Activity 6: Test Ability of Mushroom Extracts to Increase Reaction Rate

Cellobiase that breaks down the 1,4 β-glucoside linkages in cellobiose is produced by many organisms. Fungi, such as molds, yeasts and mushrooms, produce this enzyme and can excrete it to digest cellobiose to produce glucose for energy usage. Many bacteria also contain cellulytic enzymes and cellobiase to break down plant cell walls. These bacteria can be found in the second stomach (rumen) of many hoofed animals such as cows and also in the gut of termites. Cellobiase can also be found in the seeds of bitter almonds where it is known as emulsin. Emulsin is actually thought to be a combination of cellobiase and other enzymes. In this activity, you will choose a potential source of cellobiase, extract proteins from this source, and take this extract and combine it with the substrate, *p*-nitrophenyl glucopyranoside, to determine if your extract has any enzymatic activity that allows it to break down the substrate.

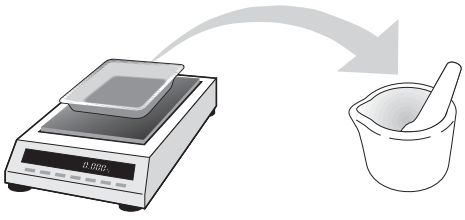
Student Workstation	**Quantity**	**(✔)**
Mushroom sample	1	❒
1.5 mM substrate	1	❒
Stop solution	1	❒
1x extraction buffer	1	❒
15 ml conical tube	1	❒
1.5 ml microcentrifuge tube	1	❒
DPTPs	4	❒
Cuvettes	6	❒
Marker	1	❒
Mortar and pestle	1	❒
Filter paper, cheese cloth, or strainer	1	❒
Beaker with deionized or distilled water to rinse DPTPs	1	❒
Stopwatch or timer	1	❒

Instructor's Workstation (Optional)	**Quantity**	**(✔)**
Spectrophotometer	1	❒

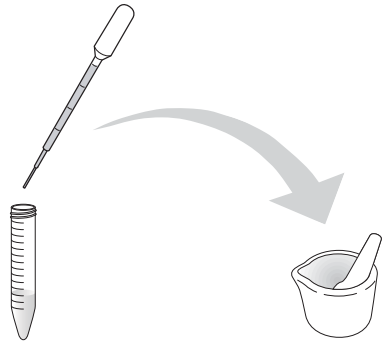
Protocol

1. Write down the name of your mushroom _____

2. Weigh out approximately 1 g of your mushroom and place it in a mortar. _____g



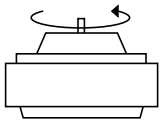
3. Add 2 ml of extraction buffer for every gram of mushroom into the mortar. _____ml



Extraction buffer

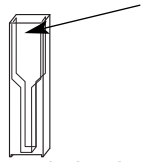
4. Using a pestle, grind your mushroom to produce a slurry.

5. Strain the solid particles out of your slurry using a piece of filter paper or cheese cloth into a 1.5 ml microcentrifuge tube. Alternatively, if you have a centrifuge, scoop the slurry into a 1.5 ml microcentrifuge tube and then pellet the solid particles by spinning at top speed for 2 minutes.

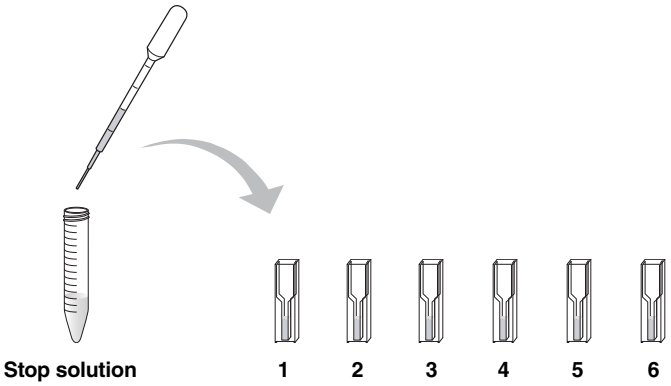


Note: You will need at least 250 µl of extract to perform the enzymatic reaction.

