The Affect of pH on Enzyme Activity

Figure 1: List of variables in experiment including errors, intervals and methods of controlling variables.

Kind of variable	*Variable / units ± error*	*Method of measuring variables (describe intervals if appropriate)*
Independent variable	Hydrogen ion concentration / pH ± 0.5	pH in this experiment was obtained by using pHydrion's "Chemvelopes" to make solutions of pH 3, 5, 7, 9 and 11. Used "pHydrion" sticks which turn color to match pH against a color key.
Dependant variable	Time / s ± 0.2	Used electronic hand timer to measure time from disk being placed at bottom of test tube until part of disc broke surface of liquid after rising as a result of enzyme in paper filter disc. When the disc did not rise within 10 minutes (600 seconds), then a time of 600 seconds was recorded in each event.
		Method of controlling variables
Controlled variable	pH	Standard pH solutions were made using pHydrion's "Chemvelopes." 10.0 cm^3 of each pH solution was added to one test tube and each test tube was labelled for its pH; ex. "pH = 5" Once the solution was made, its pH was tested with pHydrion pH sticks using a color matching system.
Controlled variable	substrate concentration	Each test tube labelled with with a unique pH received 10.0 cm^3 of 3% H_2O_2 solution (which was purchased from a local pharmacy.)
Controlled variable	enzyme amount	Enzyme was extracted from potatoes by pureeing potatoes with minimal (unmeasured) water in blender. Slurry was poured into cheesecloth and the liquid draining through the cloth was collected. A discs were dumped into liquid. Disks were taken out to place into test tube as needed. *[Mr. Reimer says - It would be best to place discs into solution for identical times and MIX solution be swirling before each disc was added.]*
Controlled variable	*temperature*	*Mr. Reimer says - This was not controlled but should be. Since temperature, substrate and pH all affect enzyme activity, students should be controlling them if possible.*

DATA COLLECTION & PROCESSING

Aspect 1: Recording Raw Data

Figure 2. Table shows the length of time it took a single, potato-juice soaked paper disc to float to the surface of the test tube. The test tube contained 10 cm^3 of 3% H_2O_2 solution and 10 cm^3 of pH solution. Time was measured on a hand stopwatch. All times of 600 seconds mean that at after waiting 600 seconds (10 minutes) the disc still did not rise, so a maximum value of 600 seconds is recorded even thought the disc did NOT rise at all.

	Hydrogen ion concentration / pH ± 0.02				
	3	**5**	**7**	**9**	**11**
Time for paper disc to rise to surface of solution / s ± 0.2	180	600	600	600	600
	73	600	600	600	144
	121	115	83	95	71
	111	173	221	600	600
	215	104	110	180	108

Figure 3. Qualitative observations related to individual pHs; see individual observations.

5	One of the “did not rise” paper discs, did accumulate a few bubble and the disk stood upright, but never rose to the surface
7	Both discs that never rose to surface did accumulate bubbles and “bounced” up from bottom of test tube, but never floated all the way to surface.
9	The pH 11 solution was stored in the incubator (set at 37º) so it felt warm to touch when we used it. The other solutions were on the table in the lab and felt cool
Potato solution	I noticed that on the second testing day, about 48 hours after the first day of testing, the potato juice looked very dark brown, not red-pink as on the first day.

Aspect 2: Processing Raw Data

Figure 4. Mean and standard deviation of times for paper discs to rise in test tubes. Note that mean times inclue "600" values, which are trials in which, after waiting 600 seconds, the disc never rose.

Time from data in Figure 2 (above)		*Hydrogen ion concentration / pH ± 0.02*				
		3	**5**	**7**	**9**	**11**
*Data **does not** include times ≥ 600 seconds*	*Mean **time** for paper disc to rise to surface of solution / s ± 0.2*	140	131	138	138	108
	***Standard deviation** of all times at a given pH*	57	37	73	60	37
Data includes times ≥ 600 seconds	*Mean **time** for paper disc to rise to surface of solution / s ± 0.2*	140	318	323	415	305
	***Standard deviation** of all times at a given pH*	57	260	260.0	260.0	270.0

Aspect 3: Presenting Processed Data:

