusions:

Should this research proposal receive funding? Explain your answer.

# **STUDENT-SCIENTIST PROJECT – POSTER GRADING RUBRIC**

\*YF = Yeast Fermentation and CV= Cell Viability

\*\*IV = Independent Variable, DV = Dependent Variable, EG = Experimental Groups, C = Constants, and CG = Control Groups

### STUDENT NAME\_

### Judge's Name\_

**Task Description**: A Poster Presentation environment similar to a Scientific Meeting is simulated for the student will act as scientist and present purpose, methods (including data analysis), results and conclusion (implications of the experiment) of your Student-Scientist project to the NCCU scientific community.

Criterion	Exemplary =4	Competent = 3	Developing = 2	No Attempt = 1	Score
	🗆 Confident	□ Timid (Nervous)	□ Hesitate (Second guessing themselves)	🗆 Unprepared	
	Knowledgeable about project	$\square$ Comfortable with the topic	$\Box$ Uncomfortable with the topic,	Do not know the	
DDECENTED	topic and accurate	and accurate	but the content is	concepts associated	
<b>PRESENTER</b> TOTAL POINTS = 12	$\square$ Answered questions with the	$\Box$ Comfortable with	questionable	with the project	
101 AL POINTS = 12	correct explanation and	answering questions but	Uncomfortable with	Does not answer	
	elaboration	little elaboration	answering questions and does	questions	
			not elaborate		
	□ Title specific and informative	Title specific, but not	$\square$ Title not specific nor	🗆 No Title	
	Each author name is provided	informative	informative	$\Box$ Two or more author	
	$\Box$ Text font size is appropriate	$\Box$ One author name is	$\Box$ Two author names are	names are omitted	
	and consist for each section	omitted	omitted	Text font size is not	
	$\square$ Presentation is organized and	Text font size is	$\Box$ Text font size is not	inappropriate	
DDECENTATION	easy to read	appropriate but not	appropriate but inconsistent	Presentation is	
PRESENTATION OF THE	$\square$ Written in clear English with	consist for each section	for each section	unorganized and	
RESEARCH	no grammatical errors	Presentation is organized	$\Box$ Presentation not easy to	difficult to follow	
TOTAL POINTS = 24	Professional Attire	and somewhat easy to	follow	Not written in clear	
101711 01013 - 24		read	$\Box$ Written in clear English with	English and not	
		$\Box$ Written in clear English	several grammatical errors	grammatical correct	
		with limited grammatical	Semi-Business Casual Attire	🗆 Casual Attire	
		errors			
		Business Casual Attire			
	Project accurately introduced	$\Box$ Project sufficiently	Project inadequately	Project poorly	
	with purpose of experiment	introduced with purpose	introduced with purpose of	introduced and does	
	□ Methods summary completely	of experiment	experiment	not contain a purpose	
	explained and accurate	Methods summary missing	Methods summary missing	of experiment	
PROJECT	$\square$ Hypothesis provided and	key components but	key components and	Methods incomplete	
DESIGN	justified	accurate	inaccurate	and inaccurate	
TOTAL POINTS = 16	Each variable clearly defined	$\square$ Hypothesis provided but	$\square$ Hypothesis with no	🗆 No Hypothesis	
	(IV, DV, EG, C and CG)	poorly justified	justification	$\square$ No defined variables	
		$\square$ One variable group not	Two or more variable not		
		defined	defined		