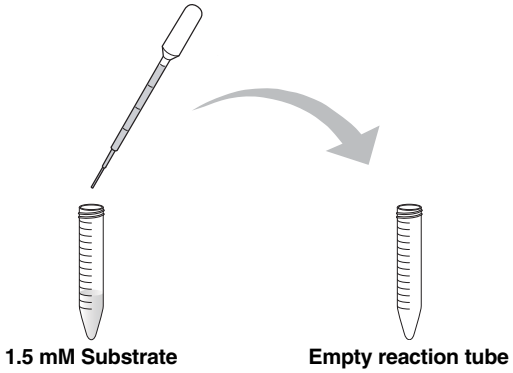
6. Label your cuvettes "1–6". Only label on the upper part of the cuvette face.



7. Using a clean DPTP, pipet 500 µl of stop solution into each cuvette. Rinse out the DPTP thoroughly with water.

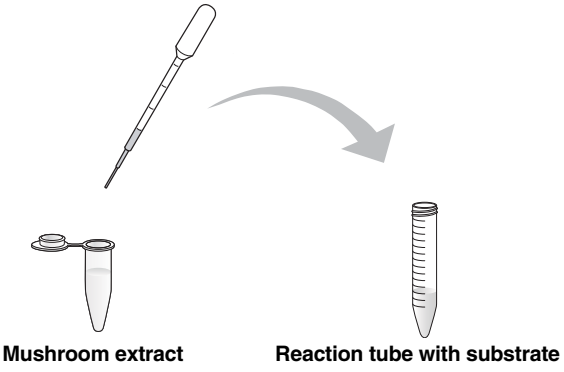


8. Label a 15 ml conical tube with the type of mushroom you are using. Using a clean DPTP, pipet 3 ml of substrate into the tube.

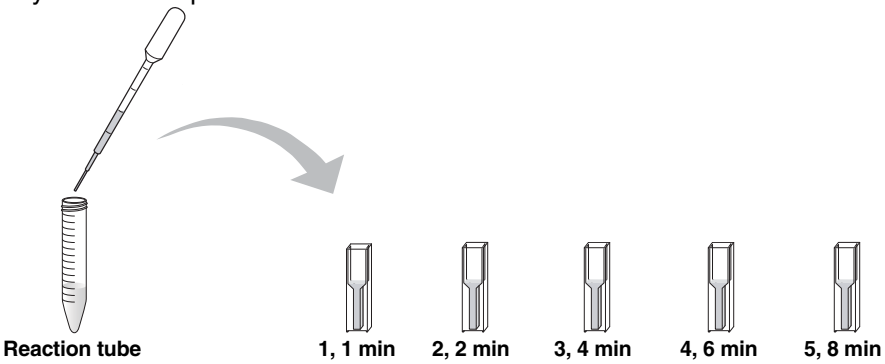


Please read and understand steps 10–11 fully before proceeding. These steps are time sensitive!

9. Using a clean DPTP, pipet 250 µl of your mushroom extract into the 15 ml conical tube containing 3 ml of substrate. **START YOUR TIMER**.

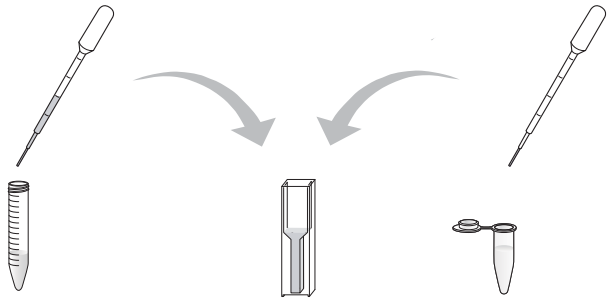


10. At the times indicated in the table below, remove 500 µl of mushroom extract/substrate mixture from the 15 ml conical tube, and add it to the appropriately labeled cuvette that already contains stop solution.



Time	Cuvette
1 min	1
2 min	2
4 min	3
6 min	4
8 min	5

11. Using a clean DPTP, add 500 µl of extraction buffer to cuvette #6. Clean the DPTP and then add one drop of enzyme extract. This will serve as the "blank" for this experiment.



12. Rinse out all DPTPs with copious amounts of water and save them for later activities. After you have finished your analysis, rinse out your reaction (conical) tubes and cuvettes with copious water and save them for later activities.

Note: Do not discard the unused stock solutions or cuvettes containing standards. They will be used for the next activity.

