The effect of an environmental factor on the activity of yeast peroxidase.

D 1

Back ground

Baker's yeast (*Saccharomyces* cerevisiae **)** is a unicellular fungus. The peroxidase enzyme is found in peroxisomes and mitochondria[1]. Peroxidase is responsible for the breakdown of the toxic bi-product of metabolism hydrogen peroxide into water and oxygen

$$H_2O_2 \rightarrow H_2O + \tfrac{1}{2} O_2$$

The activity of this enzyme can be followed by measuring the temperature change in reaction mixture as this is an exothermic reaction.

Aim: To determine the maximum velocity of peroxidase enzyme in a suspension of yeast cells (*Saccharomyces* cerevisiae).

If the concentration of the substrate, hydrogen peroxide, is increased then the reaction should proceed faster. This is because the more substrate molecules there are in the mixture the more easy it is for the enzyme to find a molecule to react with. This will happen until all the enzymes in the mixture are occupied. At this point the enzymes will be saturated and further increases in the concentration of the hydrogen peroxide will not result is a faster reaction.

Variables
Independent variable: Hydrogen peroxide concentration
Dependant variable: Rate of peroxidase reaction
Controlled variables:
- pH of mixture,
- The concentration of yeast suspension
- The initial temperature of the mixture
- The volumes of the solutions

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Materials

Apple notebook with LoggerPro installed
LabPro interface
Temperature probe
5 test tubes
50cm^3 of 5% yeast suspension in pH 7 buffer
20 volume H_2O_2
Distilled water
5cm^3 syringe
10cm^3 pipette and pump
5cm^3 pipette and pump
Marker pen
Safety glasses

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[1] Marijana Plesnicar, Walter D. Bonner, Jr., and Bayard T. Storey **Peroxidase Associated with Higher Plant Mitochondria** Plant Physiol. 1967 March; 42(3): 366–370.http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1086543

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Method

Five different concentrations of H_2O_2 are prepared by diluting 20 volume H_2O_2 using distilled water as follows

Amount of 20 volume H_2O_2 ($cm^3 \pm 0.1\ cm^3$)	Amount of distilled water ($cm^3 \pm 0.1\ cm^3$)	Final concentration (Volumes)
20.0	0	20.0
10.0	10.0	10.0
5.0	15.0	5.0
2.5	17.5	2.5
0	20.0	0

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The temperature probe is placed in the test tube with $10cm^3$ of H_2O_2 and allowed to stabilise at room temperature. The Vernier temperature probe has a sensitivity of ± 0.3°C

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The data collection is set for 1 sample per second for 300 seconds.

A syringe is prepared containing $2cm^3$ of yeast suspension.

The data collection is started and allowed to run for 20 seconds when the yeast suspension is added to the test tube and stirred in.

The experiment is repeated for each of the different concentrations of H_2O_2.

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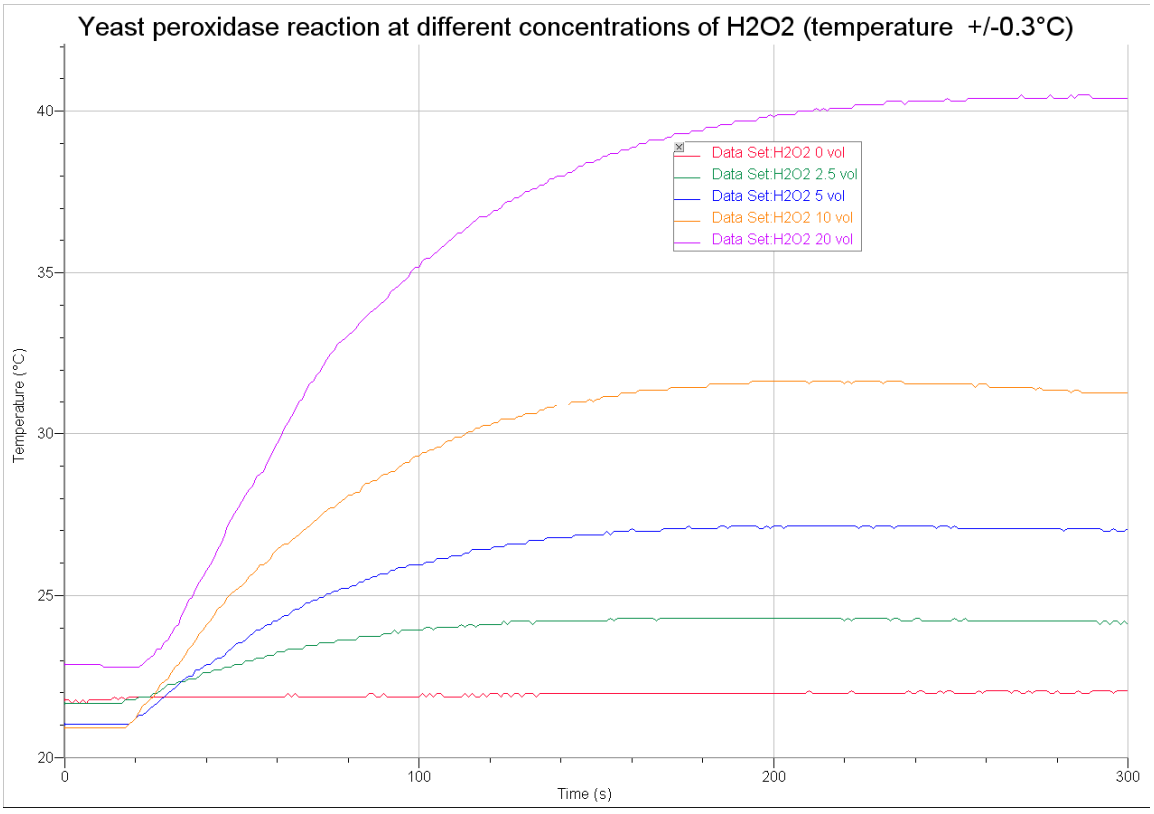

