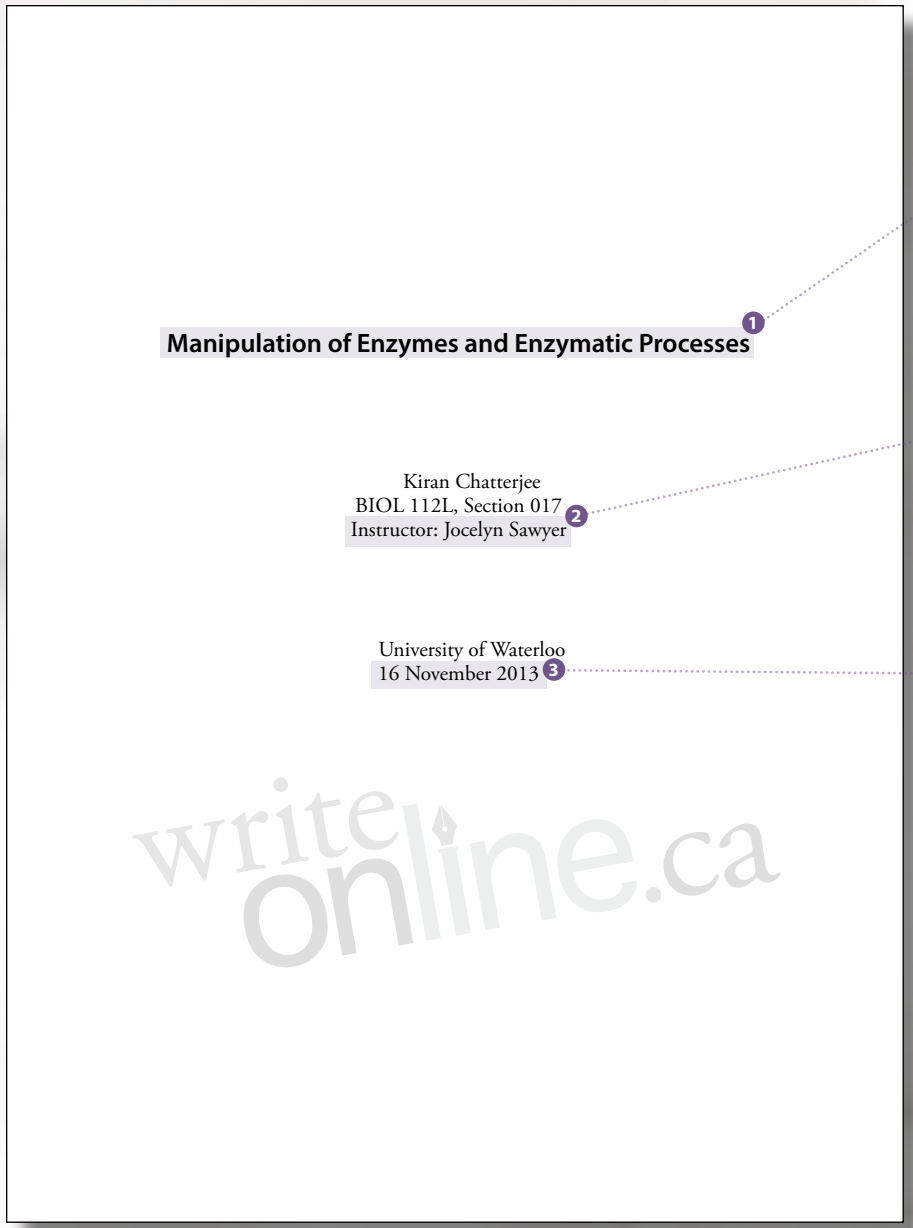
Annotated Lab Report



Manipulation of Enzymes and Enzymatic Processes 1

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University of Waterloo
16 November 2013 3

