Methods/Procedure:

Preparing the Spectrometer for kinetics experiments

Start the LoggerPro software

Calibration

1. Click on Experiment then Calibrate then Spectrometer

The Calibration dialog box will display the message

"Waiting __ seconds for lamp to warm up…"

A. While the lamp is warming up, pipet 2 mLs of pH 7 buffer into an empty cuvette. Pipet 0.5 mL turnip extract into this cuvette. Pipet up and down a few times to mix thoroughly.

2. After warmup is complete, place the cuvette in the spectrometer.

3. Click Finish Calibration

4. Click OK

Configuring the Data Collection for a Kinetics Experiment

1. Click on the Configure Spectrometer icon (found in the toolbar, looks like a graph with rainbow colors below the graphed line). This should open a dialog box.

2. Under the Set Collection Mode area, click on Abs vs. Time.

On the right hand portion of the screen, under the title Select Wavelengths, select 490 nm.

3. Click OK to close the dialog box.

4. Click Experiment then Data Collection. The duration settings should be changed to 90 seconds.

5. Click Done.

Performing a Trial Run Enzyme Assay

1. With the sample used for calibration still in the spectrometer, click the play button to start the collection

2. Into the cuvette still in the spectrometer, pipet 1 mL substrate. Quickly pipet up and down to mix thoroughly.

3. Rinse out your pipet with water between assays (to avoid contamination).

4. When the assay is finished, highlight the steepest 30 seconds of the line. To do this, click and drag the cursor along the steepest 30 seconds of the line. The highlighted region should appear gray in color.

4. Click the Linear Fit icon (found on the toolbar, it shows a straight line with curved ends and has R= in the lower corner). A box will appear on the computer screen. The slope of this line will be indicated as m(Slope): The slope of the line measures Absorbance per second. Since we are interested in Absorbance per minute, multiply the slope by 60. Then record this number in Table I.

Because variation in sampling techniques occurs with each reaction, we like to perform the same experiment multiple times and average the results.

Take an empty cuvette and repeat the test for a second trial run. You do not need to save your graph after you have recorded your number on your table.

<u>Sugar effects on enzyme activity</u>

Preparing samples

Repeat the following steps 1 and 2 for each sugar concentration:
(5%, 10%, 15%, 20%, 25%, 30%)

1. Pipet 4 mL of the pH 7 buffer and 1mL turnip extract at your lab bench into 1 test tube.

2. For the sugar % solution, add 1 mL of that concentration to the tube from the step above. [% solution = (1/5)(% in g) / 20mL]

Measuring absorbance

3. Pipet 3 mL of the buffer/extract/sugar mix into an empty cuvette. Place the cuvette into the spectrometer.

4. Follow the directions from the start of lab to calibrate the spectrometer again.

(NOTE: you can skip the warmup this time)

5. After calibration is complete, pipet 1 mL of substrate into the cuvette. Quickly pipet up and down to mix thoroughly.

6. Click Collect. If you already have a graph on the screen, it will be necessary to click Erase and Continue.

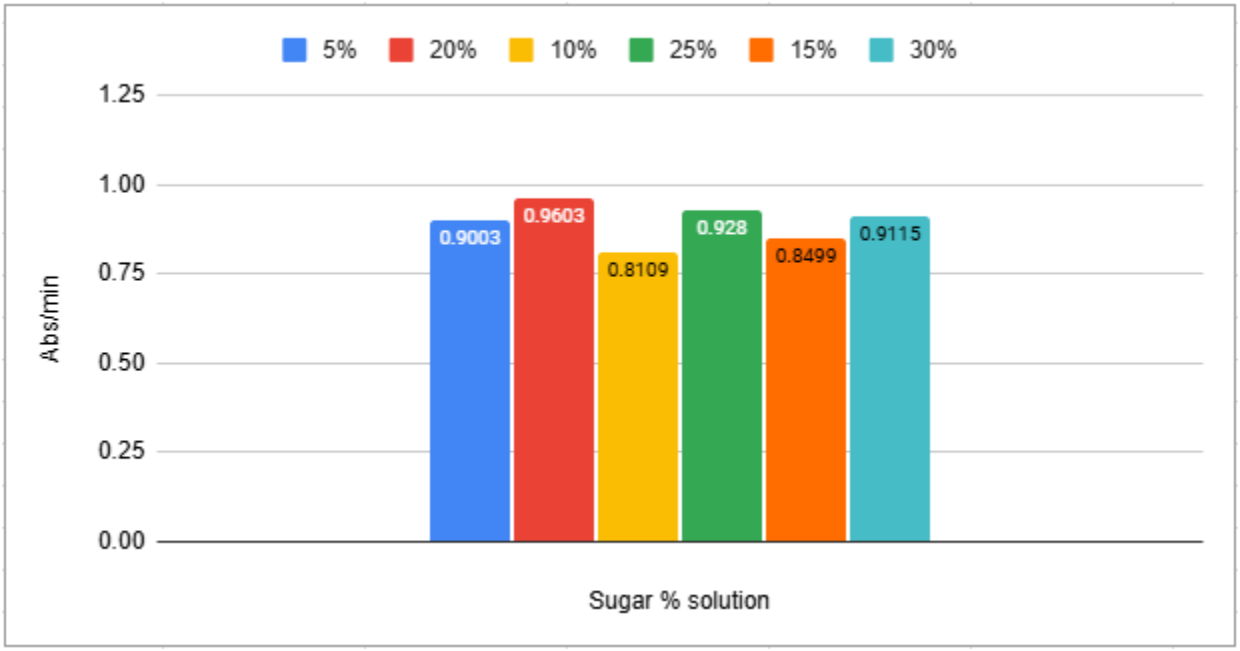
7. When the assay is finished, highlight the 30 second time period when the slope of the line is the steepest. Move the cursor to the start of this time point and drag the mouse to highlight this region. The highlighted region should appear gray in color.