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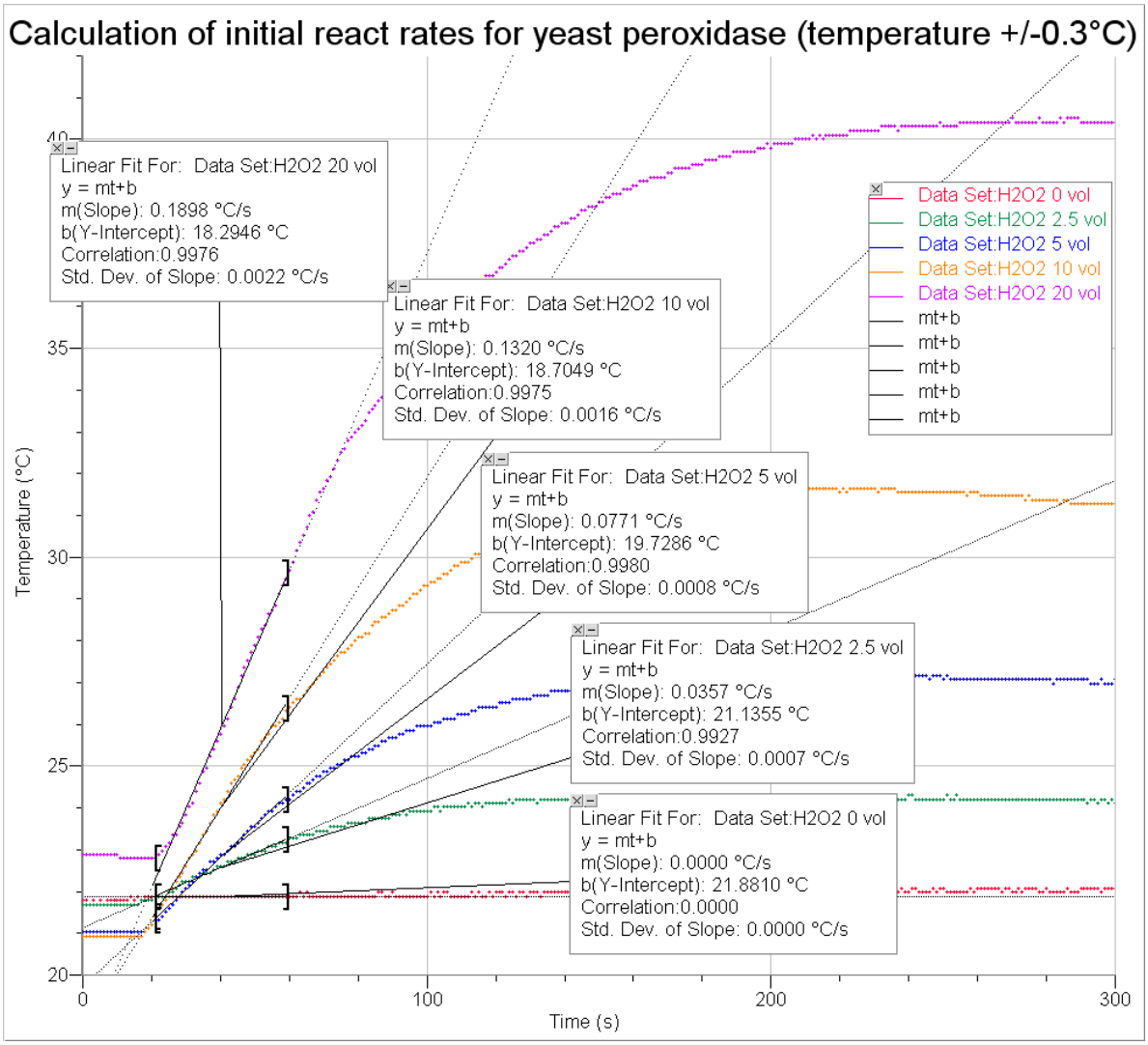
Data processing