1 The title is a single line and written as a phrase.

2 The writer includes her name and the name of the instructor.

3 The date is included on the title/cover page.

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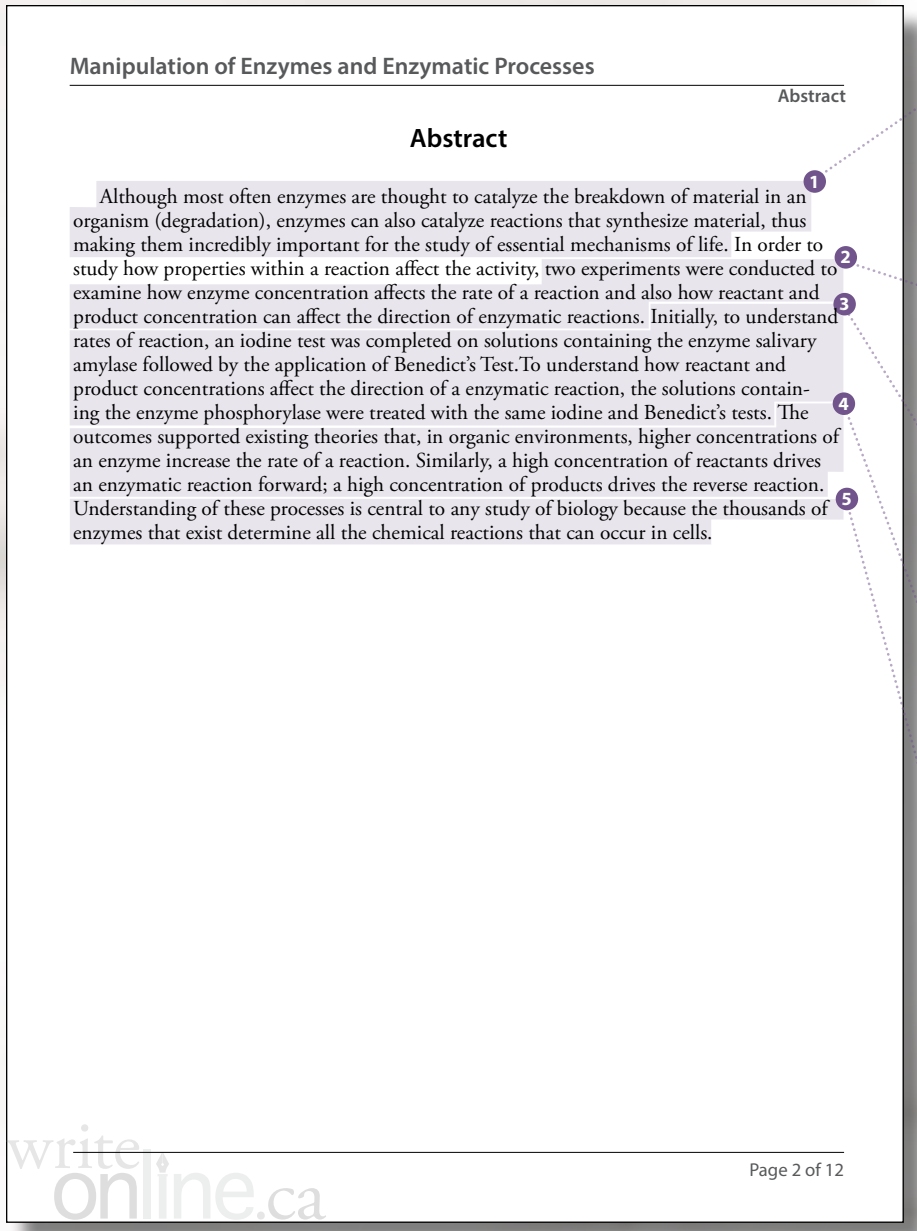

Manipulation of Enzymes and Enzymatic Processes

Abstract

Abstract

Although most often enzymes are thought to catalyze the breakdown of material in an organism (degradation), enzymes can also catalyze reactions that synthesize material, thus making them incredibly important for the study of essential mechanisms of life. In order to study how properties within a reaction affect the activity, two experiments were conducted to examine how enzyme concentration affects the rate of a reaction and also how reactant and product concentration can affect the direction of enzymatic reactions. Initially, to understand rates of reaction, an iodine test was completed on solutions containing the enzyme salivary amylase followed by the application of Benedict's Test.To understand how reactant and product concentrations affect the direction of a enzymatic reaction, the solutions containing the enzyme phosphorylase were treated with the same iodine and Benedict's tests. The outcomes supported existing theories that, in organic environments, higher concentrations of an enzyme increase the rate of a reaction. Similarly, a high concentration of reactants drives an enzymatic reaction forward; a high concentration of products drives the reverse reaction. Understanding of these processes is central to any study of biology because the thousands of enzymes that exist determine all the chemical reactions that can occur in cells.

