INQUIRY & INVESTIGATION

A Simple Microscale Setup for Investigating Yeast Fermentation in High School Biology Classrooms

RECOMMENDED FOR *AP Biology*

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ABSTRACT

High school students often find the concept of respiration difficult. Yeast, a readily available resource, offers promising material for studying the topic. This article describes a low-cost, microscale setup for investigating yeast fermentation. The observations in the practical activity are visually appealing to learners. The article also illustrates how this setup can be used to promote student engagement with scientific ideas by prompting students to (1) predict what they will observe in the activity and (2) link what they actually observe in it to the underlying scientific ideas, in the context of studying the effects of different sugar substrates on yeast fermentation. The simple setup can be easily modified for various scientific investigations related to yeast fermentation and, hence, represents a promising teaching tool for teaching this difficult-to-learn topic in high school biology classrooms.

Key Words: *Yeast fermentation; yeast; demonstration assessment.*

Introduction

High school students often find the topic of respiration difficult (Bahar et al., 1999; Yip, 2000). Because experiments with yeast can often be conducted without posing a serious health risk, yeast is a promising material for helping students gain insights into the elusive metabolic process related to anaerobic respiration (Reinking et al., 1994; Myers et al., 2005). For example, practical work on yeast may be used to illustrate the scientific idea that increasing the sugar substrate (carbon) or yeast (enzyme) increases the rate of fermentation (Bartlett, 2002). While practical work can be a useful means to help students make sense of the scientific ideas related to yeast respiration, many studies have illustrated that practical work, as practiced by teachers, is not really effective (e.g., Hodson, 1990; Abrahams & Millar, 2008). This is because teachers often fail to engage students in *thinking about* the scientific ideas behind their observations. In view of the above, this article describes a simple microscale setup for investigating fermentation in yeast. It also illustrates how this setup can be used to help students actively think about their observations and connect what they observe in the practical work to the underlying scientific ideas in order to promote meaningful learning of the topic.

This is because teachers often fail to engage students in thinking about *the scientific ideas behind their observations.*

A Simple Microscale Setup

A variety of setups have been devised to study yeast fermentation. Some involve the use of test tubes (Taylor, 1987; Johnson, 1998; Bartlett, 2002), others the use of burettes and pipettes (Yurkiewicz et al., 1989) and/or Erlenmeyer flasks (McElroy, 1976; Tatina, 1989). Still others make use of materials that are common in the contemporary molecular biology laboratory (e.g., 15 mL centrifuge tubes and Eppendorf tubes; Reinking et al., 1994; Grammer, 2012). The setup described here utilizes plastic transfer pipettes commonly found in molecular biology labs. It is a microscale version of the traditional respirometer assembled using pipettes (e.g., Yurkiewicz et al., 1989). Microscale practical work is common in the domain of chemistry but much less so in biology (but see Delpech, 2005; Bradley & Ovens, 2006; Weise, 2006). Microscale work offers several advantages over the traditional approach, including lower cost (for both equipment and chemicals) and less class time. In particular, the time saved in setup/preparation can be channeled into promoting engagement with scientific ideas. The protocol described here is distinct from the standard lab protocols for yeast respiration in that the practical activity gives a distinct visual experience to students (i.e., a beautiful color change). The appealing visual experience should help students visualize ideas about yeast fermentation. The assembled setup is shown in Figure 1.

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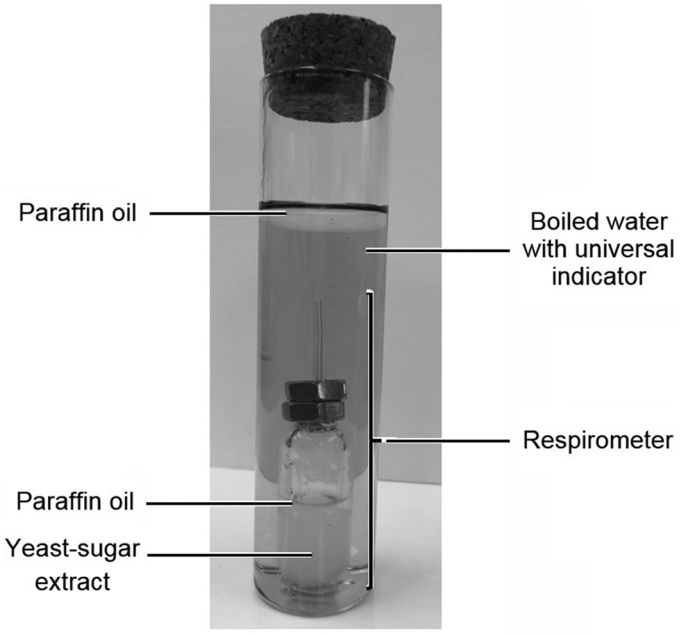

Figure 1. The microscale setup for investigating yeast respiration.