8. Click the Linear Fit icon (found on the toolbar, it shows a straight line with curved ends and has R= in the lower corner). A box will appear on the computer screen. The slope of this line will be indicated as m(Slope): The slope of the line measures Absorbance per second. Since we are interested in Absorbance per minute, multiply the slope by 60. Then record this number in Table I for the sugar concentration.

9. Run each % solution again and record the number in Table I, then take the average of the two assays.

Sugar concentration	**Absorbance/minute**		
	First assay	**Second assay**	**Average of both assays**
5% solution			
10% solution			
15% solution			
20% solution			
25% solution			
30% solution			

Results (Graph):



Conclusion:

1. Summarize data

In our lab we found that for each percent sugar solution we did, each of their absorbance/min were within 0.1 of each other. This showed that increasing or decreasing the amount of sugar did not affect the enzymes activity and remained relatively constant for each sugar concentration. The full table of data can be seen below in Table 1.

Sugar concentration	Absorbance/minute		
	First assay	Second assay	Average of both assays
5% solution	0.8334	0.9672	0.9003
10% solution	0.837	0.7848	0.8109
15% solution	0.8532	0.8466	0.8499
20% solution	0.9330	0.9875	0.9603
25% solution	0.9192	0.9368	0.9280
30% solution	0.9108	0.9122	0.9115

Table 1

2. State the hypothesis (4 parts).

In this lab we will study the effects of sugar on enzyme activity. We will prepare 6 different percent solutions of a sugar solution starting at 5% and stepping up by 5% each time. We'll combine the ph 7 buffer and our sugar solutions then add substrate to it and record on logger pro what happens in terms of enzyme activity for each. After recording each then we can make a graph or chart to represent visually how the different percent solutions of sugar affected the enzyme activity. We expect that increasing the sugar concentration will decrease the enzyme activity, because it will act as a competitive inhibitor.

3. Does your data support or not support your hypothesis? Why or why not?

Our data did not support our hypothesis. We expected the sugar to act as a competitive inhibitor and decrease enzyme activity, but our results shows that there was

no effect on enzyme activity for differing sugar concentrations. The lowest concentration and the highest were about the same.

4. Draw inferences

It would seem, based on our data, that changing the concentration of sugar does not effect enzyme activity.

5. Identify things that went good and things that went bad with your experiment. How would you change your experiment to make it better?

Things that went well for our experiment were that we were able to correctly create the different concentrations of sugar solutions and measure the absorbance/min of each after adding the substrate. Despite our hypothesis being incorrect, we did correctly execute the experiment and were able to show how our hypothesis was incorrect. To make this experiment better, we could change it to include a baseline to measure adding sugar against. Meaning to include a test tube with no sugar added to see what adding sugar does as well as what changing the concentration did.

Look a Little Deeper

1. What is another experiment that could utilize a spectrometer? What are spectrometers used for outside of BIO 180 Lab?

Another experiment that could utilize a spectrometer could be measuring the concentration of bacteria cells in a sample.

Outside of BIO 180 Lab, a couple examples of what spectrometers can be used for is in astronomy by telling different things about stars and planets or environmental things like measuring pollution in water or air.