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Enzymes: Strange Brew – How Effective is Amylase?

Objective: to determine how quickly amalyse can break down starch

An important step in beer production is the breakdown of starch from grain into sugars that are used for fermentation. This process is called malting and companies generally use enzymes to help speed up this process. A local brewery found a cheaper source of amylase, a major enzyme in this production. This would help decrease the cost of production, but before using this, the brewery would have to figure out if amylase did break down the starch and how efficiently it worked.

My general approach was to create a blank using Tris and Lugol's reagent. To make a standard curve of concentration vs. absorbance a 1-2 dilution series will be made using a starch solution and Tris. This standard curve will determine how long it would take for a sample of amylase enzyme to metabolize starch into sugar. The absorbency of these dilutions will be tested with the spectrophotometer. A mixture of amylase and starch will be made and timed in order to stop reaction rates at certain intervals up to 5 minutes. Each test tube of starch and amylase will be tested for its absorbance and concentration determined. A concentration vs. time graph will then be made to find out the rate of reaction for how amylase breaks down starch.

First, we created a blank solution, negative control, in a test tube using 4mL of Tris and 1mL of Lugol's Reagent. We were then able to set the absorbance of the spectrophotometer using this blank as a calibration. There were three big test tubes so in the first one we put 15mL of starch and 15mL of Tris. Since we were given a 0.2g/L concentration and wanted a 0.1g/L concentration, we then diluted 2-fold with Tris. This came out to be; 4mL of this solution with 0mL of Tris, 2mL of this solution with 2mL of Tris and 1mL of this solution with 3mL of Tris. In the second big test tube (experimental) we put, 14.7mL of Tris, 0.3mL of Amylase (which we only added right before we were about to start the experiment) and 15mL of starch. We added 1mL of Lugol's to 5 test tubes and then added 4mL of solution in big test tube 2. Since Lugol's stopped the reaction we had to set a timer so we knew when exactly to add it. At 0 minutes we added 4mL of this solution into one of the 5 test tubes with Lugol's in it. At 1 minute we added 4mL of this solution to Lugol's and continued with this pattern until the 5th test tubes reaction was finished. After the 5 test tubes were filled, we got their absorbance from the spectrophotometer. The third big test tube was used to make sure that amylase was actually breaking down the starch. We put 15mL of starch, 0.3mL of amylase and 14.7mL of Tris into the third big test tube. Then repeated what we did for the second big test tube, however no amylase was added. We placed 4mL of test tube 3 solution into a test tube of 1mL of Lugol's at 0 minutes and then 4mL again at 1 minute and continued on until the 5 test tubes were filled.

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Results

Big Test Tube 1:
Dilutions:
We used the formula $M_1V_1=M_2V_2$ to find the final concentration of starch in the starch/tris solution

(0.2g/L)(15mL) = (x)(30mL) x= 0.1M

Dilutions in 5 test tubes:
Test tube 1 – (0.1g/L)(4mL) = (x)(4mL) x=0.1M
Test tube 2 – (0.1g/L)(2mL) = (x)(4mL) x=0.05M
Test tube 3 – (0.1g/L)(1mL) = (x)(4mL) x=0.025M
Test tube 4 – (0.1g/L)(0.5mL) = (x)(4mL) x=0.0125M
Test tube 5 – (0.1g/L)(0.25mL) = (x)(4mL) x=0.00625M

Big Test Tube 2:
We used the formula $M_1V_1=M_2V_2$ to figure out how much of each substance to put in this test tube. We were told to use a final concentration of 0.1mg/mL for both amylase and starch.
How much amylase to use:
(10mg/mL)(x)=(0.1g/L)(30mL) x=0.3mL of amylase

How much starch to use:
(0.2g/L)(x)=(0.1g/L)(30mL) x=15mL

The final volume is 30mL so 30= 15 + 0.3 + x(amount of Tris) which comes out to be 14/7mL of Tris.

Absorbance Values
Starch/Tris dilutions from the 5 test tubes:
For Standard Curve

Concentrations (g/L)	Absorbance/nm
Blank	0.0
0.1	1.29
0.05	0.578
0.025	0.258
0.0125	0.109
0.00625	0.033

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Test Tube 2 (starch/tris/amylase solution). At every minute we added 1mL of Lugol's

Time/minutes	Absorbance/nm	Concentration from standard curve
0	1.16	0.1041
1	1.005	0.091
2	0.840	0.076
3	0.712	0.063
4	0.662	0.058

Test Tube 3 (starch/tris/NO amylase) this one contains only Tris and starch with 1mL of Lugol's being added every minute

Time/minute	Absorbance/nm	Concentration from standard curve
0	1.36	0.1221
1	1.31	0.119
2	1.33	0.121
3	1.31	0.119
4	1.32	0.120

We were able to find the concentrations of the solutions with the amylase and without the amylase by using the recorded absorbance values. We looked at our standard curve and traced from where the absorbances were located until it fell upon our best-fit line. This showed us what the concentrations were.

The point of this lab was to find the rate at which the enzyme amylase broke down starch. The concentrations for the starch/tris/amylase solution were 0.1041 at 0 minutes, 0.091 at 1 minute, 0.076 at 2 minutes, 0.063 at 3 minutes and 0.058 at 4 minutes. These results demonstrate that amylase is breaking down the starch. This is further reinforced by the fact that there is no change in the concentration of the starch/tris solution that contained no amylase. The concentration of this solution at 0 minutes was 0.1221, while at 4 minutes it was 0.120. This is very little change compared to the starch/tris solution that contained amylase. We did this by using the formula: later concentration – earlier concentration/time interval. Which comes out to be: (0.058g/L – 0.1041g/L)/4 minutes = -0.012g/L/min

Based on the results that we got, it shows that the concentration of the starch decreases over time in the presence of amylase. We concluded that amylase does break down starch and would function as a good enzyme to metabolize starch into sugar in the brewing of beer. Before the company switches to amylase, however, they should compare these results to what enzyme they are currently using to determine if it would be a beneficial switch.