Quantitative Analysis of the Amount of Product Formed at Different Substrate Concentrations

1. Blank your spectrophotometer with the blank (cuvette #6) at 410 nm. Measure the absorbance values for your five cuvettes and record the absorbance values in column 2 of Table 17.

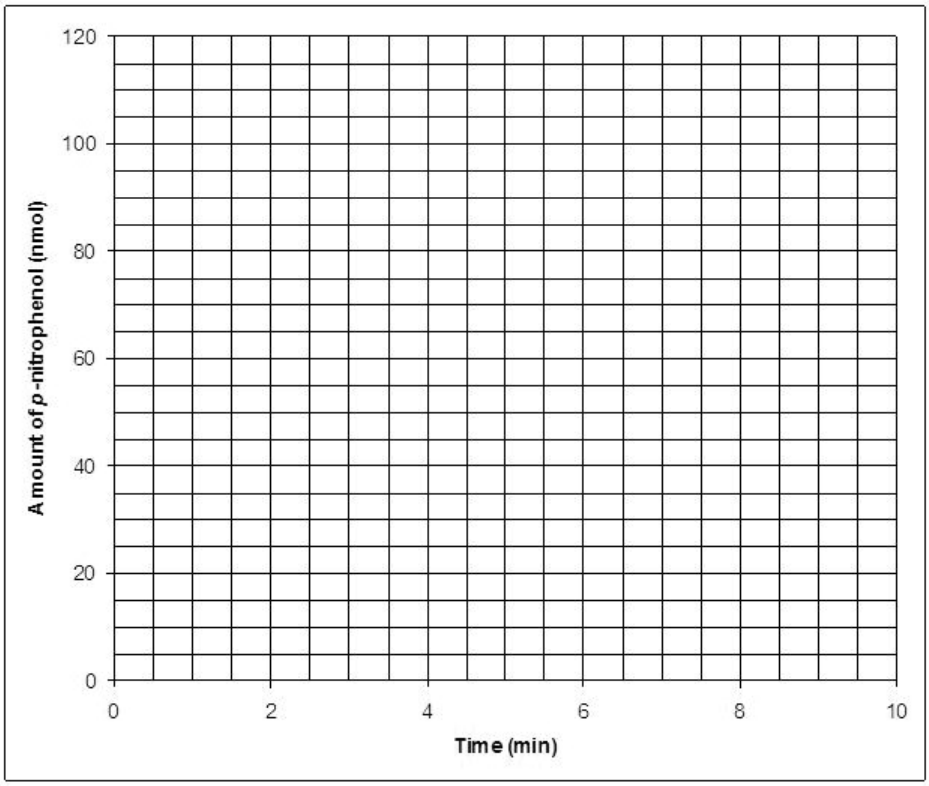
2. Using the protocols you learned in Activity 1, calculate the amount of *p*-nitrophenol formed in all of your samples and record it in column 3 of Table 17.

Table 17. Determination of *p*-nitrophenol produced by the mushroom extract breaking down the substrate based on a standard curve (similar to the one shown in Figure 8).

Cuvette	Absorbance at 410 nm	Amount of *p*-Nitrophenol Produced (nmol)
1		
2		
3		
4		
5		
6	0.00	

Analysis of Results

1. Plot the amount of product produced over time by your mushroom extract on the graph below.



2. Calculate the initial rate of reaction for mushroom extract

Initial rate of reaction = _____ nmol/min

Activity 6 Analysis Questions

1. Did your mushroom extract break down the substrate (that is, produce any yellow product)?

2. Why did we use a blank for this experiment that was different from the one used in earlier experiments? **Hint**: What would be the effect on your absorbance readings if a mushroom naturally had some yellow color to it?

3. Compare the initial rate of reaction of your mushroom extract to the enzyme included in this kit. From what you have learned about the effect of pH, temperature, and enzyme concentration, can you explain some factors that might influence your enzyme extract's initial rate of reaction?

4. Scientists are constantly looking for sources of enzymes that can be used in industrial processes. If you were going to pick a source of cellobiase for ethanol production for biofuels, what type of organism might you look for as a source of this enzyme? **Hint**: The production of glucose to be converted to ethanol in biofuel production requires the reactions to occur at high temperatures and low pH.