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1. The first sentence presents the basic, theoretical background for the lab.
2. A second sentence highlights the lab's purpose.
3. The third and fourth sentences present the lab's objectives.
4. The fifth sentence presents the findings and their relationship to existing theories.
5. The last sentence clarifies the importance of the lab work.

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Introduction

Enzymes are an important protein in living organisms essential for the existence of life. Their role is to speed up chemical reactions that are the foundation bodily functions, including digestion, cell formation, and even waste disposal. Without enzymes these chemical reactions would occur too slowly to support life. Thus, understanding enzymatic reactions and how they affect chemical processes is crucial to better understanding how many of our bodily functions happen.

An enzymatic reaction refers to a reaction in which an enzyme acts as a catalyst (Alberts et al, 2014). An enzyme is a specialized protein that increases the rate of a specific chemical reaction by lowering the activation energy. Activation energy is the energy a molecule requires to begin a chemical reaction (Alberts et al., 2014). An enzymatic reaction occurs in two steps (Artioli, 2008). The enzyme first binds the substrate, a reactant, at its active site to form a substrate-enzyme complex (Artioli, 2008). The substrate-enzyme complex then reacts (Artioli, 2008). The binding provides better chemical conditions to activate the reaction and, in turn, lowers the activation energy (Artioli, 2008).

The purpose of this lab was to observe the effect of enzyme concentration on the reaction time of an enzymatic reaction, as well as the effect of the concentration of reactants and products on the direction of enzymatic reactions. Because an enzyme's role is to speed up a reaction, a useful hypothesis is that providing a greater concentration of an enzyme to a substrate (reactant) should increase the rate of reaction. However, enzymes only act when they bind to a substrate (Beals, Gross, & Harrel, 1999). Thus, when the concentration of enzymes exceeds the amount of substrate, these "extra" enzymes cannot act as catalysts. At this "saturation" point, increasing the concentration of enzymes should not affect the rate of reaction (Beals, Gross, & Harrell, 1999).

Salivary amylase catalyzes the reaction, acting on starch as the substrate [the other reactant] (Barrass, 1981). During the reaction, the alpha-1, 4 linkages between glucose units in starch are hydrolyzed (Sanderson & Walker, 1999) to form units of maltose, a disaccharide and reducing sugar (Rostogi, 2005). This maltose becomes a source of energy for the body. The aforementioned reaction occurs in the forward direction (meaning that the reactants, water and starch, collide to produce products) and is written as follows (Barrass, 1981).

By using different concentrations of salivary amylase, the effect of enzyme concentration on the reaction time can be observed. To confirm the presence of starch, a positive iodine test shows a change of colour, from blue to black (Harisha, 2006). The reaction's end point is confirmed by a negative iodine test result, shown by the solution remaining the yellow colour of the original iodine solution (Harisha, 2006). Following these tests, a positive Benedict's test confirms the presence of maltose, a reducing sugar (Kumar, 2007). The initial solution for Benedict's test is blue in colour. A precipitate ranging in colour from green, yellow, brown to red then indicates the presence of maltose. If the solution remains the original blue colour

1. In-text citations appear throughout the report, especially in the introduction, discussion, and conclusion sections.
2. Writer discusses scientific concepts and background information for the lab.
3. Refers to previous research on the subject.
4. Writer presents the purpose of the lab.
5. Writer includes reasons for her hypothesis.

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of Benedict's solution, the test for presence of maltose is negative, meaning a reaction did not occur (Toole & Toole, 2004).

Another enzyme used for this experiment was phosphorylase, an enzyme found in plants that, like salivary amylase, also degrades starch (BeMiller & Whistler, 2009). The enzyme is crucial for a phosphorolysis reaction to occur. During this type of reaction (which is analogous to hydrolysis) phosphoric acid, rather than water, acts as a reactant to break down complex starch molecules into simpler subunits of glycosyl (Brody, 1999). The enzyme phosphorylase catalyzes a reaction between the starch and inorganic phosphate to remove single glucosyl units from the starch (BeMiller & Whistler, 2009).

