Big Question:

How do you maximize the production of CO\textsubscript{2} in yeast?
Overview & Background Information

This module is designed to give students experience in designing experiments. In particular, the students should 1) recognize the function of a control experiment, 2) recognize the merits of only focusing on one variable at a time, and 3) understand the importance of running several trials within the experiment.

The context of this module is optimizing yeast activation and growth using a given protocol of collecting CO$_2$ produced. Students will work in research groups of 6-7 students and you will be their Advisor. As their Advisor, you will lead, teach, assess verbally, facilitate and help the students complete and present their experiments.

As their Advisor, you should know as much about yeast and baking bread as possible. Yeast are a type of single-celled eukaryotic organism that are members of the fungi kingdom. Eukaryotes are distinct from prokaryotes (e.g. bacteria) in that eukaryotes have organelles (membrane-bound compartments within cells, e.g. nucleus, mitochondria, etc.) and linear DNA. Prokaryotes have circular DNA and are typically about ten times smaller than eukaryotes. Eukaryotic cells are 10-100 microns in diameter; a human hair is about 100 microns ($10^6$ meters) wide. All cells (prokaryotic and eukaryotic) have cell membranes and use DNA to replicate.

All cells need to harness energy in order to grow and replicate. The chemical reactions that take place to sustain a cell are broadly termed metabolism, but this lab focuses on a specific subset of metabolism referred as `respiration. Proteins called enzymes help carry out (catalyze) the metabolic reactions inside cells.

**Aerobic Respiration** (requires oxygen, O$_2$)

- Reactants = sugar (generally speaking, an energy-rich, carbon-containing compound; specifically, glucose) and oxygen
- Products = carbon dioxide (CO$_2$), water, chemical energy in the form of ATP (adenosine triphosphate)
- Overall reaction = C$_6$H$_{12}$O$_6$ + 6 O$_2$ $\rightarrow$ 6 CO$_2$ + 6 H$_2$O + energy
- The rate of respiration is proportional to the amount of available food (sugar), the amount of yeast cells, the temperature, and the rate at which reactants meet each other (i.e. the stirring rate).
Bread Baking Basics

1. “Activate” the yeast. In order for the yeast to become active, they need to be warmed up and given a food source. This is achieved by putting the yeast in warm water with some sugar. You know it’s activated once it starts bubbling.
2. Mix in flour and salt (and seasonings if you’re fancy); stir.
3. Knead the dough.
4. Let the dough rise (i.e., let the yeast produce the desired amount of CO₂). This is achieved by putting the dough in a container and letting it sit in a warm (just above room temperature) place.
5. After allowing the dough to rise the desired amount, put it in a suitable pan and bake it in the oven. The yeast will continue to respire for the first few minutes, making the bread rise even more. Pretty soon, however, it will become too hot and the yeast will die.
6. Continue baking so that the starches are broken down into simple sugars and the proteins are broken down into amino acids.
7. Remove the bread before it burns; eat when cooled.

To learn more than you would ever want to know about baking bread visit:

DAY 1   Microscopy, Indicator Dye

Introduction

First, the SciTrek program will be introduced. The lead of the period will introduce themselves, then the volunteers will follow. State your name, what year you are in school, and what you are studying.

The lead will then give an introduction to yeast. They will go through if yeast is a eukaryote or a prokaryote and what that means to us. The discussion will go somewhat as follows:
“Is yeast more like us or the bacteria that causes you to have an ear infection.”
It is more like us. Yeast have organelles like us, which help us to learn more about ourselves.
“What is yeast used for”
Bread, Beer, etc.

Then we will relate yeast to us, through cellular respiration.
“So when we breath in oxygen, what do we breath out?”
Carbon Dioxide.
“And in order to breathe in oxygen, what do we need. What allows us to go running or to go out and play sports?”

Food which gives us energy.

“Right, so just like us, yeast need energy, which allows them to take in oxygen and breath out carbon dioxide.”

At this point, the students will split into small groups, where they will start to explore yeast further.

**Yeast Under a Microscope**

The intent of this section is to provide context and engage the students. Most students do not understand what yeast cells are.

Since finding the yeast under a microscope is very difficult and will take the students a long time to find a single cell, we will instead give them printed out pictures of each of the microscopic images. They will paste those pictures in their notebooks and make observations about them to the side of the pictures.

