Can life gain energy from fuel without oxygen?
FERMENTATION LAB: **TEACHER GUIDE**

RATIONALE:

This lab can be useful in various contexts and different ways. As written here, it is as a follow up to our model of cell respiration that explores the question of how the earliest organisms on Earth were able to access energy from food when we know there was no O_2 available. However, it could be used prior to building the cell respiration model as a phenomenon – behold an organism doing something to glucose that results in energy being released! The introduction would need to be rewritten however to fit that scenario. Either way it gives students the opportunity to directly observe organisms rearranging glucose to obtain energy. It establishes that glucose contains a lot of energy, and that organisms have ways of accessing that energy.

In follow-up discussion it is important to reinforce the connections between the data and the ideas of the chemical reaction model, namely 1) products are simply the rearranged atoms of reactants, 2) these rearrangements always result in a change in energy, and 3) in this case, the fact that energy (heat) was released tells us that glucose has more energy than the products (CO2 and alcohol).

Another important take-away – the reaction only happens with the living (unboiled) yeast, not the dead (boiled) – so something in the living yeast made the reaction happen. If you have already discussed enzymes you can make that connection here, but if not, you can come back to it later.

It is also important to discuss why the reaction stopped. Hopefully at least some students will come up with the idea that all the glucose was rearranged, though many will think the yeast died or we "ran out of yeast". We need to facilitate the discussion until all agree on the former, but it's better if the idea originally came from students.

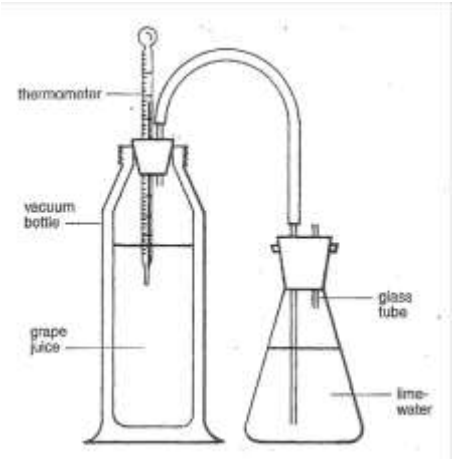
Another important point to address in discussion after the lab is the KIND of energy yeast cells can actually use. We have evidence that heat was released but can yeast use heat to do the work of living? Can humans? How you handle this will depend upon what you have or haven't discussed prior to this about ATP. Since we do this after cell respiration we have already introduced ATP, so we simply need to establish that in addition to heat, ATP was given off and this is what the yeast can actually use. If you haven't already built the cell respiration model or introduced ATP, this can provide a nice segue into the topic.

NUTS AND BOLTS: How to do it!

Because of the equipment and space involved, this lab is best done as a demonstration – but have students do the actually setting up! We set up a Flask A and Flask B with each class but due to equipment limitations we do just one Flask C (grape juice only) and have all classes share the data.

THE DAY BEFORE: Prepare all the of solutions (see Consumables on last page). Set up stations at front of the room for preparing the flasks. You'll need beakers for mixing yeast and juice together, graduated cylinders for measuring (phenol red, grape juice, yeast solution), and stir rods. You will also need 3 small (100 ml.) beakers and droppers into which you will put a small sample of each solution for students to observe under the microscope.

DAY 1 (one 55-minute period): We recommend enlisting teams of student volunteers to follow the directions and set up each flask as the rest of the class watches. The amounts below are for a 1-pint thermos, however shapes of thermoses vary so you will want to try it out ahead of time. Make sure that once the solution is in the thermos, the thermometer reaches INTO it, but the glass tubing that connects it to the PR flask does not. See Fig.1.



Flask A:
290 ml grape juice
50 ml boiled yeast solution

Flask B:
290 ml grape juice
50 ml unboiled yeast solution

Flask C:
340 ml grape juice

Before sealing each flask, pour a very small amount into a 100 ml beaker for microscopic examination later. Once all are set up, have the class come up closer and examine. Make sure they are convinced that no O_2 can get into the thermoses.

