

LABORATORY REPORT

Activity: Enzyme Activity

Name: Student

Instructor: Instructor

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Predictions

1. Sucrase will have the greatest activity at

pH 6

2. Sucrase will have the greatest activity at

40 °C (104 °F)

3. Sucrase activity

increases with increasing sucrose concentration until a plateau is reached

Materials and Methods

Effect of pH on Enzyme Activity

1. Dependent Variable

amount of product (glucose and fructose) produced

2. Independent Variable

pH

3. Controlled Variables

temperature, amount of substrate (sucrose) present, sucrase + sucrose incubation time

Effect of Temperature on Enzyme Activity

1. Dependent Variable

amount of product (glucose and fructose) produced

2. Independent Variable

temperature

3. Controlled Variables

pH, amount of substrate (sucrose) present, sucrase + sucrose incubation time

Effect of Substrate Concentration on Enzyme Activity

1. Dependent Variable

amount of product (glucose and fructose) produced

2. Independent Variable

amount of substrate (sucrose) present

3. Controlled Variables

temperature, pH, sucrase + sucrose incubation time

4. Describe what is measured as an indicator of sucrase activity and why this is an indicator of sucrase activity.

The amount of product (glucose and fructose) formed is used as an indicator of sucrase activity. The higher the sucrase activity the more product formed.

5. Explain why denatured sucrase was used as a control.

Denatured sucrase is inactive and cannot catalyze the chemical reaction sucrose to glucose and fructose. Therefore amount of product formed.

6. Addition of DNS at the end of the incubation period stopped the reaction by denaturing sucrase. Explain why it is important to denature sucrase before measuring product concentration.

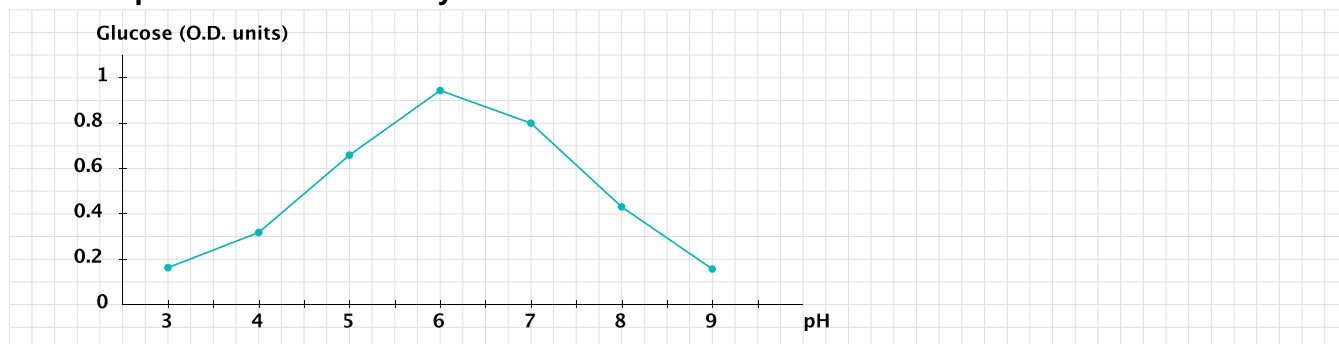
By denaturing sucrase, DNS can be used to stop the chemical reaction at a specific incubation time. This enables comparison of amount of product produced under different conditions.

Results

Table 4: Effect of pH on Sucrase Activity

	Optical Density						
	ph 3	ph 4	ph 5	ph 6	ph 7	ph 8	ph 9
1	0.152	0.320	0.645	0.931	0.820	0.428	0.150
2	0.178	0.323	0.670	0.951	0.767	0.427	0.156
3	0.159	0.311	0.661	0.951	0.814	0.438	0.166
average	0.163	0.318	0.659	0.944	0.800	0.431	0.157

Effect of pH on Sucrase Activity



1. State the optimum pH for sucrase activity and describe how sucrase activity changes at more acidic and more alkaline pH values.

Optimum sucrase activity occurs around a pH of 6. Activity of sucrase decreases as pH becomes more acidic or more alkaline than a pH of 6.