**Observe Activated and Inactivated Yeast**

You will set up two yeast samples for your entire group: one beaker with sugar and one without. The one with sugar will be properly “activated” and should produce bubbles after a few minutes, while the beaker without a food source should remain essentially unchanged. Allow the beakers to sit for 10 minutes or so while you proceed with the next demo.

The instructions for this activity are in their notebooks and should be easy to follow.

**Indicator for CO₂ – Class Activity**

**Bubbling Blue CO₂ Indicator**

This is a fun way to show that indeed CO₂ is being produced by the yeast. The indicator being used here is ‘Bromothymol Blue’ which is pH sensitive. Weakly acidic and basic solutions are blue, whereas more strongly acidic solutions (pH < 3) are yellow. **Bromothymol Blue is a mild skin irritant and slightly harmful if digested.** The students will not be handling the indicator, however it is important that they know about the different materials they are seeing. Before starting this demo, tell your students what an indicator is. They probably will not understand that an indicator changes color with the pH of a solution, so instead tell them that the indicator changes color when it comes in contact with CO₂. Remind them of the discussion that we had earlier about what we breathed in and out. In order to make sure this demo is as safe as possible, the volunteers will be performing it. We don’t want any students to
accidently ingest the indicator. The volunteers will use a straw to bubble CO₂ (their breath) into water with the Bromothymol Blue. The indicator will change colors. To make it more interesting for the students, have them count how long it takes their lead to change the color of the water. Make it a race for the volunteers.

Like stated before, this demo gets the students thinking about how yeast are similar to us. They can see that we, like yeast, breathe out carbon dioxide. This is also a demo to get the students excited. One big emphasis of SciTrek is to get the students excited about science. Sometimes that happens through a simple demo.

The second part of this demo shows that yeast is also producing CO₂ in the presence of sugar. This demo involves warming a 100 mL of diluted solution of bromothymol blue in a 125 mL Erlenmeyer flask to 37 °C on a hot plate in front of the class. The lead will then add 1 gram of sugar to the flask and allow it to dissolve before adding 1 gram of yeast. Once yeast has been added, swirl the flask and wait as the solution turns from blue to yellow. This experiment is used to show the comparison between human’s CO₂ production and the CO₂ production in yeast.

**Assemble & Discuss the Experimental Apparatus**

The students will then split up into their small groups with their volunteers. The volunteers will show them the picture of the experimental set up and challenge them to recreate the setup. Let them work together to try to figure out what their experiment will look like. If they are able to do it themselves this time, it will be easier for them to figure out later on. Call out individual students and see if they can name each piece of equipment as you put it together. Don’t forget to explain how it works. This is important, because the students will be working with this basic set up for the rest of the module and need to be able to assemble it on their own. By the time you finish setting up and explaining how it works, students will probably have started their sketches of the apparatus. Don’t be afraid to point to parts of students’ sketches and ask what that part is called. If they forgot already, give them a friendly reminder. This is a great chance to teach and reinforce scientific vocabulary.
DAY 1  What is yeast and what does it do?

Microscopy

Place Microscopy Here

Observations:

Activating Yeast

Instructions: Weigh out 3 grams of yeast into two separate beakers. Add 1 gram of sugar to one of the beakers (label this one “beaker 1” and the one without sugar “beaker 2”), and then add 50 mL of warm (37 °C) water to each of the beakers. Swirl the beakers and then let them sit for 10 minutes. Record your observations below.

Beaker 1  The beaker is slightly warm. There is a layer of bubbles that are CO₂ bubbles that the yeast produced.

Beaker 2  The beaker is not warm. There is no CO₂ production.

CO₂ Indicator

Instructions: Follow the directions on the “Bubbling Blue” demo sheet. Record your observations below.

Name of indicator dye: Bromothymol Blue

Observations: The liquid goes from blue to yellow as more CO₂ comes into the liquid.
DAY 2   Literature Review

The students will read some articles and use the internet to investigate yeast. They will brainstorm some ideas for ways to increase the amount of CO₂ that yeast produce. Make sure you also understand the different variables that will affect the yeast so that you can answer their questions. The lead and volunteers will not be there for this activity, so this is up to the teachers to make sure that they know what different variables may affect how much CO₂ yeast produces.