Criterion	Exemplary =4	Competent = 3	Developing = 2	No Attempt = 1	Score
	$^{\square}$ Results clear, consistent and	Results vague but	Results confusing but	$\Box$ Results are	
	accurate	accurate	accurate	contradictory and	
	□ Includes all statistics (mean, std.	Missing some statistical	$\Box$ Only statistics for either yeast	inaccurate	
RESULTS	dev. and p-values) for YF and CV	results but both YF and CV	fermentation or cell viability	$\square$ Statistics missing for	
TOTAL POINTS = 12	Significant differences	Significant differences	are discussed	both YF and CV	
	between the groups are	between the group are	Significant differences	□ Significant differences	
	clearly indicated (both YF and CV)	vague for YF and CV	between the groups indicated	are not explained or	
		5	for either YF or CV	poorly explained	
	□ Overall study is clearly	□ Overall study is vaguely	Overall study inadequately	Overall study summary	
	accurately summarized	summarized	summarized	not provided	
	□ Major implication of research	□ Major implications of	□ Major implications of	□ Major implications not	
CONCLUSIONS	stated	research are vague	research incomplete	stated	
AND FUTURE	Hypothesis accepted or	□ Hypothesis accepted or	□ Hypothesis inadequately	□ Hypothesis accepted or	
DIRECTION	rejected clearly stated	rejected is vaguely stated	accepted or rejected	rejected not stated	
TOTAL POINTS = $20$	□ Clearly describe limitations	□ Vaguely describe	□ Inadequately describe	□ No limitations	
	□ Suggest a clear and accurate	limitations	limitations	□ No future research	
	future research question	$\Box$ Suggest a vague question	□ Random, unrelated future	question	
	future research question	for future research	research question	question	
	Neat and easy to read	□ Neat, but unclear	Neat and Confusing	□ Not neat and confusing	
	□ Accurate descriptive titles for	□ Accurate descriptive titles	□ Inaccurate descriptive titles	□ No descriptive titles for	
	both figures	for one figures	for one or both figures	figures	
	□ X and Y axis labeled clearly	$\square$ X and Y axis labeled	□ X and Y axis not labeled	$\square$ X and Y axis without	
FIGURES	with the proper units.	clearly without the proper	clearly but contains the	labeled axis and units	
TOTAL POINTS = 16	□ Mean & error bars accurately	units.	proper units	Graphs do not	
	displayed for both YF and CV	□ Mean & error bars	Mean accurately displayed but	accurately represent	
	displayed for both fr and CV	accurately displayed only	error bars missing	the data	
		on one figure	error bars missing	the tata	
		0			
	□ Neat and easy to read	Neat, but unclear	□ Neat and Confusing	□ Not neat and confusing	
	n-values provided and correct	n-values provided but	n-values provided by not	☐ Missing n-values	
	□ Mean, Stdev & SEM accurately	partially correct	correct	□ Mean, Std. dev & SEM	
TABLES	displayed with units (both YF & CV)	Mean, Std. dev. & SEM	□ Mean, Std. dev & SEM	not accurately	
TOTAL POINTS = 16	$\Box$ p-value table with descriptive	accurately displayed but	accurately displayed with	displayed on one or	
	title (both YF and CV)	no units (both YF & CV)	units only on one table	both YF & CV tables	
		$\Box$ p-value table without	$\square$ p-value table for either YF or	Missing or incomplete	
		descriptive title (both YF & CV)	CV	p-values	
EXTRA CREDIT	$\square$ Microscope slide pictures	□ Microscope slides with	$\square$ Microscope slides without	Microscope slide	
TOTAL POINTS = $4$	with total magnification and	total magnification but a	total magnification but a	without a title or total	
	descriptive title	poorly written title	poorly written title	magnification	
Additional Comm	ients:			TOTAL POINTS	S
					/11



# Soda Sugar Effects on Fermentation Rates

Department of Biological & Biomedical Sciences, North Carolina Central University, Durham, NC



#### INTRODUCTION

Yeast are single-celled eukaryotic organisms that reproduce asexually by budding in aerobic or anaerobic environments. Yeast need a carbon source for energy such as glucose. Glycolysis is used to breakdown the molecule of glucose into the two pyruvate molecules with the net of the two ATP produced during this cycle.

molecules with the net of the two ATP produced during this cycle. Under anaerobic conditions yeast carries out the metabolic process that converts sugar into acids, gases, or alcohol. Saccharomyces cerevisiae, commonly known as Baker's yeast is used to carry outfermentation to produce wine, beer, bread, etc. In this study the sugar content effect on yeast was

In this study the sugar content effect on yeast was investigated. The sodas ginger ale, coke and sprite were used to supply the sugar source for the yeast to carry out fermentation process called yeast fermentation. Yeast fermentation was used to determine which soda would produce a higher carbon dioxide volume based off its their sugar content.

#### **OBJECTIVES**

The objective of this experiment is to determine the effect of The sugar content in ginger ale, coke, sprite on yeast fermentation (CO<sub>2</sub> volume produced during 50 minutes monitoring period) and cell viability(percentage of live cells after 30 minutes).