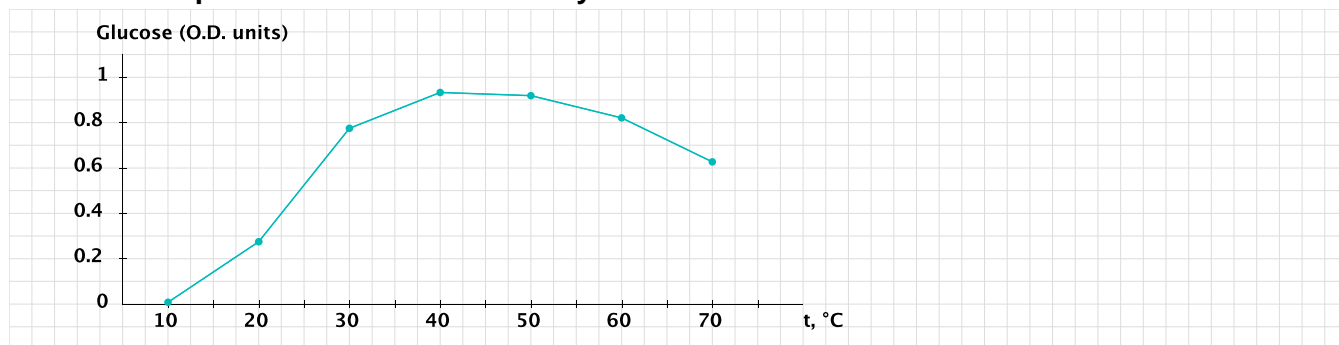
2. Was the rate of increase of sucrase activity higher at a pH of 8.5 or a pH of 5.5?

Sucrase activity increased more above a pH of 5.5 and then decreased above a pH of 6.

Table 5: Effect of Temperature on Sucrase Activity

	Optical Density						
	10 °C (50 °F)	20 °C (68 °F)	30 °C (86 °F)	40 °C (104 °F)	50 °C (122 °F)	60 °C (140 °F)	70 °C (158 °F)
1	0.006	0.254	0.759	0.946	0.902	0.818	0.610
2	0.009	0.279	0.776	0.925	0.947	0.803	0.646
3	0.009	0.291	0.790	0.929	0.909	0.841	0.626
average	0.008	0.275	0.775	0.933	0.919	0.821	0.627

Effect of Temperature on Sucrase Activity



3. State the optimum temperature for sucrase activity and describe how sucrase activity changes at lower and higher temperatures.

Optimum sucrase activity occurs around 40 °C. Activity of sucrase decreases as the temperature decreases below 40 °C or increases above 40 °C.

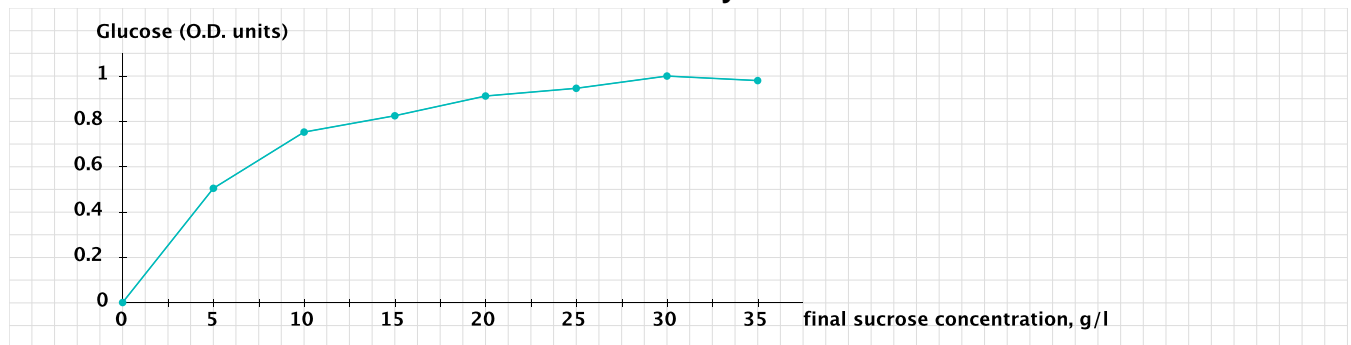
4. Was sucrase activity higher at 25 °C or 55 °C?