○ Suggested Lesson Sequence

The lesson sequence is aimed at prompting students to actively link the observations in the practical work and the underlying scientific ideas. It draws on the ideas of demonstration assessment, a pedagogical strategy used to engage students in explaining the scientific ideas behind their observations (Radford et al., 1995; Ramsey et al., 2000). In demonstration assessment, students are shown a short demonstration and are asked to record as many observations as possible. They are then asked to explain what they observed and, in doing so, *explicitly* link what they have observed to the underlying scientific ideas. Similarly, the lesson plan requires students to explain their observations. However, there are two distinct differences between the strategy described in the lesson plan and demonstration assessment. First, it is not a demonstration. Second, it goes beyond demonstration assessment by requiring students *first* to plan for what they will observe *before* the practical work and then actively record their observations *during* it. They are then required to explain their observations fully (for an example of instructional material, see the Appendix). As in demonstration assessment, students are then assessed on how well they can correctly utilize their knowledge to explain their observations. It is important to note that the suggested lesson plan allows plenty of time (more than half of the suggested time) for students to *think* and *talk about* the scientific ideas behind the practical activity. This includes *making predictions* about what they may be able to observe in the practical work as well as explaining what they can actually observe. As such, this activity promotes the engagement of scientific ideas behind observations. In the suggested lesson plan (Table 1), students work in pairs to study the fermentation of one type of sugar substrate by yeast (e.g., sucrose). They then compare their results with those from another pair of students working on a different sugar substrate (e.g., fructose). Teachers are encouraged to modify the suggested time given to students and also the grouping, based on the ability and prior knowledge of the students.

Table 1. Flow chart of the 100-minute practical activity.

Lesson Sequence	Duration	Lesson
• Introducing the objective and the background of the practical work	10 minutes	1
• Showing students the apparatuses and materials (Figure 1)		
• Asking students to read the procedures of the practical work (students are allowed to raise questions about the procedures)	10 minutes	
• Discussing the precautions and the rationales behind the procedures/setup with students	10 minutes	
• Students discussing in pairs how to take as many observations in the practical activity as possible	20 minutes	
• Students conducting and recording their actual observations	20 minutes	2
• Students completing a worksheet that prompts them to link their observations to the scientific explanation	30 minutes	
• Students discussing their results with another pair working on another sugar substrate		

○ Materials & Apparatus

A universal indicator (a type of pH indicator) is used in the protocol to help students *visualize* the carbon dioxide produced by the fermentation of yeasts. For many students, this will draw on knowledge acquired in previous science classes: the universal indicator changes color depending on the pH of the solution; carbon dioxide dissolves in water to form carbonic acid; carbon acid increases the acidity of the solution, which means that it decreases the pH of the solution; and so on. The appealing color changes provide a memorable visual experience, allowing students to connect the abstract ideas to what they can see in the practical work. The required materials and equipment are listed in Table 2 and shown in Figure 2.

○ Protocols

The protocol is presented in Table 3. The ratio of yeast to sugar and the concentration of yeast/sugar in the protocol have both been optimized such that the yeast will not overflow from the transfer pipette

Table 2. Materials for the practical activity (one of each is needed for each student group).

• 50 mL flat-bottomed boiling tube
• 45 mL water (in 50 mL falcon)
• Glass dropper
• 450 µL universal indicator (in 1.5 mL microcentrifuge tube)
• 2 nuts
• Timer
• 3 mL plastic transfer pipette
• 1 mL 15% yeast extract (in 1.5 mL microcentrifuge tube)
• 1 mL 0.6 M sucrose/fructose solution (in 1.5 mL microcentrifuge tube)
• 300 µL paraffin oil (in 1.5 mL microcentrifuge tube)
• 1.5 mL paraffin oil (in 1.5 mL microcentrifuge tube)

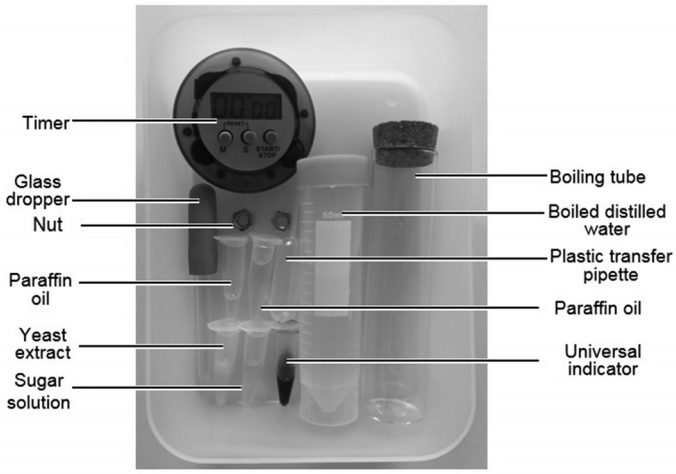

Figure 2. Materials and apparatuses.