Figure 5: Graph of times required for filter paper disc soaked in potato juice (in which, we are assuming, is active catalase enzyme) and placed in different pH solutions of hydrogen peroxide, to rise to the surface of the test tube. Note that this data set does not include trials in which the disc did not rise (see caption for Figure 3 for details.)
[***Error bars*** *on x-axis represent error in pH; error bars on y-axis represent standard deviation of times - but these values omit times of 10 minutes (in which the disc NEVER rose while being observed*)]

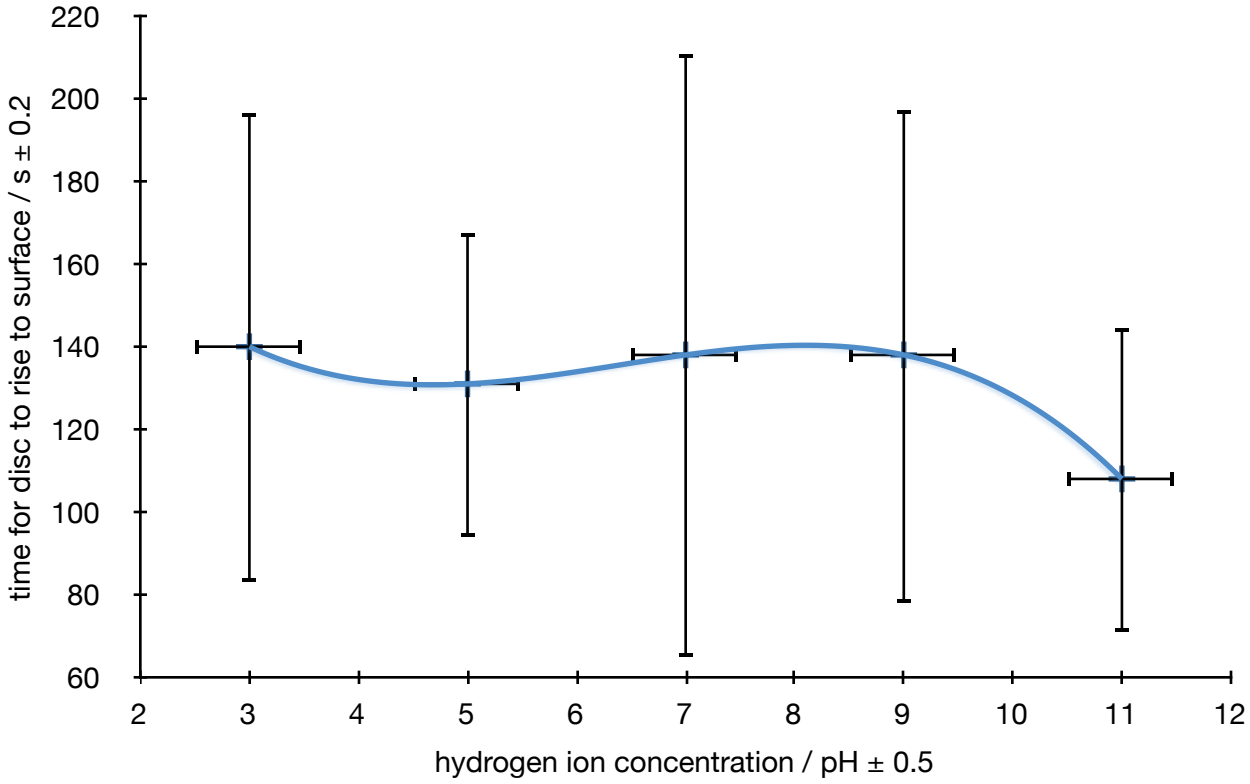
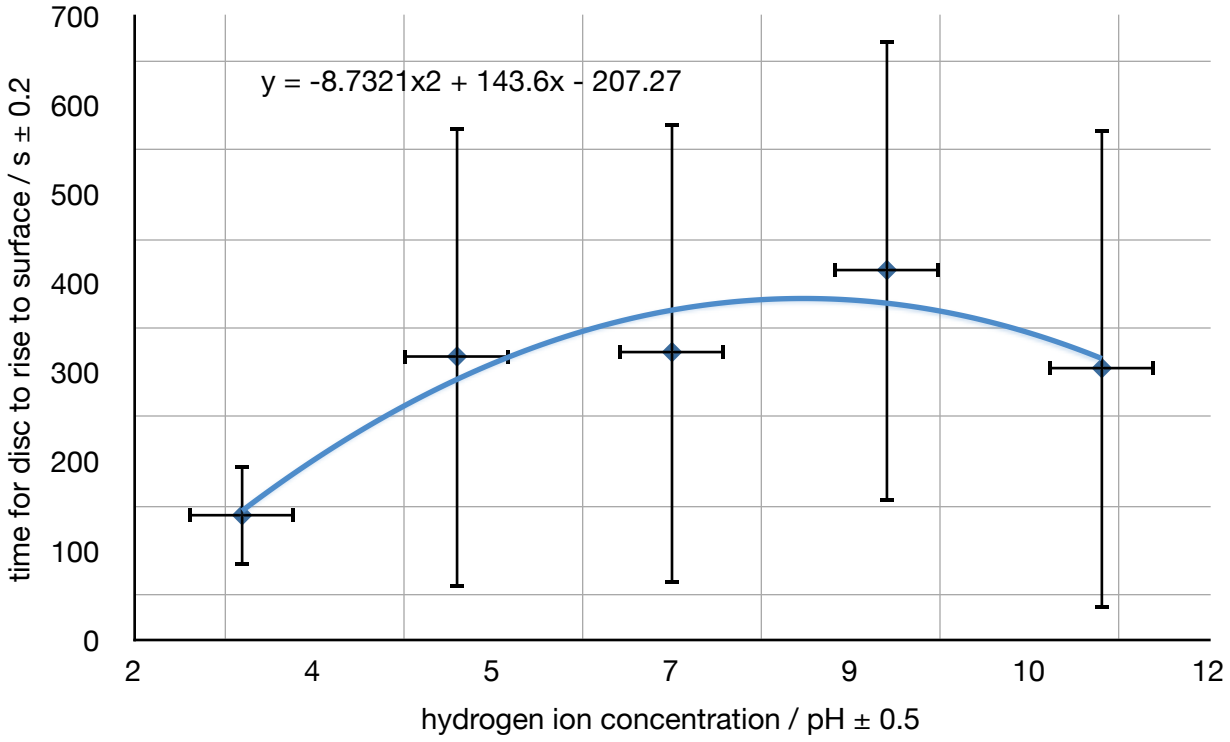