Teachers have options on how to discuss the variables that effect CO₂ production in yeast.
1. Teachers may give students prechosen articles (preferred)
2. Students may search online for articles themselves or in groups (preferred)
3. Teachers may go over variables that effect CO₂ production without articles (not preferred)

We prefer students use options 1 and 2 because learning how to do accurate research is an important part of the scientific process. Option 2 gives the students an opportunity to practice these skills while option 1 gives the students a model for what good research articles

Articles can be distributed to the students via google classroom.
DAY 3  Control; Maximize CO₂

Using the information that they gathered the previous day, students will design their own experiments. The lead will first remind students what they did the previous day that the volunteers were there. They will do a quick run through of what yeast is, and how they are similar and different from us. It is always important to make sure that the students are reminded of what they did with SciTrek the previous day. These students have many classes each day they are in school, and it is beneficial to them to give them a quick refresher of what was done before.

“Hey everyone, welcome back. So can anyone remind me of what we are going to be studying for the next few days.”

Yeast

“Right, and are yeast more like us, or more like bacteria.” More like us

“Correct. And just like us yeast breathe in what?” Oxygen.

“And we both breathe out…” Carbon dioxide.

After the lead has this discussion, they will quickly go into the literature search that was done the previous day.

“Alright, so yesterday you did a literature search with your teacher and you went through some different thinks that may affect how much carbon dioxide is released. What types of things did you come up with?”

Stir speed, temperature, amount of sugar, type of sugar (food), amount of yeast, time.

The lead will then go through each

Stir speed: “So if I run around a lot, I’m going to be breathing heavier right? Just like us, stir speed affects how much carbon dioxide yeast releases.”

Temperature: “What is your normal body temperature?” 98.6 degrees Fahrenheit. “Right, and when you get sick, you may get a temperature. That is your body trying to fight off any infection. So just like us, yeast have a temperature that allows them to function perfectly.”

Amount of Sugar: “Just like us, yeast need food to survive. In this experiment, we are giving them that food in the form of sugar.”

Type of food: “After you eat something really greasy and fatty, do you ever feel tired? That can happen to yeast to. Yeast work best if they have a certain type of food.”

Amount of yeast: “If you have a lot of yeast, there will be more carbon dioxide released, right?”

Time: “Eventually, the yeast will run out of food, but if you allow to yeast to produce carbon dioxide for the longest time possible, then you will have more carbon dioxide.”

Control Run

Help your research group assemble and run the control experiment. Follow the protocol carefully. Have the students fill out the data table in their handbook to record control run conditions. Ask the students why doing a control experiment is important.
A control experiment is important so you know how effective your experiment actually was. If you don’t have anything to compare it to, how are you supposed to know if your experiment was successful.

One part of running this experiment that becomes complicated in the classroom is the temperature of the water bath. We want the temperature to be constant throughout the experiment, so it is usually the lead's responsibility to walk around with a beaker of hot water and give each group a little more if their temperature drops. This becomes difficult when the lead needs to run a group as well. In this case, the volunteers should make sure they have a beaker of hot water at their tables. These beakers should be very hot, so you won’t have to add much to the water bath to increase the temperature. Because of this hot temperature, the students should not pour the hot water and the volunteer or lead should always be using a fire proof glove to protect their hand.

After all groups have finished their control experiments, the lead/teacher will write each group’s data on the board and determine the class average. Student’s will write this average into their notebooks and compare it to their result. This is a good opportunity to discuss the possibility of outliers and how they can affect your data. If the students know that they made a mistake during their procedure (i.e. letting their temperature drop, not having the stir bar spinning for half the experiment, etc.), then their data should be removed from the average. Mention to these groups that another way of testing if their data was an outlier is by reproducing the experiment. However, this will not be done during module because of time constraints.

**Experiment Design #1**

Challenge your group to maximize the amount of CO2 produced. Have them brainstorm all the variables that they can explore. Let them try a run with as many variables changed as they want. It will make you cringe, but we need them to try multiple variables at the same time in order to make our point when trying to unravel their results.

At first, allow them to choose as many variables as they want. At first they will want to change anything in the flask and see what happens. After they do this for their first experiment, they won’t know what has exactly caused the change in carbon dioxide production. This will help guide their thinking. After they realize they don’t know exactly what affected the carbon dioxide production, ask them to choose one variable they hope will cause the most carbon dioxide production and have them change just that variable.