Similar to rates of enzymatic reactions, concentrations of the substrate and the products of an enzymatic forward reaction should also have an effect on the reaction. In this case, following theories of degradative and synthetic reactions, a useful hypothesis is that an excess of product will encourage the building of substrate (synthesis); in contrast, an excess of reactant will encourage the breakdown of the substrate (degradation). By using different concentrations of starch, either in excess or in the primer form, the effect of the concentrations of reactants and products on the direction of the enzymatic reaction can be observed. Similar to the experiment with salivary amylase, an iodine test can confirm the presence of the longer starch – the synthetic reaction. In this case, the solution colour changes from yellow to blue-black (Harisha, 2006). A negative iodine test, shown by a yellow colour, confirms the presence of the shorter starch (starch primer) – the degradative reaction (Harisha, 2006).

6 Writer presents hypothesis for the experiment.

7 Writer details the lab's objectives (what will occur in the lab).

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Methods and Materials

The following description of the methods and materials for two experiments, salivary amylase and phosphorylase, can be found on pages 39 to 45 of the fall 2014 Biology 130 [Biol 130L], (Department of Biology, 2014) lab manual for the materials and procedures of this lab. All steps were followed with no deviations.

Note: The steps listed in this section have been intentionally shortened for the purposes of demonstration. An actual Lab Report would contain more accurate detail.

Salivary Amylase.

The following steps were followed to conduct the salivary amylase experiment.

Step 1: preparing test tubes and beakers. In the first step for this experiment, test tubes and beakers were labelled to ensure accurate identification. Twenty test tubes were labelled #1 through #20; two 50ml beakers were labelled #1 and #2; one 100ml beaker was labelled #3; and two 250ml. beakers were labelled #4 and #5. After all equipment was labelled, water was added to the beakers by, first, filling 200ml. of tap water into beaker #5 and then transferring (with the use of measuring cylinders) the beaker #5 water into the other beakers as follows: 9ml in beaker #1, 19ml. in beaker #2, 49ml. in beaker #3, and 99ml. in beaker #4.

Step 3: administering the iodine and Benedict's tests on a starch suspension control. In test tube #5, 2 ml. of a 1% (0.25% NaCl) starch suspension was added. Using a new spot plate, the iodine test was carried out followed by the Benedict's test (as prescribed in step 3). The results of each test were recorded.

Step 4: preparing test tubes for reaction rate tests. Using a 10ml graduated cylinder, 2ml. of water from beaker #5 was placed in test tube #10. Then, 2ml. each of 1%, 2%, 5%, and 10% salivary amylase solutions were placed in test tubes #9, #8, #7, and #6, respectively. To test tubes #11-#15 were added 2ml. each of the 1% starch solution and McIlvaine's buffer (to maintain an optimal pH). Once test tubes #6-#15 were prepared, they were placed in a rack and then in a 37 degree water bath and left for 5 minutes. During this time, two spot plates were prepared by adding one drop of iodine solution to the wells.

Phosphorylase.

Step 1:pre-lab preparation. The following activities were prepared by teaching assistants for the labs. Six hundred grams of potatoes were peeled for 32 students. The potatoes were cubed and then, using a blender, the potatoes were homogenized with 400ml. of .01N sodium fluoride. The mixture was filtered through a cheesecloth and then centrifuged at high speed for 5 minutes. The mixture was given an iodine test to ensure that no starch from the potatoes was transferred with the enzyme.

Step 2: prepare test tubes. In order to assess the effects of varying concentrations of substrate and product on the enzymatic reaction, various test tubes were prepared with different combinations of substrate, reactant and product. Eight clean test tubes were labelled #1 to #8. In test tube #8 was placed 4ml. of fresh phosphorylase.

1. Headings and subheadings help to organize the methods described in the lab.
2. Materials are introduced as each step in the method is presented.
3. Important details are included to ensure repeatability.