#### **Hypothesis**

Under anaerobic conditions, if saccharomyces cerevisiae is Under after Udit. Controlling, in section onlyces cereaters is added to the different sodas (ginger ale, coke and sprite), then the soda with the highest sugar content would produce a faster fermentation rate and a higher cell viability. Sprite contains the highest sugar content than the other sodas therefore, it will produce a faster fermentation rate and a higher cell viability.

#### EXPERIMENTAL DESIGN

Independent variable: The sugar substrates in the different types of soda solutions. Dependent variables: CO<sub>2</sub> concentration when the samples are

Dependent Variables: Cory Concentration when the samples are formenting after being put in the incubator set at 30°C and cell viability when using a microscope. Standardized Variables: The amount of time spent while the former being and the spectrum in the bunchespectrum back and the 2005

fermentation apparatuses were in the incubator set at the 30°C, the type of yeast used and the amount of solution for the experimental variables and controls

Control groups: the positive control is glucose and the negative control is water. Experimental groups: Ginger ale Soda, Coke Soda, and Sprite Soda

#### METHODOLOGY

3mL of 20% yeast solution was added to individual 15mL tubes containing 10mL of the control solutions (water and glucose) and the sodas (ginger ale, coke, and sprite). The 15mL tubes containing the yeast mixtures were then inverted into a individual 50 mL tube to assemble fermentation apparatus and to simulate an anaerobic environment. The fermentation apparatuses were then placed in an incubator set at 30°C so they can maintain a constant temperature.

This incubation period was 50 minutes. Every 10 minutes the fermentation apparatuses were taken out to measure its CO<sub>2</sub> volume In incubation period was 50 minutes, tvery 10 minutes the fermentation apparatuses were taken out to measure its Cu<sub>2</sub> volume. After 30 minutes, 10 microliters was taken out of each yeast mixture from each apparatus and placed into the microcentrifuge tube containing the corresponding dilution solution. 5 microliters was taken out of each microcentrifuge tube and placed on an individual microscope slide and stained with 5 microliters of methylene blue. Each slide was placed under a compound microscope to determine the survival percentage of the yeast cells. Statistical **Methods**: standard deviation, mean, variance, and other functions in Microsoft Excel were used to present and interpret the data. Graphpad was used to conduct a t-test of the p-values for comparing the to the sodas to each other and to glucose.

#### **RESULTS AND DISCUSSION**

The yeast fermentation rates and cell viability of the different types of sodas were compared based on there sugar content. On average coke produced the highest CO, volume(1.38m  $\pm$  0.46m). Therefore sprite soda produced the lowest CO, level(0.83m  $\pm$  0.13m). When analyzing the cell viability of the soda samples by using a microscope and we were able to see that Ginger Ale on an average was higher for the amount of live cells being (13.50%  $\pm$  12.02%) and Sprite soda was the lowest being (5.50%  $\pm$  3.54%). When calculating the p values of sodas, it was determined they were not significantly different to each other because they value showed value higher than 0.05.

Yeast fermentation of data table yeast mixtures for glucose and sodas. (Table 1)

Yeast Mixtures	Mean & Standard Deviation(mL)	SEM
Ginger Ale	1.10mL ± 0.28mL	0.20
Coke	1.38mL ± 0.46mL	0.33
Sprite	0.83mL ± 0.18mL	0.13
Glucose	1.90mL ± 0.99mL	0.70

Yeast fermentation t-test and sodas(mL). (Table 2) results of the yeast mixtures for glu

	Ginger Ale	Coke	Sprite	Glucose	
Ginger Ale		0.5460	0.3639	0.3864	
Coke			0.2550	0.5665	
Sprite				0.2697	
Glucose					
	ability t-test ( sodas(%). (Ti		ie yeast mix	tures for	
	Ginger Ale	Cok	e Spr	ite Glu	cos
Ginger		0.67	03 0.46	519 0.2	98

0.3445

0.3040

9922 N

g

Col

Sprit

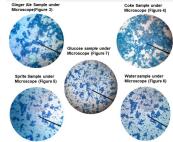
Gluce

Yeast cell viability data table of the yeast mixtures for glucose and sodas(%). (Table 3)





