

Modifying a simple hydrolysis of sucrose experiment to measure michaelis menten e...



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How can the Michaelis-Menten constant for the dextrase content of yeast be calculated with simple experiment on hydrolysis of sucrose?

Introduction:

In anaerobic conditions yeast cells break down sugar molecules into ethanol and produce carbon dioxide. The process is called alcoholic fermentation. The equation of this process is: $C_6H_{12}O_6 \rightarrow 2 C_2H_5OH + 2 CO_2 \uparrow + E$. The process consists of a series of reactions catalyzed by specific enzymes. The enzymes present in yeast cells are specific, i. e. they will catalyze the fermentation of certain sugars but not others.

Michaelis-Menten kinetics is one of the simplest and best-known models of enzyme kinetics. The model takes the form of an equation describing the rate of enzymatic reactions, by relating reaction rate to , the concentration of a substrate S. Its formula is given by

([http://en.wikipedia.org/wiki/Michaelis-Menten_kinetics](http://en.wikipedia.org/wiki/Michaelis%E2%80%93Menten_kinetics))

Independent variable: Different concentrations of sucrose(%)(range: 0. 0% (Distilled water), 0. 5%, 1. 0%, 5. 0%, 10. 0%, 20. 0%)

Dependent variable: Height of the suspension-sugar level(cm)(range: 0~)

Controlled variables: Temperature(°C), heat treatment length(seconds), concentration of yeast suspension(%)

Hypothesis:

Hydrolysis of different concentration of sucrose and graph's tangent would make able to predict the calculation of the rate of reaction.

Materials and methods:

Materials:

6 fermentation tubes

6 test tubes

Saccharomyces fungus suspension (20%)

Different concentrations of sucrose: 0. 0% (distilled water), 0. 5%, 1. 0%, 5. 0%, 10. 0%, 20. 0%

Stirring rod

Glass marker

Semco pipette

Water bath

Ruler

Stopwatch

Methods:

Label 6 fermentation tubes 1 – 6 with a wax pencil so that experimenters can distinguish clearly.

Using a clean pipette in each case, transfer the following into each fermentation tube.

(1 mL of distilled water, 0.5% sucrose, 1% sucrose, 5% sucrose, 10% sucrose, 20% sucrose)

For each tube, add 1 mL of yeast and top up with distilled water.

(Mix the yeast by swirling the flask before putting the yeast to each tube)

Push each fluid-filled fermentation tube to end of each test tube with pencil.

Tap the outer tube firmly to release any bubbles trapped at the mouth of the inner tube.

Once all the fermentation tubes settings are completed, record the height of the liquid in mm in each one using a ruler.

Place all the test tubes in a water bath at 38.9°C.

When it reaches every 4 minutes, measure the height of each liquid in fermentation tubes in mm.

Repeat the step 8 up to 16 minutes.

Data collection:

Table. 1

Individual student raw data

The table shows the height changes of the suspension-sucrose mixture in fermentation tube with different concentrations of sucrose in each test tube containing 20% yeast suspension kept in heating bath (38.9°C) over 12 minutes. 0.0% concentration of sucrose means that the solution is plain distilled water without any sucrose concentration. The height has been measured with a ruler with uncertainty $\pm 0.05\text{cm}$ every 240 seconds. I expect about 10 seconds of uncertainty between each time intervals. Because to avoid accidents such as breaking the test tubes, I placed 2 test tubes into water bath at a time which would have caused some time differences between each test tubes, and also, I took 2 test tubes out from water bath at a time, which would also have caused additional time differences between each test tubes. The results are from the first trial.

Individual student raw data

The table shows the height changes of the suspension-sucrose mixture in fermentation tube with different concentrations of sucrose in each test tube containing 20% yeast suspension kept in heating bath (38.9°C) over 16 minutes. . 0.0% concentration of sucrose means that the solution is plain distilled water without any sucrose concentration. The height has been measured with a ruler with uncertainty $\pm 0.05\text{cm}$ every 240 seconds. I expect about 10 seconds of uncertainty between each time intervals. Because to avoid accidents such as breaking the test tubes, I placed 2 test tubes into water bath at a time which would have caused some time differences between each test tubes, and also, I took 2 test tubes out from water bath at a time, which would also have caused additional time differences between each test tubes. The results are from the second trial

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Height change of solution over time($\pm 0.05\text{cm}$)

Sucrose concentration

Individual student raw data

The table shows the height changes of the suspension-sucrose mixture in fermentation tube with different concentrations of sucrose in each test tube containing 20% yeast suspension kept in heating bath(38.9°C) over 16 minutes. 0.0% concentration of sucrose means that the solution is plain distilled water without any sucrose concentration. The height has been measured with a ruler with uncertainty $\pm 0.05\text{cm}$ every 240 seconds. I expect about 10 seconds of uncertainty between each time intervals.