Table 3. Procedures of the practical activity.

Activation of yeast (to be prepared by the technician):

1. Add 0.5 g sucrose/fructose into 15% yeast extract (15 g in 100 mL boiled water).
2. Activate the yeast for 30 minutes.

Setting up the respirometer:

1. Transfer 450 μL universal indicator into 45 mL boiled water using the glass dropper.
2. Pour the solution into a 50 mL flat-bottomed boiling tube.
3. Carefully squeeze out the air in the 3 mL plastic transfer pipette.
4. Suck up 1 mL of 15% yeast extract.
5. Invert the transfer pipette and let the solution run down into the bulb.
6. Carefully squeeze out the air in the 3 mL plastic transfer pipette (with the yeast extract within the bulb).
7. Suck up 1 mL 0.6 M sucrose solution (Version A)/0.6 M fructose (Version B).
8. Invert the transfer pipette and let the solution run down into the bulb.
9. Mix the yeast extract and sucrose/fructose solution in the transfer pipette by squeezing the bulb gently.
10. Carefully squeeze out the air in the 3 mL plastic transfer pipette.
11. Suck up 300 µL paraffin oil.
12. Invert the transfer pipette and let the solution run down into the bulb.
13. Put two nuts over the neck of the transfer pipette.
14. Put the setup (Figure 1) into the boiling tube until the transfer pipette is fully submerged.
15. Add a layer of paraffin oil.

when undergoing fermentation. Using the protocol, color changes can be observed within a reasonable time-frame (within 5 minutes for sucrose and 10 minutes for fructose). Video of the setup and the color changes can be found at https://youtu.be/IU3CpVvb9J0 and https://youtu.be/1H6d3-PkbqM. An example of the worksheet for the activity is provided in the Appendix. The generic rubrics for assessing student work are in Table 4.

○ Discussion

This article illustrates how a low-cost, microscale setup can be used to promote student engagement with scientific ideas in the context of studying the effects of different sugar substrates on yeast fermentation. The setup presented here has several strengths. First, students are able to observe the visually appealing color change caused by carbon dioxide during fermentation. Second, the ease of assembling the setup (it normally takes <5 minutes) also means that more time can be left for discussion and negotiation of scientific ideas, both among students and with teachers. Moreover, this simple setup can be easily modified for various scientific investigations related to yeast fermentation (e.g., the effect of sugar concentration or temperature on the rate of fermentation of yeast; Collins & Bell, 2004). It can also be used for discussion of more advanced scientific ideas (e.g., Pasteur effect, substrate specificity) if more advanced learners are the target population (see Reinking et al., 1994).

In terms of the pedagogical design of the practical activity, similar to demonstration assessment, it explicitly prompts students to connect what they *observe* in the practical activity to the underlying *scientific ideas*. It goes beyond demonstration assessment by requiring students to apply the scientific ideas they have learned before to *predict* what they will observe. It is believed that this will further enhance students' engagement with scientific ideas. Students also need to draw on their prior knowledge about pH indicators to explain their observations in the practical activity. With these

Table 4. Scoring rubric for the practical work.

Score	Accomplishments
1	Make incorrect observations or no observations.
2	Describe *one* correct observation (e.g., gas bubbles were evolved from the respirometer during the practical activity; the color of the boiled water turned from green to yellow).
3	Describe *one* correct observation and correctly link the observation with the correct scientific idea(s) (e.g., in the absence of oxygen, yeast carries out fermentation. The carbon dioxide produced appears as gas bubbles under water; the carbon dioxide produced by fermentation dissolves in water to form carbonic acid; this lowers the pH of the boiled water from neutral to acidic).

design features, this simple setup – together with the suggested lesson activities – offers students a meaningful practical work experience that goes beyond the mere verification of scientific ideas.

○ Conclusion

In summary, this article not only describes a simple microscale setup for investigating yeast fermentation but also illustrates how this setup can be used to promote hands-on and minds-on engagement with ideas about fermentation. This practical activity is a simple alternative way for high school biology instructors to teach this difficult-to-learn topic.