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Results

The following tables and figures show the results for tests performed on the two enzymes, salivary amylase and phosphorylase. In Part A, the results of the iodine test and Benedict's test on salivary amylase show the increased rate of reaction as the concentration of salivary amylase is increased. Part B presents the results of the iodine test performed on phosphorylase. These results indicate that synthesis using fresh phosphorylase requires a starch primer and glucose-1-phosphate. Boiled phosphorylase produced a synthesis only when an excess of starch and potassium phosphate were added.

Part A: Salivary Amylase Results. Table 1 shows the results of the initial iodine and Benedict's tests performed on control samples, varying percentages of salivary amylase concentrations. A positive result for the iodine test (starch is present) was a colour change ranging from violet to black; a negative result (no starch) was the yellow colour of the iodine solution.

Iodine and Benedict's Test Results for Control Salivary Amylase Solutions

Test Tube # / Solution	Appearance for Iodine Test	Iodine Test (+/-)	Appearance for Benedict's Test	Benedict's Test (+/-)
10% Salivary Amylase	Solution remained yellow colour of iodine solution.	-	Orange brown precipitate formed.	+
5% Salivary Amylase	Solution remained yellow colour of iodine solution.	-	Green brown precipitate formed.	+
2% Salivary Amylase	Solution remained yellow colour of iodine solution.	-	Solution remained blue colour of Benedict's solution.	-
1% Salivary Amylase	Solution remained yellow colour of iodine solution.	-	Solution remained blue colour of Benedict's solution.	-
1% Starch Suspension	Blue-black colour change occurred.	+	Solution remained blue colour of Benedict's solution.	-

Table 2 illustrates the results of the iodine test at different time intervals after mixing an amylase solution of varying concentrations with a 1% starch suspension. It is important to note that, within the contents of each, 2ml of McIlvaine's buffer was added to maintain an optimal pH for the enzymatic reaction. A blue-black colour change (a positive result) suggests the presence of starch. A yellow colour, or negative result, indicates a lack of starch in the solution and, thus, the completion of the reaction. Table 2 shows that the reaction time – the time needed for starch to be degraded – decreased as the concentration of salivary amylase increased.

1. Summary of the overall findings.
2. Tables and figures are numbered and have clear, descriptive titles.
3. Introductions to tables and graphs highlight important observations.
4. Tables include clear headings.

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Results of the Iodine Test at Differing Time Intervals After Mixing (Tn)

Test Tube Combination	Solution Mixed with 1% Starch Solution and McIlvane Buffer	Reaction Time When Iodine Test Turned Negative (s)
#9 - #14	1% Salivary Amylase	660 seconds
#8 - #13	2% Salivary Amylase	300 seconds
#7 - #12	5% Salivary Amylase	135 seconds
#6 - #11	10% Salivary Amylase	55 seconds
#10 - #15	Water	N/A

Figure 1 shows the downward slope of change for increasing concentrations of salivary amylase. Time is plotted on the ordinate; the concentration of salivary amylase in the solution is on the abscissa. Note that as the concentration increased, the time needed to complete the reaction decreased.

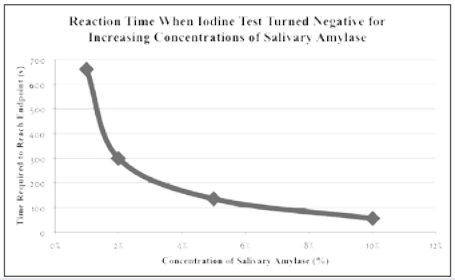

Figure 1 Reaction time for solutions with increasing concentration of salivary Amylase (%)

The final step was to apply Benedict's test to the different concentrations of salivary amylase mixed with the 1% starch solution. When Benedict's test is applied, a green, yellow, orange, red or brown solution indicates a positive test and the presence of reducing sugars. If the solution remains blue, the test is negative, indicating the absence of these sugars. It is important to note that the same percentages of salivary amylase were used in the Benedict's test as were used for the iodine test (shown in Table 2). Table 3 presents the results of the Benedict's test on these solutions. Table 3 reveals that only one solution – the tube without any salivary amylase – tested negative for reducing sugars.