#### CONCLUSIONS



Our study shows that yeast fermentation rates and cell viability Constantly flows for the peak remains the second state of the second states varied at differently in ginger ale, coke and sprite. In conclusion our hypothesis was rejected because coke actual produced a higher fermentation rate and cell viability even though coke did not contain as much sugar as sprite. These ise coke actually findings are important because people who enjoy making homemade sodas can find it very useful for natural carbonation. A homemade sodas can tind it very useful for natural carbonation. A limitation for this experiment would be not using a high amount of samples. If more samples were used results and data would be more accurate to analyze. Future Studies: Research shows that by making homemade sodas can be temperature dependent on the yeast fermentation. The same can said with the production of wines.

#### **REFERENCES & ACKNOWLEDGEMENTS**

https://www.fatsecret.com/calories-nutrition/sprite/sprite-(12-oz)

http://www.thekitchn.com/summer-recipe-fresh-peach-sodarecipes-from-the-kitchn-192849

We would like to acknowledge the funding from HHMI Precollege and Undergraduate Science Education Program (Grant # 52007553.



# The Best Substrate For Fermentation

Department of Biological & Biomedical Sciences, North Carolina Central University, Durham, NC

#### INTRODUCTION

 Yeast fermentation is an anaerobic process where glycolysis is yielded, allowing a yeast solution to produce ethanol and carbon dioxide.

•The three natural sweeteners used were agave, honey, and

The uncertainty activity of the second secon

Include glucose and rutoses, sucrose. - Agave stems from the cattus agave tequilana in Mexico. The carbohydrate composition is 70% frutose. - The three natural sweeteners can be used in the manufacturing of beer and julices as a sweetener substitution. - The Gi (glycomic index) is jouver in substrates that avoid crystallization and are in a loose or raw form. All three cubettark share a law Gi. Gi. Lark drawning how those I wurk.

substrates have a low GI. GI rates determine how blood levels

elevate due ton glucose in the blood stream levels

#### **OBJECTIVES**

 The purpose of the experiment is to determine which natural sweetener will have the greatest effect on yeast fermentation in saccharomyces cerevisiae; in addition to calculating cell viability of substrates after a 30 min interval has passed

#### HYPOTHESIS

·If the substrate honey has a higher carbohydrate composition of glucose then more carbon CO<sub>2</sub> bubbles will be produced during fermentation.

#### EXPERIMENTAL DESIGN

There was both a positive and negative control used during the experiment. The positive control is glucose and this is because there needs to be a presence of sugar in the experiment. The negative control is H\_O because it breaks down glucose. The independent variables of this experiment are the substrates maple syrup, honey, and agave. Our dependent variables are the cell viability,  $CO_2$  production, and fermentation. The experimental group includes substrates will be tested in order to see which will affect the yeast fermentation the tested in order to see which will affect the yeast fermentation the tested in order to see which will affect the yeast fermentation the tested in order to see which will be are time allowed during the tested in order to see which will be set of more the oldered during the tested in order to see which will affect the yeast fermentation to see the set of the process. The standardized variables are time allotted during the incubation process. Methylene blue and the amount of the sweeteners used to conduct the experiment are also standardized variables.

### METHODOLOGY

First, use a micropipette to transfer 3mL of yeast culture into a 15mL conical tube. In that same tube there will be 10mL of each substrate mixture. Pour yeast/experimental mixture into a 15mL tube with holes in the cap. Place each tube upside down into a fermentation apparatus. Mark along the sides to indicate the volume. Place the tubes into a tube rack and place the rack into an incubator set to  $30^{\circ}$ C. You will check the tubes every 10 minutes for a total of 50 minutes and collect the volume of how much CO<sub>2</sub> is produced. After 30 minutes, create a wet mount for each substrate mixture. You will add 5mL of the dilution solution and substrate mixture onto the slide and 5mL of methylene blue. You will check the cell viability of each wet mount. At the end of the three week period, we will normalize our data and average out the two sets of data collected from two groups. We averaged out our cell viability as well as yeast fermentation data collected over the 50 minute trial. Microsoft Excel was used to calculate descriptive statistics and Graphpad was used to determine the P-values of both the yeast fermentation and cell viability.