- Be sure to save time at the end to talk to your whole group. Discuss each of their results (3 mini groups will be reporting out). Facilitate a discussion around how to best interpret the results – lead them to understand that testing only one variable at a time is the best way to work on optimizing the system.
- You can record their results on a white board or piece of paper to help the students visualize their results.
- Review with your group the concepts learned from the multiple variable experiment. As a group, decide on a variable or variables they think worth pursuing to optimize CO2 production. The amount of yeast must remain constant, i.e. the same amount as the control.
- The students can explore any variable besides the following: amount of yeast, time, and amount of liquid.

### DAY 3  Control Exp. and Maximize CO2

#### Control Run

Instructions: Follow the directions on the “Lab 1 – Yeast Control” sheet. Record your data and observations below.

<table>
<thead>
<tr>
<th>Temperature of water bath</th>
<th>Amount of yeast</th>
<th>Amount of sugar</th>
<th>Stir speed</th>
<th>Amount of water collected</th>
</tr>
</thead>
<tbody>
<tr>
<td>31°C</td>
<td>3g</td>
<td>1g</td>
<td>1</td>
<td>5mL</td>
</tr>
</tbody>
</table>

Observations: The water is pushed from the middle of the flask into the graduated cylinder as CO2 is produced by the yeast.

Class average: 7mL

Discussions Question:

Was your experimental result consistent with the classes average? Was your data an outlier?

#### Maximize

Instructions: Using your knowledge from your literature search and from the control experiment, try to maximize the amount of CO2 produced by the yeast. Be sure to record any changes you make to the procedure and record your results below. When you are finished collecting data, answer the questions that follow.

<table>
<thead>
<tr>
<th>Temperature of water bath</th>
<th>Amount of yeast</th>
<th>Amount of sugar</th>
<th>Stir speed</th>
<th>Amount of water collected</th>
</tr>
</thead>
<tbody>
<tr>
<td>37°C</td>
<td>3g</td>
<td>4g</td>
<td>3</td>
<td>17mL</td>
</tr>
</tbody>
</table>

1. Were you successful with your first trial? How do you know?
   - Yes, we collected more water in the graduated cylinder.

2. Which variable is directly responsible for your success? How do you know?
   - Either amount of sugar or stir speed was responsible because these were the two that were changed.

3. As a group, which variables would you like to explore further?
   - Stir speed is what we want to explore.


**DAY 4**  
Procedure Practice

On this day, teachers get the option to have their students perform a procedure-writing activity. The goal of this activity is to remind students that the procedures they write should be clear, descriptive, and specific.

First, the class will be split into two groups and each group will be shown video of a task (either folding a paper airplane or tying shoes). The students are challenged to write a procedure of the task in their video. As students finish their procedures, they are paired together with a member from the other group. One at a time, the students will share their procedure with their partner and observe them as they try to complete their task. If the student’s partner fails, then the student should be given the opportunity to revise his procedure and try again. Once the student has completed the task or attempted twice, then that student will share his procedure with the other student and repeat the cycle.

You should emphasize that each step should be taken literally, and no steps should be implied. For instance, how will you tie your shoe if you do not first bend downwards?

If there is additional time left in the class, students can improve a “bad” procedure for the CO₂ collection experiment that was done on day 3. This procedure could use more explicit steps and reorganization of steps, giving them a chance to review the experiment through a challenge.

Teachers may elect to give this segment of the module electronically, rather than on paper.

**DAY 5**  
Refining the Experiment

Review concepts from the last meeting, such as what the purpose of a control is, how many variables should change in a single experiment, etc. Go over the variables that the group as a whole wants to explore.

**Variable List**
The following is a list of potential variables that the students can explore:

- Sugar amount
- Sugar type
- Stir speed
- Water bath temperature
- Salinity
- Acidity/Alkalinity

Each experiment must leave the amount of yeast, the total solution volume, and the reaction time constant, but all other variables are free to be changed as much or as little as possible.
As an advisor, help the students organize their data tables. They can be fashioned after the ones used for Day 2, but make sure that they record the new quantity for the variable that they are testing. However, they will only have time for 2 experiments per day, and we would also like them to repeat their trials to check for consistency. On this day, have each group run the same experiment twice. Students will probably be anxious to try a new value for their variable, so remind them how important repeatability is in science. If students get very different results for the same experimental conditions, talk to them about possible sources of error. These groups should start day 6 by doing one more trial. If a group gets reasonably consistent results, they can test a different value (still same variable) on day 6.

While the students are conducting their experiments, constantly review equipment vocabulary, assess verbally whether they understand the process that is occurring, and why they are collecting data on water volume and not gas. They should also be comfortable enough to assemble and run the experiments on their own the next day.

