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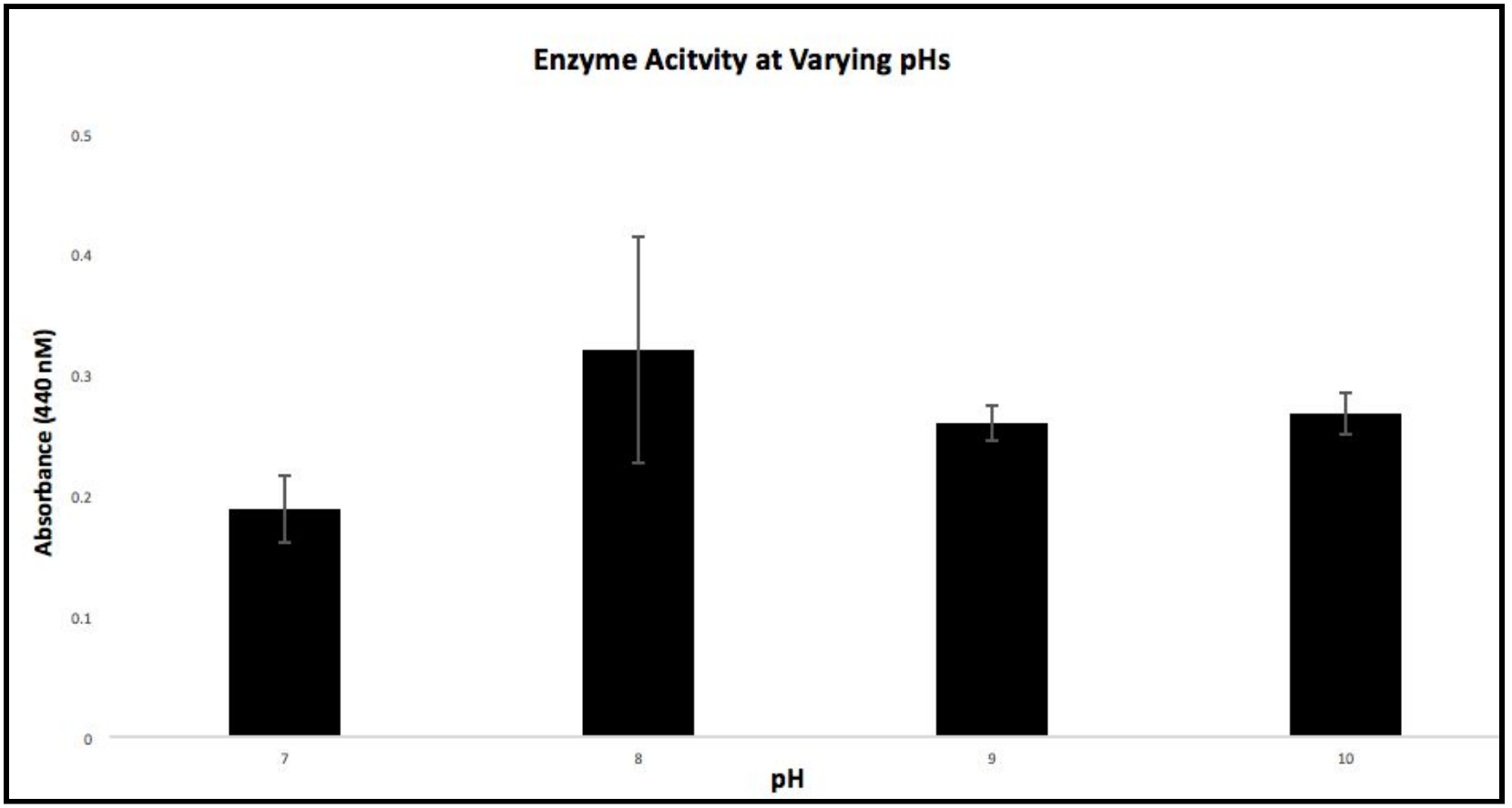
Introduction

Trypsin, a proteolytic enzyme, is found in the small intestines and breaks down proteins into amino acids. Trypsin is first produced in its inactive form, trypsinogen, in the pancreas. When it enters the small intestines through the common bile duct, it is then converted into active trypsin. For digestion, a catalytic pH range of 7 to 9 is required. Our hypothesis is the optimal pH where trypsin is most active is pH 9.