Figure 6: Graph of times required for filter paper disc soaked in potato juice (in which, we are assuming, is active catalase enzyme) and placed in different pH solutions of hydrogen peroxide, to rise to the surface of the test tube. Note that this data differs from Figure 4 since Figure 4 data does not include time ≥ 600 s (10 minutes) but this data set includes times of "600 seconds" as the measurement for all filter paper discs which never rose to surface of test tube during the 600 second trial length (see caption for Figure 3 for comparison of data sets.)
[***Error bars*** *on x-axis represent error in pH; error bars on y-axis represent standard deviation of times - but these values omit times of 10 minutes (in which the disc NEVER rose while being observed*)]



CONCLUSION AND EVALUATION

Aspect 1: Conclusion

There *did not appear to be a consistent pattern* in the data. I expected there to be an optimal pH - a pH at which enzyme would be most active causing paper disc to rise the fastest - above and below which the enzyme activity would be less[1]. However, Figure 5 suggests optimal pH is 11 and Figure 6 suggests optimal pH is near 3. This conclusion contradicts published data[2], my own expectation, and even some of the other results in this experiment. Maximum enzyme activity in Figure 5 occurred at pH 11 to maximum enzyme activity in Figure 6 at pH 3.

This contradictory data may result from insufficient controls. Some other variable besides the independent variable must have caused this difference, since Figure 1 shows that at pH 11 the disc rose quickest (71s) of all tests and slowest (two times of "more than 600 s"). If a test tube had a controlled amount of enzyme, substrate, temperature and pH, then the principles of enzymes suggest there should be some degree of uniformity in the enzyme activity, but these results do not show that.