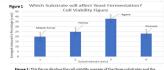
#### **RESULTS AND DISCUSSION**

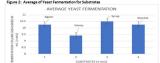
T-Test P-values	Maple Syrup	Honey	Agave	Glucose
Maple Syrup		0.0505	0.0001	0.0451
Honey			0.0001	1.0000
Agave				0.0001
Glucose				

#### 2: Descriptive Statistics Of Substrate

Descriptive Statistics	Agave	Honey	Syrup	Glucose
х	10mL	8.75mL	9.25mL	11mL
х	10.5mL	9.75mL	11.75mL	11.5mL
х	10.25mL	9.75mL	10.5mL	8.25mL
Average	10.25mL	9.42mL	10.5mL	10.25mL
STDEV	.25mL	.60mL	1.25mL	1.75mL
Standard Error	0.14mL	0.33mL	0.72mL	1.01mL

AGAVE	HONEY	SYRUP	GLUCOSE
	0.0433	0.7084	1.000
		0.1703	0.8239
			0.1703





Descriptív e Statistics	Agave	Honey	Syrup	Glucose
Week 1	36	24.5	20	22
Week 2	37.4	23	21.5	24
Week 3	36	24	21.5	22
Average	36%	24%	22%	23%
Standard Deviation	1.72240	1.16904	1.632993	1.04880 9
Standard Error	0.70316	0.47726	0.666667	0.42817



Howard Hughes

Medical Institute

 Using the bakers yeast, the results show that maple syrup goes through the fermentation process the quickest (10.5mL) while the honey goes through fermentation the slowest (9.42mL). With agave and glucose in the middle (10.25mL) • The hypothesis was incorrect. Maple Syrup had the most

amount of  $CO_2$  bubbles by 1.08mL from honey which was the least amount. This is because maple syrup has the highest carbohydrate composition of glucose These results are important because a natural sweetener such as maple syrup can be used instead of the processed sugar we use today.

 Limitations with the experiment was not having the incubator at 30°C the entire time through the intervals. This is because there were other groups using the incubator. Another limitation was not having the exact measurements while using the micropipettes during the transfer process to do the microscope slides because the substrate would get stuck on the side of the tube. This limited the results because not all of the sample would be on the microscope slide. So the cell viability may have been thrown off a little.

•FUTURE STUDIES: Instead of doing a 50 minute trial, we would test it for a hour so that we can get a better representation of the CO<sub>2</sub> production. If we conducted this experiment for a month would that change our results

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#### Appendix 18: Example of SSP categorized as "Developing"



#### How Does Various Temperatures Effect Yeast Fermentation and Cell Viability. Howard Hughes Medical Institute

Department of Biological & Biomedical Sciences, North Carolina Central University, Durham, NC

#### INTRODUCTION

Saccharomyces cerevisiae otherwise known as baker's yeast, is used in winemaking, baking, and brewing. Saccharomyces cerevisiae is not only used for food and drink consumption but it can be used as over the counter medicine for common colds and flu symptoms

•This yeast ferments differently when it is under different temperatures. The research question of this experiment is: How does various temperatures effect yeast fermentation and cell viability? Saccharomyces cerevisiae was used in this experiment. When temperatures are high, yeast tend to ferment quickly but when temperatures are low yeast takes a longer time to ferment, temperatures also plays an effect on the cell viability rate. If temperatures are too high, or too low the cells may be killed. It is imperative to know the effect temperatures have on yeast fermentation, because it will allow you to know what environment is best to produce yeast in.

 This experiment was conducted for the purpose of determining whether yeast fermented faster in hot(60°C), cold(9°C), or room temperatures(22°C).

#### **OBJECTIVES**

#### Our objective is to find the optimal temperature for yeast

We are researching this to see how various temperatures affect the st fermentation p These various temperatures being an ice bath(9°C), room

temperature(22°C), and a hot bath(60°C)

#### **Hypothesis**

If the yeast is fermented at various temperatures being 9°C, 22°C, 60°C and they all have different outcomes, then it can be assumed that the yeast ferments better in one temperature than the other based off the average fermentation rate, and cell viability. There are optimal growing temperatures that they as thrive in which is between (20°C-30°C). Outside of those optimal temperatures the yeast fermentation process will be hindred. If the temperature shoe too low around (10°C), then the enzyme reaction will slow to a point where it work' work. If the temperature becomes to high around (40°C), then the enzyme swill denature and unfold and becomes inactive.