Don’t let students start another trial if you don’t think they will finish in time. When students are finished have them start cleaning up.
DAY 6  Collecting More Data

Students run experiments on their own with teacher and SciTrek Lead. Groups that obtained consistent results should proceed to try a different amount of the variable in question. They should try 2 different values on this day – we won’t have time to do repeat trials.

DAY 7  Graphing Practice: Experimental Data

Students will practice graphing bar and line graphs. They will be given data sets that they can use to create SULTAN bar graphs. After they’ve reviewed graphing with a few data sets, the students will then attempt to graph their own data. The data should be presented with the changing variable on the x-axis, while water collected should be presented on the y-axis. Students should choose an appropriate name for this graph, considering that it will be presented on day 8.

If time permits, then the teacher may discuss with the class what the optimized variable values for each group were. If not, this can be done at the beginning of day 8 with the help of the volunteers.

DAY 8  Finish Data Collection; Begin Poster

Start by having each group share with the others what the optimal value of their variable was. This should be facilitated by using the graph that the students made on day 7. If the group did not find an optimal value (e.g. results are totally inconsistent) help them make an educated guess. Suppose that a group finds that more of a certain variable increases the output of CO₂, and they don’t find an upper limit (a point where adding more of the variable actually decreases CO₂ production). They do not have to pick the value with their best results. They could pick a higher value. Caution them to not go very much beyond their highest value, since they can’t be sure that the trend will hold. Write down the group’s combination of optimized conditions on a white board or piece of paper. Students should write down these optimized conditions in their notebook as well. Have each group run an experiment with all optimized variables. Because each group is doing the same experiment, we can teach them about uncertainty and error when doing science. Small variability in results is a normal part of science! If results of the same experiment are very different from each other, then it is likely that one of them is not correct. The teacher may go through finding the optimized class experiment on the day before the SciTrek volunteers come in. That is okay. The class with the best optimized carbon dioxide outputs out of all the classes will be the winner.

After analyzing your group’s results, help them get started on their posters. Each student will work on a particular part of the poster, and they will eventually share their poster as a whole.
with the rest of the class. Help the students decide who does each part, and make sure that each student knows what to do for his/her part.

**DAY 8** Conclusions and Write Up

**Conclusions**

Based on your class results, describe what conditions would produce the most CO₂ with yeast:

> My results showed that a higher stir causes the yeast to produce more CO₂. Other groups found that more sugar also causes the yeast to produce more CO₂.

Now that you have a hypothesis for what the best conditions to produce CO₂ with yeast are, try it for yourself. Make a table below with all your experimental values and take note of how much water you collect. Once each group has finished their experiment, your teacher will help you find the class average.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Amount of Sugar</th>
<th>Amount of Yeast</th>
<th>Stir speed</th>
<th>Sugar Type</th>
<th>Amount of water collected</th>
</tr>
</thead>
<tbody>
<tr>
<td>37°C</td>
<td>5g</td>
<td>3g</td>
<td>6</td>
<td>2</td>
<td>70mL</td>
</tr>
</tbody>
</table>

Optimized experiment class average: 74 mL

**Discussion Question:** Was your group's experimental value consistent with the class average? If not, what could have caused this error? Explain below.

The amount of water my group collected was slightly below average, but we think it was caused by our temperature decreasing over the experiment.
DAY 9  Finish PowerPoint Presentation
Student’s from the same group will work together to complete a PowerPoint presentation that discusses their control results, their experimental results, and their optimized results. These will be presented on day 10.

DAY 10  PowerPoint Presentation
Groups will be presenting their PowerPoint presentations today. The first 5 minutes should consist of the volunteers checking their groups presentation. After which, groups will present in front of the class. Presentations should take about 5 minutes. There should be a 2-3 minutes question period after presentation. Volunteers are encouraged to start the questioning process if none of the students choose to do so. Communicating your results with peers is an important of how science works, so try to have fun.😊
SciTrek is an educational outreach program that is dedicated to allowing 2nd-8th grade students to experience the scientific process first hand. SciTrek partners with local schools to present student-centered inquiry-based modules that not only emphasize the process of science but also specific grade level content standards. Each module allows students to design, carryout, and present their experiments and findings.

For more information please feel free to visit us on the web at http://www.chem.ucsb.edu/scitrek/ or contact us by e-mail at scitrekadmin@chem.ucsb.edu.

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