Because to avoid accidents such as breaking the test tubes, I placed 2 test tubes into water bath at a time which would have caused some time differences between each test tubes, and also, I took 2 test tubes out from water bath at a time, which would also have caused additional time differences between each test tubes. The results are from the third trial

Height change of solution over time($\pm 0.05\text{cm}$)

Sucrose concentration

Table 4 shows the processed data from Table 1. To find the Michaelis-Menten constant, I included the value of biggest change in height for each concentration of sucrose. The uncertainty of the value (± 0.1) was calculated by adding two uncertainties (± 0.05) together, thus getting ± 0.1 as the answer.

Table 5 shows the processed data from Table 2. To find the Michaelis-Menten constant, I included the value of biggest change in height for each concentration of sucrose. The uncertainty of the value (± 0.1) was calculated by adding two uncertainties (± 0.05) together, thus getting ± 0.1 as the answer.

The value of V_{max} was found instantly because it is close to the maximum value of the height changes in different concentrations of suspension-sucrose mixture. V_i was found by dividing V_{max} by 2 and K_m was acquired by following the point where V_i and the best fit line for the data overlaps. The value of K_m is known as the Michaelis-Menten constant. The data used in graph is from table 6 which is a processed data from the third trial. The data from the third trial has been chosen because compared to those previous trials, the first and second trial, the third trial has shown the complete reliable data. The software called “GraphPad Prism” has been used to present Michaelis-Menten Graph.

Michaelis-Menten constant

Conclusion:

The aim of experiment is to investigate about classic enzyme kinetics with different concentrations of substrate, sucrose, attempting to calculate the Michaelis-Menten constant for the Dextrase content of *Saccharomyces cerevisiae*. After I carried out the experiment, I found out that the value of Michaelis-Menten constant is close to 0. The Michaelis-Menten constant represents the rate of enzyme reactions in relation to concentration of the

substrate and lower value of it means that the enzyme has a higher affinity
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(Kimball, John W. "Enzyme Kinetics."). According to Graph. 1, the Michaelis-Menten constant of dextrase is quite low, and I found out that the constant is lower than many other enzymes (Kimball, John W. "Enzyme Kinetics."), it indicates that dextrase has higher amount of affinity compared to other enzymes.

Evaluation:

There were some weaknesses I had while carrying out the experiment. First of all, when putting the fermentation tube into the test tube in an inverted direction, some solution leaked out from the fermentation tube thus making height readings a bit inaccurate. Second, when putting in the test tubes into the water bath for heat treatment, the time interval will most likely to be differ by few seconds since putting them all in at once might cause test tubes to break. Not putting those test tubes into the water bath at the same time might cause wrong data. Third, measuring the height levels of each suspension-sugar solution with ruler is not a best way to measure it because it is not very accurate and it is hard to measure them because sometimes the fermentation tubes are covered with the solution went out which would make it hard to read. Forth, the limitation of space for water bath for everyone to carry out the experiment caused us to have very limited amount of trials and sometimes, I had to wait until somebody take out their test tubes, which could've cooled down the solution, causing unreliability in data, and making us unable to have more data to make data reliable. Last of all, as you can see from Table. 2, the solution with 10% concentration of sucrose hasn't shown any changes after 960 seconds. It is occurred because the tube

with 10% concentration of sucrose has broken by human mistake. So it might have brought a bit of inaccuracy data.

Ways of improvement:

While carrying out the experiment, I found that when I had my test tubes in the water bath, some people moved my test tubes around. This might also cause some errors in my results, so if this problem can be fixed, the data collected will be more reliable. This problem can be solved by having more space in room available for more water baths so each person can use their own water bath.

In my opinion, further investigations can be performed by making controlled variables listed above such as the concentration of yeast suspension changeable and perform the experiment, using that as the independent variable. This might bring out some interesting and different results than the experiment.

References:

Kimball, John W. " Enzyme Kinetics." Kimball's Biology Pages. 31 Jan. 2011. Web. 13 Apr. 2011. .