Also have them think about why we have the tube connecting to the PR flask, and why we need the open tubing in the PR flask (so it doesn't explode!). Our students have used PR before to indicate CO_2 but even if yours haven't, they can probably surmise that a gas may be formed. Let students come up with these ideas.

Have students read the starting temperatures in the 3 flasks and record. If you are going old-school (using thermometers) you need to set up a schedule of student volunteers to come in to read and record the temperatures every hour during school day over the next 48 hours. Even though there will be big gaps in the data during the overnight hours, the pattern will still be clear. If you are fortunate enough to have probeware (Fig. 4), set it to read the temp automatically every hour.

Once students have recorded the initial temperatures, have them observe a drop of each solution under the microscope. Caution students to stir each solution before taking a drop. Emphasize that they should pay attention to the size and density of yeast cells in the field. They may observe movement, so it's a good idea to tell them ahead of time that movement is irrelevant - yeast cells can't move under their own power. The water itself may move as a result of pressure on the cover slip, so it may appear that the yeast are moving, but really they are just along for the ride.

DAY 2 (about 15 minutes): Students record data from the previous 24 hours and observe the PR flasks to see if any have changed. Discuss whether or not there is any evidence of chemical change in any of the flasks. You may need to probe to get them to recall just what that might be. They should come up with 1) evidence of new substances formed and 2) evidence of energy change. The answer should be NO for A and C, but YES for flask B. The PR should have turned to yellow (evidence of a new substance) and the temperature has increased significantly (energy change). Since room temperature does have an effect there may be small fluctuations in A and C, so you need to address that here.

DAY 3 (one 55-minute period, if you allow time to work on graphs): Students record the remaining temperature data and final PR color. Open the flasks and pour small samples into 100 ml beakers w. droppers so students can examine under the microscope.

- When students come up to get their samples have the thermoses there so they can smell (by wafting) the contents. We also allow students to taste (if they are brave!) by putting one drop on their pinky finger. This provides evidence of an additional new substance formed (alcohol!).
- When finished with microscope observations, we let students work together to set up graphs and work on discussion questions. This enables us to troubleshoot students who need help with the

graphs, and also allows students a chance to process what they have observed by talking to classmates.

DAY 4: Collect lab and discuss. See "RATIONALE" for considerations to guide discussion.

EQUIPMENT AND SUPPLIES NEEDED (Per Class)

Fig 2

NOTE: List below is materials needed for one Flask A and one Flask B per class. Besides what is listed below, you need one set up (thermos, Erlenmeyer flask, stoppers, tubing, probe or thermometer etc.) for Flask C that will be shared by all classes.

- 2 one-pint narrow-mouth thermos bottles
- 2 #7 two-hole rubber stoppers for thermoses
- 2 - 125ml Erlenmeyer flasks
- 2 #5 two-hole rubber stoppers for flasks
- 2 thermometers (unless you are using Pasco probes or something similar) – should extend INTO the grape juice (Fig. 1)
- Glass tubing (see Figs. 2-4):
  - 2- 6mm x 14cm (long enough to extend into PR solution in 125ml flask)
  - 4 – 6mm x 6cm (should be ABOVE surface of liquid in each flask)

Fig 3

- rubber tubing:
  - 2 – ¼" x 16"

Optional:

- bubble wrap to wrap around each thermos for additional insulation
- 2 electronic data collection devices (like Pasco Sparks – Fig. 5) with thin temp probe.

Fig 4

Consumables (amounts per class):

Grape juice – about 1 qt

Phenol red – 75 ml

Preparation: dissolve .1g powder in 1 L distilled water to make stock solution. Dilute for lab: 7 tap water: 1 stock soln.

Yeast - 1 package Active Dry (not "rapid rise")

Advance preparation: Prepare the night before so boiled and unboiled solutions will be at the same temp the day of the lab. Mix each package of yeast with about 100ml tap water. Set aside a little more than half of this to be used for Flask B ("unboiled yeast"). Add about 25 ml more water/package to the remaining yeast solution (because some boils off) and boil it 15-20 minutes.

Fig 5