#### EXPERIMENTAL DESIGN

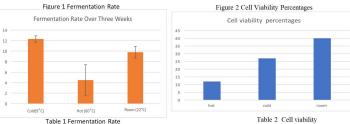
The control group for this experiment is the room temperature deionized water. The experimental group and the independent variable for this experiment is the 9° Cold bath, 22° croom temperature and the 6°C heated bath. The dependent variable for the experiment would be the fermentation rate of the 20% yeast and 5% glucose solution. The standardized variables would be the amount of time to ferment the 20% yeast, 5% glucose, dilution solution and deionized water.

#### METHODOLOGY

- Use a transfer pipette to transfer 3 mL of the 20% yeast solution directly into the 15 mL conical tube containing 10 mL of 5% glucose solution. Shake and pour 20% yeast and 5% glucose solution into 15 mL tube with holes in cap After pouring solution into the sing conclat tube over and inver tube Take initial volume and place fermentation apparatus in incubator, refrigerator, and heated bath. Every 10 minutes mark the volume of the rate of fermentation for 50 minutes After 30 minutes of fermentation make a wet mount from the fermentation apparatus that was place in the incubator, refrigerator, and bath. Using 10 µJ of the yeast solution and 10 µJ of the dilution solution. Mix and take 5 µJ of that solution. And stain with 5 µJ of 0.1% me blue
- ne blue Place experimental solution back in its appropriate place and examine wet mounts under the microscope During this experiment, we used measured the fermentation rate of saccharomyces cerevisiae and the cell viability of fermented omyces cerevisiae.

saccnaromyces cerevisias. • The website that we will be using to analyze the data is graphpad. We will be using graphpad to conduct the test which will compare the means of the experimental group and the control group and calculate the p-values. We will also be using microsoft excel to, we will be using microsoft excel to calculate the mean, stand card deviation, standard error of the mean, and to create graphs.

#### **RESULTS AND DISCUSSION**



experimental Sample experimental variables Mean Stdev Std Error Cell viability variables size Cold(9°C) 6 12.3 0.6 .3 Cold 27% Hot(60°C) 4.5 2.9 12 Hot 12% 6 Room Room 6 9.8 1.1 .5 40% temperature temp(22°C)

- Figure 1 is a bar graph that shows the results of the yeast fermentation in different temperatures. Table 1 is a table that shows the summary of the data collected over the three week period Figure 3 is a bar graph that shows results of the cell viability from the total amount of live and dead cells from the three weeks of research. Table 2 displays the percentages of the cell viability in the samples The hot temperature samples had an average of 12.5 ml yeast fermentation after 3 weeks of collecting data. The cold temperature samples had an average of 4.5 ml yeast fermentation after 3 weeks of collecting data.

The experimental groups were extremely significantly different from the control. The p-values were less than 0.05

### **CONCLUSIONS**

 Overall results show that yeast ferments fa (60°C). Room temperature (22°C) had an average of 9.75ml fermentation, cold temperature (9°C) had a average of 4.45ml, and the hot temperature (60°C) had a an average of 12.25ml. Results prove that the higher temperature worked as an enzyme, while the colder temperature worked as an inhibitor. This is proven because the higher the temperature the faster the yeast fermented, but for the low temperature the yeast barely fermented. For cell viability, results showed that yeast is mostly likely to have a higher cell viability rate in showed that yeast is mostly likely to have a higher cell viabilityrate in room temperature, which is understandable because the optimum temperature for yeast is 20-30°C. Room temperature (22°C) had a cell viability rate of 12 %, and cold temperature (92°C) had a cell viability rate of 27%. Room temperature had a higher cell viability rate than the other two experimental virables, because it was placed in the optimum temperatures, or the optimum and cold temperature able to thrive. The hot temperature, and cold temperature have not the appropriate environment for the yeast of they were killed.

Our hypothesis was accepted because the he data showed that one of the temperatures in fact did act more as an inhibitor than the other two different temperatures causing for a lower fermentation nate and lower cell viability in the yeast cell. This is because the the temperature would have acted as an enzyme and slowed the reaction down.

These results are important because they can be used to efferment yeast. These results will allow bakers, and anyone what temperature yeast ferments the best, and the worst.