Sucrase activity was higher at 55 °C

Table 6: Effect of Sucrose Concentration on Sucrase Activity

	Optical Density							
	35 g/L	30 g/L	25 g/L	20 g/L	15 g/L	10 g/L	5 g/L	0 g/L
1	0.973	1.003	0.950	0.912	0.827	0.745	0.496	0.001
2	0.984	1.003	0.944	0.901	0.823	0.757	0.517	0.001
3	0.984	1.009	0.945	0.924	0.826	0.756	0.503	0.003
average	0.980	1.000	0.946	0.912	0.825	0.753	0.505	0.002

Effect of Sucrose Concentration on Sucrase Activity



5. State how sucrase activity changes with increasing sucrose concentration.

Sucrase activity increases with increasing sucrose concentration until a plateau is reached at about 30g/l final sucrose concentration.

6. Was the rate of increase of sucrase activity greater when sucrose concentration went from 2.5 to 7.5 g/l or when it went from 22.5 to 27.5 g/l?

The rate of increase of sucrase activity was greater when sucrose concentration went from 2.5 to 7.5 g/l

pH Along the GI Tract

Organ	mouth	stomach	duodenum (first section of small intestine)	jejunum (second section of small intestine)	ileum (third section of small intestine)	colon
pH	6.35 - 6.85	1 - 3	6	7.5	7.5	7.5 - 8

Discussion

1. Referring to the table above, specifically state where in the intestine sucrase is likely most active.

Since the optimum pH for sucrase is 6.0, sucrase is likely to be most active in the duodenum.

2. Compare optimal temperature for sucrase activity to body temperature.

The optimal temperature for sucrase activity occurs at 40 °C which is a little higher than normal body temperature.

3. How would a slight fever affect sucrase activity?

Because sucrose is more active slightly above normal body temperature, a slight rise in temperature due to fever should increase sucrose activity.

4. Explain how pH of intestine and temperature of body together affect enzyme activity.

Body temperature will not change along the G.I. tract but as the pH in the intestine becomes more alkaline, sucrose activity will decrease

5. Explain how increasing amount of sucrose in food affects sucrase activity.

As the sucrose content of food increases, the ability of sucrase to break it down increases until the amount of sucrose saturates the enzyme (enough sucrose molecules present to bind to active site of all sucrose molecules). Since the body absorbs glucose and fructose through the small intestine but not sucrose, increased sucrase activity enables the body to absorb more glucose and fructose as the sucrose concentration in the intestine increases.

6. In the results section you were asked if rate of increase of sucrase activity was greater when sucrose concentration went from 2.5 to 7.5 g/l or when it went from 22.5 to 27.5 g/l. Explain why the rate of increase was greater for this range.

Sucrase was more effective at the lower sucrose concentrations because there was more unoccupied active sites on the sucrase molecule. At the higher concentrations many of the active sites were occupied.

7. In this experiment, if the sucrose concentration were increased to 70 g/l would you expect sucrase activity to be significantly higher than the activity at 35 g/l. Explain your answer.

Since the activity of sucrase levels off at the higher sucrose concentrations, then it would be predicted that sucrase activity would not change at even higher sucrose concentrations.

8. Restate your predictions that were correct and give the data from your experiment that supports them. Restate your predictions that were not correct and correct them, giving the data from your experiment that supports the corrections.

The answer to this question depends on students predictions. If predictions were erroneous they should be corrected to the following: Sucrase will have the greatest activity at pH 6. Sucrase will have the greatest activity at 40 °C. Sucrase activity increases with increasing sucrose concentration.

Application

1. Myosin ATPase is an enzyme that is involved in muscle contraction. Athletes do warm-up exercises prior to athletic performance. Explain why warm-up exercises increase myosin-ATPase activity.

The optimal temperature for most enzymes in the body is above normal body temperature. The increase in temperature that occurs during warm-up exercises brings body temperature closer to optimal temperature. Myosin ATPase breaks down ATP, releasing energy that is used for muscle contraction. Increasing myosin ATPase activity increases energy available for muscle contraction.

Describe how increasing myosin-ATPase activity affects muscle contraction.

Increasing enzymatic activity would result in greater concentrations of ATP and therefore increased energy for muscle contraction.

2. Salivary amylase, an enzyme in saliva that breaks down starch, has an optimal pH of 6.7-7.0. Explain why salivary amylase is active in the mouth, but becomes inactive in the stomach.

Salivary amylase is most active at the typical pH values in the mouth (pH 6.35-6.85) but would decrease activity in the much lower pH values of the stomach.