5 On figures, axes are clearly labelled.

6 Tables and figures are numbered and have clear, descriptive titles.

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Discussion

Chemical reactions within cells are aided by enzymes that increase the rate at which reactions take place (Alberts et.al, 2010 p.90). Enzymes are biological catalysts that work by binding themselves to substrate molecules and lowering the activation energy of a reaction (Alberts et.al, 2010 p.90). Enzymes are highly specific and highly efficient which make them essential for life as it exists (Wiseman, 1971, p.31). The fundamental purpose of this lab was to examine how enzyme concentration affects the rate at which an enzymatic reaction takes place. It was also our purpose to examine how the concentration of a substrate, a product and an enzyme can affect the direction of an enzymatic reaction.

Salivary Amylase.

First experiment. In the first experiment conducted, the authors used salivary amylase to examine how changes in concentration of this enzyme affect the rate of reaction with starch. Salivary amylase is a digestive enzyme found in saliva, which degrades starch by breaking off maltose molecules. This enzymatic reaction requires the consumption of water molecules. Thus, the reaction is called a hydrolytic reaction and undergoes a process called hydrolysis.

To determine the presence of starch in a substance we relied on the iodine test for starch and glycogen. Five solutions, 10% salivary amylase, 5% salivary amylase, 2% salivary amylase, 1% salivary amylase and 1% starch solution, were initially tested for the presence of starch and maltose through the iodine test. Varying concentrations of salivary amylase -- 10%, 5%, 2%, and 1% solutions -- all had a negative result for the initial iodine test, indicating an absence of starch. These outcomes were predicted, as no starch elements were introduced in these solutions. Salivary amylase is an enzyme, a specialized protein (Sanderson & Walker, 2009). It does not contain starch.

However, a 1% starch solution had a positive result for the initial iodine test. A 1% starch solution does contain starch; thus the positive result (a black-purplish colour) was expected. The color change that occurs when iodine is administered to solutions containing starch is caused by the reaction between amylose, a component of starch, and iodine. Iodine is not very soluble in water and it is made soluble by dissolving the iodine reagent in water in the presence of potassium iodide (UC Davis, 2003). This soluble iodine forms a trioidide ion complex and will form a dark purple color when it coils with amylose in starch (UC Davis, 2003). Starch is made up of 20% amylose and 80% amylopectin (UC Davis, 2003).

1. Explanation and significance of the findings.
2. Summary of the lab's purpose.
3. Summary of the findings, expected and unexpected.
4. In-text citations appear throughout the report, especially in the introduction, discussion, and conclusion sections

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Second experiment. In a second control experiment, the authors used Benedict's test to determine the presence of reducing sugars in a solution of salivary amylase. (Kumar, 2007). The presence of reducing sugars would indicate the completion of the enzymatic reaction with starch. The authors added 4ml of Benedict's solution to four test tubes containing only varying concentrations of salivary amylase in water -- 10%, 5%, 2%, and 1% solutions, respectively. Similar to the iodine test, a fifth test tube contained no salivary amylase but instead a 1% starch solution. The authors predicted that none of the solutions of salivary amylase would have a positive Benedict's test because no source of reducing sugars was present in the test tube. Salivary amylase is a specialized protein, and Benedict's solution consists of cupric sulphate, sodium citrate, and sodium carbonate (Chatterjae & Shinde, 2012). However, the 10% and 5% salivary amylase solutions did have positive results (for the 10% solution, an orange brown precipitate formed, and a green-brown precipitate formed for the 5% salivary amylase solution). These results were not expected.

These positive results likely occurred because the authors used a manufactured version of the enzyme salivary amylase. This manufactured enzyme contained lactose, a reducing sugar (Kumar, 2007), in trace amounts. However, since the enzyme contained only trace amounts of lactose, only the solutions with a high concentration of salivary amylase had a positive result, indicating the presence of the sugar. Thus, the other two solutions with lesser concentrations of salivary amylase, 2% and 1%, respectively, tested negative, indicating no presence of reducing sugars.

Third experiment. To determine the effect of varying concentrations of the enzyme on the rate at which starch degrades to maltose, the authors again applied the iodine test. Varying concentrations of salivary amylase in water (to instigate hydrolysis) -- 10%, 5%, 2%, 1% -- were mixed with a 1% starch solution, and a McIlvane Buffer. The buffer was added to maintain the appropriate pH for the enzymatic reaction. The authors expected that the salivary amylase would act on the starch, the substrate, to catalyze the reaction to form maltose (Sanderson & Walker, 2009). Thus, at the end of the reaction, no starch should be present. All solutions had an initial positive iodine test, as starch was present in the 1% starch solution. As time progressed, the solutions turned yellow, indicating the absence of the original starch, as expected. The solutions of starch had been hydrolyzed to maltose (Sanderson & Walker, 2009).