Limitation during this experiment includes the incubator being open, timers not being set, the hot bath was not prepared, the thermometer was broken for the cold samples.

At what room temperature degrees does yeast ferments the best. W would like to perform the same experiment with the variable being room temperature at different degrees.

#### **REFERENCES &**

#### ACKNOWLEDGEMENTS

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We would like to acknowledge the funding from HHMI Precollege and Undergraduate Science Education Program (Grant # 52007553.

#### Appendix 19: Student self-reflection responses about student-scientist curriculum

QUESTION: "Did you find the mock review panel of final posters from previous semesters activity useful?"

#### Yes (96%)

- I found it useful because it made me see what should and shouldn't be included in the poster. It
  gave me kind of a sense of structure when approaching the mini posters/final posters.
- The mock review panel was very useful. I was able to see two examples of a mini poster. One poster was an effective example and the other one was an ineffective poster. I was able to judge both posters and see what is expected of us on our own mini posters. One poster provided too much information while the second one provided enough and was better to understand.
- I can see the mistakes made by other people and ensure that those same mistakes are not made
  on mine. As well as, I can see an example of what it should like and see the perspective of the
  grader, which should help my score tremendously.

QUESTION: "Did you feel like a student-scientist while completing your research proposal?"

# Yes (68%)

- This is the first "real" project where we are carrying out the experiment by ourselves rather than following set guidelines and rules.
- Lessons were learned such as learning to research background info like a regular scientist would.
- I did feel like a student scientist. I read background information and worked on conducting our own experiment. It was very hands on.

# Somewhat (9%)

- I believe that I still need more experience to feel like a student scientist.
- I'm just starting to do such experiments. I'll wait until I get in my junior year to confirm that.

# No (23%)

- I was just trying to finish the work.
- I was confused and I am really lost. Luckily my group members help me out when I need it.
- I felt like a panicked college student.

QUESTION: "Did you feel like a student-scientist while completing your research experiment?"

# Yes (77%)

- I was not only conducting an experiment, but I was also following the scientific process, like as scientists.
- Completing my experiment on my own allowed me with the opportunity to be independent and collecting the results from the experiment which we created.
- You have to have critical thinking in order to process what is really happening. A scientist student project is more challenging than a regular project. In our previous experiment, you gave us the data but on this one we had to build it on our own.

# Somewhat (13%)

- We did deal with experimental equipment, but the setting did not feel scientific.
- I sort of felt like a scientist it definitely showed me how scientist do what they do. I don't feel that being a scientist is something I'm interested in.
- Sort of because we normalized data, and used a micropipette

### No (10%)

- During the research experiment, I was more focused on trying to finish than trying to understand what I was doing.
- This was more so just for a grade.
- My group members helped me a lot. I'll prefer if the teacher demonstrated how to carry out the experiment before we do it ourselves.

**QUESTION**: "After completing the student-scientist project, what are your thoughts about executing the scientific process and conducting a research project?"

# Positive (58%)

- This project has made me look at science in a different way. If asked to do this project again, I would quickly do so.
- It has given me confidence to conduct research projects in the future.
- My thoughts are clearer on how to carry out an experiment. I understand there is a lot of work to do for student scientist projects, but I am looking forward to doing more.

## Neutral(43%)

- It requires patience and more importantly background knowledge about the experiment.
- The scientific process and conducting a research project takes a lot of time and planning for it to be successful.
- I think it requires much more writing and paying attention to detail than I first thought.

### Negative(15%)

- · It is a lot of work.
- It is a long and tedious process that requires a lot of work.
- · That project was stressful.

QUESTION: "Would you recommend a student-scientist curriculum to your friends?"

### Yes <u>(78%</u>)

- My friends would benefit from this lab. Although the lab required lots of patience, students were able to gain knowledge, presentation skills and research skills.
- It is a good experience. Especially, if you've never took part in a lab.
- I would because you learn some great skills that you may not gain from a traditional lab.

### Somewhat (22%)

- It was an exhausting.
- It is too stressful and takes a lot of time from other classes.
- It is stressful, extremely time consuming, and ask for too much effort and hard to do

## Somewhat (4%)

 Slightly, however, I still feel as only experience will benefit me better.