Aspect 2: Evaluating procedures

The *variability in measurement during this experiment is so large* that it brings the validity of the data into question. In Figure 4 the standard deviation of the times without "including 600 second" data is about 30% of the range of measured values. At pH 5: (standard

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Note: these footnotes are done in CSE format.

[1] Allott A, Mindorff D. Biology Course Companion. 2nd ed. Oxford: Oxford University Press; 2010. Page 76-81. Book. (IB Diploma Programme Course Companion.)

[2] Worthington Biochemical Corporation [Internet]. Lakewood, NJ (USA); c2011 [cited 2011 Feb 5]. Website. Available from: http://www.worthington-biochem.com/introBiochem/effectspH.html

deviation was 37 s) / (mean time of 131 s) ≈ 30%. In the same table, the standard deviation for measurements which "include the 600 second" values is 30% of mean time at best, and sometimes almost equal to the mean. In other words the standard deviation sometimes equals the measurement. This is like saying my height is 2 m ± 2; a meaningless conclusion. Figure 4 shows that at pH 11 the standard deviation of 270 seconds was 89% of the mean time value of 305 seconds; that variability is very large!

In this experiment it is important to control: (1) amount of enzyme in the filter paper discs, (2) amount of substrate, and (3) amount of buffer used to maintain pH[1]. The huge variation in measurements means that something was not adequately controlled in the experiment.

The range of pH measurements was probably not a problem; pH 3 - 11 covers the center 60% of the pH range. Wider pH intervals may have given more pattern to the data, but the variation of times in a single pH suggest that the solution or timing was a problem more than the pH range.

Apparently during some measurements when the disc did not rise, the enzyme was present and active as shown by gas in paper disc; see in Figure 3. However there was not enough activivity to cause a measurable effect inside the maximum window of 10 minutes. This happened in two trials in pH 5, 7 and 11 and three times in pH 9. The variability in each trial points to ineffectively controlled variables in individual test tubes. It seems likely that either pH, substrate concentration or enzyme concentration inconsistencies caused "times to rise" to vary widely.

Aspect 3: Improving the investigation

Although I intended to control pH, perhaps this was focused on too much and neglected to adequately control other variables. I know that some of the trials were done on Monday when solutions were standing on the bench, at room temperature, but on the second day the pH solutions were stored in the incubator at 37^{o} C. The temperatures should have remained on or near the same for all trials since temperature is one of the things that affects enzyme activity[3]. 37^{o} C is optimum temperature for many enzymes and a water bath or incubator could easily maintain uniform temperature during testing.

pH was checked with pH sticks, but using a pH meter from Vernier would be an easy way to validate solution's pH.

Sometimes, the watching as very boring and ten minutes is a long time to wait. Assuming pH is appropriately controlled, the substrate concentration should be increased to increase enzyme activity and reduce wait time.

The existence of the enzyme in the potato solution was ambiguous. Clearly the enzyme existed since some bubbles formed, but the variability in results at a single pH suggest the enzyme was inconsistently incorporated. Dumping all discs into the potato liquid at the beginning and fishing them out when they were required led to some discs soaking for longer and possibly absorbing more enzyme. It would be better to swirl the potato juice as a mixing technique, then place discs into juice for a fixed length of time after which they are retrieved and placed into the test tubes for testing.

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[3] Campbell NA, Reece JB et al. Biology. 7th ed. Benjamin Cummings: Pearson Education; 2005. Page 152-154. Book.

Lastly, some tests were done on the first day and some on the second lab day, 48 hours later. Other than the issue with pH solution temperatures, I also noticed that the potato juice was much darker on the second day than the first day (see Figure 3). Mr. Reimer[4] said this may be due to "oxidizing" of the enzyme. If this is like oxidizing molecules, then losing electrons (or gaining oxygen) may cause a change in enzyme structure and therefore a change in conformation and ultimately function[3]. This change in enzyme from one experiment to the next may be a large factor in different enzyme activities. The enzyme that is used should be extracted from potato and used in a single block of time. In my experiment the enzyme was used one day then again 48 hours later and it may have been substantially changed by oxidation during that time.

[4] Mr. Reimer is my DP biology teacher. This comment was made in answer to my question about the change in potato color over the two days of experiment.