5 Comparison with original hypothesis and theories.

6 A discussion of weaknesses of the experiment.

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Conclusion

In conclusion, the purpose of this experiment was to conduct two experiments in order to examine how enzyme concentration affects the rate of a reaction and also how reactant and product concentration can affect the direction of an enzymatic reaction. These goals were accomplished by conducting the salivary amylase experiment and phosphorylase experiment, respectfully.

The experiment with salivary amylase revealed that increasing the concentration of an enzyme decreased the time needed for the reaction to reach its end point. This understanding of how enzymes affect reactions in organic material, specifically how salivary amylase helps break down starch, is useful for better understanding of and treatment for a variety of conditions. Salivary amylase is most associated with the breakdown of starch in carbohydrates, making it a centerpiece of study for diabetes and obesity research. Additionally, salivary amylase also binds to bacteria in the mouth and on teeth, which has implications for dentistry, like excess plaque and the development of cavities in the teeth (Scannapieco, Torres, & Levine, 1993).

The experiment with phosphorylase also showed how further research into its properties has the potential to enhance medical research. The experiments confirmed that the direction of an enzymatic reaction depends on the concentration of the reactants and products (Starr, Evers, & Starr, 2011). A high concentration of reactants drives the reaction forward; a high concentration of products drives the reverse reaction (Starr, Evers & Starr, 2011). Additionally, factors such as temperature and pH level also impact whether or not a reaction occurs. The ability of phosphorylase to both degrade and synthesize starch has been a source of research for the development of a variety of treatments such treatments as phosphorylase inhibitors for type 2 diabetes, and treatments for fetal lung maturation (Rannels, Rannels, Sneyd, & Loten, 1991).

1. First paragraph summarizes the goals of the lab.
2. A summary of the outcomes of each experiment are given.
3. Writer notes the larger implications of the new knowledge.
4. In-text citations appear throughout the report, especially in the introduction, discussion, and conclusion sections.
5. A summary of the outcomes of each experiment are given.
6. Writer notes the larger implications of the new knowledge.

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References [1]

Alberts, B., Bray, D., Hopkin, K., Johnson, A., Lewis, J., Raff, M., & Walter, P. (2014). Essential cell biology (4th ed.). New York, NY: Garland Science.

Artioli, Y. (2008). Enzymatic processes. In Encyclopedia of Ecology (Vol. 1, p. 1377). Oxford, UK: Newnes.

Barrass, R. (1981). Human biology: Made simple. Great Britain: Elsevier. 111-112.

Please Note: References intentionally shortened for the purposes of this sample. A lab report must include all citations.

1 Full references are listed on a separate page, after the conclusion.

2 Full references are alphabetized according to the author's last name.

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Appendix A

Conservation of Mechanical Energy in An Oscillating Pendulum

All data was recorded in table 1 below.

Table 1

Conservation of Mechanical Energy in An Oscillating Pendulum

Time	Rate of change (R)	Vector vx	Vector x	Vector y	Vector yy	Velocity (v)	Potential Energy (U)	Kinetic Energy (K)	Mechanical Energy €
0.05	0.173	-0.281	-0.168	0.029	0.107	0.301	0.285	0.045	0.33
0.1	0.153	-0.156	-0.187	0.036	0.059	0.167	0.357	0.014	0.371
0.15	0.153	-0.041	-0.188	0.037	0.016	0.044	0.358	0.001	0.359
0.2	0.153	0.001	-0.187	0.036	-0.001	0.001	0.357	0	0.357
0.25	0.155	0.007	-0.185	0.036	-0.003	0.007	0.348	0	0.348

Please Note: References intentionally shortened for the purposes of this sample. A lab report must include all citations.

1. Each item in the appendix is placed on a separate page and labelled alphabetically.
2. The appendix item is given a title.
3. Tables and other figures in an appendix should be labelled clearly, as they would be inside the text of the report.