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APPENDIX

Practical Works and Scientific Inquiry (Biology)

Practical Work Task Sheet Version A/B

Name (in full): English Name:
Class No: Date:

Objective:
To compare the effect of two different sugar substrates on the fermentation of yeast using a microscale respirometer

Background:
Yeast is a fungus. Fungi are chemoheterotrophs and they depend on organic carbon as energy source. Sugars supply yeast energy. In the absence of oxygen, yeast undergoes fermentation to obtain energy.
In this practical, you will work in groups of two to study the effect of two different respiratory substrates (sucrose and fructose) on the fermentation of yeast using a microscale setup. Your group is required to assemble a microscale setup and *make observations.* You will also need to *explain* all the observations your group listed by linking the observations to the relevant scientific concept(s) or principle(s). After your group has collected data on yeast fermentation using the sugar substrate assigned to your group, your group will then compare your observations and results with those of another group working on another sugar substrate, to compare the effect of the two sugar substrates on the fermentation of yeast.

Materials and apparatus:

- 50 mL flat-bottomed boiling tube X1
- 45 mL water (in 50 mL falcon) X1
- Glass dropper X1
- 450 µL universal indicator (in 1.5 mL microcentrifuge tube) X1
- Nut X2
- Timer X1
- 3 mL plastic transfer pipette X1
- 1 mL 15% yeast extract (in 1.5 mL microcentrifuge tube) X1
- 1 mL 0.6M sucrose/0.6 M fructose solution (in 1.5 mL microcentrifuge tube) X1
- 300 µL paraffin oil (in 1.5 mL microcentrifuge tube) X1
- 1.5 mL paraffin oil (in 1.5 mL microcentrifuge tube)

Procedures:
[See Table 3]

Q.1 Label *Figure 1* to indicate the major components of the yeast mini-respirometer
Q.2 Explain the purpose of
  (a) using boiled water
  (b) using water cooled to room temperature rather than boiling water
  (c) adding the paraffin oil to the setup.
Q.3 Discuss with your peers to plan for the observations you will make in this practical work by making notes in the column provided.
  Note: You are advised to observe the setup for around *20 minutes* (use the timer to count the time).

	Notes
1. Transfer 450 μL universal indicator into 45 mL boiled water using the glass dropper 2. Pour the solution into a 50 mL flat-bottomed boiling tube 3. Carefully squeeze out the air in the 3 mL plastic transfer pipette 4. Suck up 1 mL of 15% yeast extract 5. Invert the transfer pipette and let the solution run down into the bulb 6. Carefully squeeze out the air in the 3 mL plastic transfer pipette (with the yeast extract within the bulb) 7. Suck up 1 mL 0.6 M sucrose solution (Version A)/0.6 M fructose (Version B) 8. Invert the transfer pipette and let the solution run down into the bulb 9. Mix the yeast extract and sucrose/fructose solution in the transfer pipette by squeezing the bulb gently 10. Carefully squeeze out the air in the 3 ml plastic transfer pipette 11. Suck up 300 μL paraffin oil 12. Invert the transfer pipette and let the solution run down into the bulb 13. Put 2 nuts over the neck of the transfer pipette 14. Put the set up (*Figure 1*) into the boiling tube until the transfer pipette is fully submerged 15. Add a layer of paraffin oil	

Results:

Record the observations your group made in the table in the *Group Task Sheet*.

Discussion questions:

Q.4 (a) Explain the observations you and your group member made in the *Group Task Sheet*.

(b) Did your group observe any color change in the setup? If so, describe and explain the color change with reference to the process of yeast fermentation.

Q.5 Based on what you have done, what precaution(s) might you have taken to reduce errors in the practical work?

Q.6 *Predict* what you can observe if you are to continue this practical work for another hour. Explain your prediction.

Q.7 Compare your group's results with those of another group working on a different sugar substrate. Identify the difference in observations. (*Hint:* You may compare the time it takes for color change)

Conclusion:

Q.8 Based on your answer in Q.4, write a concise conclusion with reference to the objective of this practical activity.

Further question:

Q.9 Based on the conclusion, research on the reason why there is a difference in use of the two sugar substrates for fermentation by yeast.

Practical Works and Scientific Inquiry (Biology)

Practical Work Group Task Sheet

Group members: _____

Sugar substrate your group used: _____

Directions:

Write down the observations and the explanations for the observations. You will score points when the observations are correctly related to the appropriate scientific concept(s)/principle(s). Your group will compare the observations with those from another group working on a different sugar substrate.

Observations and Explanations:

Observation 	**Explanation for the observation**