Materials and Methods

1. Add 450 uL reaction buffer 0.15 M NaCl and 20mM Tris-HCl ph's 6 through 9 to centrifuge tubes
2. Add 500 uL Azocasein
3. Add 50 uL Trypsin in a clean centrifuge tube
4. Incubate for 20 minutes in 37 degrees Celsius
5. Add 500 uL 10% TCA in the tube
6. Centrifuge each test tube at maximum speed in the clinical centrifuge for 2 minutes
7. Transfer supernatant to clean cuvette
8. Blank the spectrophotometer with a water filled cuvette at 440 nm
9. Read Absorbance in the spectrophotometer at 440 nm
10. Record results for each reading
11. Repeat steps 1-10 for 3 trails

Results


Enzyme Acitvity at Varying pHs
Absorbance (440 nM)
0.5
0.4
0.3
0.2
0.1
0
7
8
9
10
pH

T-test Results

Null Hypothesis	P-value	Result
The enzyme activty at pH 9 is not significantly different than the enzyme activty at pH 7	0.018277836	Significantly Different
The enzyme activty at pH 9 is not significantly different than the enzyme activty at pH 8	0.338544572	Not Significantly Different
The enzyme activty at pH 9 is not significantly different than the enzyme activty at pH 10	0.593069521	Not Significantly Different

Key Results

Among our average amount of enzyme activity for the experimental pH groups, pH 8 had the highest amount of absorbance at 0.320. pH's 9 & 10 had averagely the same amount of absorbance at 0.26 and 0.27. pH 7 had the lowest average amount of absorbance at 0.189.

In the experimental groups our standard deviation (variability) for pH's or 7, 8, 9, and 10 were 0.028, 0.094, 0.015, and 0.017 respectively. For the negative controls the standard deviations were 0.008, 0.004, 0.036, and 0.005 respectively. These results indicate very low variability among all of our results, but the variability or our experimental pH 8 is higher than most. The pH 9 error bar overlaps with both the pH 8 and pH 10 error bars so there is no difference in enzyme activity between pH 8, 9, and 10.

Our t-tests indicated that pH of 7 was the only pH where enzyme activity was significantly different than enzyme activity at a pH of 9. P was less than 0.05 and the null hypothesis was rejected. The enzyme activity at pH 9 is not significantly different than the enzyme activity at pH 8 and pH 10. The P value for these results were greater than 0.05 and failed to reject the null hypothesis.

Discussion

Our results did did not support the hypothesis because our hypothesis stated that trypsin would be most active at pH9. The average enzyme activity of pH 9, 0.26, is not the highest in our data but our t-test showed that the average enzyme activity at a pH of 9 was not significantly different from the average enzyme activity at a pH of 8 and 10 even though pH 8 seemed to be the highest. Comparing the enzyme activity of pH 9 and 8 the p value was 0.34 and comparing the enzyme activity of pH 9 and 10 the p value was 0.59.

Our control variable conditions could have resulted in the highest possible rate of enzyme activity but there was experimental error when it came to pH 8 which caused variability. According to the results of Enzyme Poster Pres Group F, pH 8 should've had the most enzyme activity. Their daya supports their hypothesis that enzyme activity is greater at a pH of 8 than other pH level. Their t-test also showed that every pH they tested was significantly different than absorbance at a pH of 8.

In conclusion, based on the results from our experiment, we know that pH 9 is not the pH where trypsin is most active. From comparing our results to Enzyme Poster Pres Group F, we can conclude that the optimum pH where trypsin is most active is pH 8. The ideal conditions to grow bacteria is a pH of 8 and temperature of 37 degrees celsius.

References Cited

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