The initial reaction rates were taken by using the linear plot facility of the Loggerpro program. The period from 20 to 60s was considered the appropriate interval for this. The reactions are working at their maximum rates, they have not yet started to slow down.

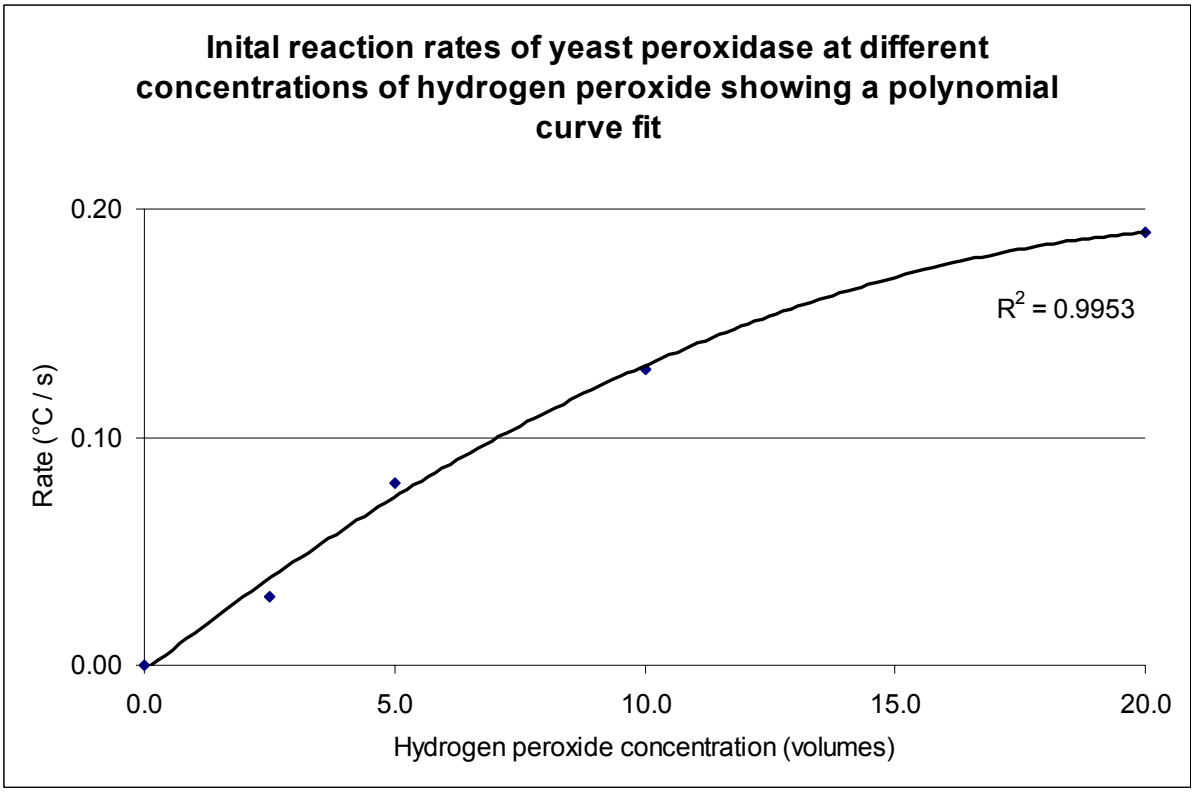


The initial reaction rates obtained are taken from the graph and put into Excel.

Initial rate of reaction of yeast peroxidase at different substrate concentrations

H_2O_2 concentrations (volumes)	Initial rate of reaction (°C / s)
0.0	0
2.5	0.04
5.0	0.08
10.0	0.13
20.0	0.19

DCP 3



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Conclusion
The data shows that the reaction rates do increase with the concentration of the hydrogen peroxide. The curve fit shows a very good correlation, R^2 is very close to 1.0. The curve looks as though it would reach a plateau at about 0.2°C / s for this experiment but it is still rising at 20 volumes H_2O_2.

CE 2

Evaluation
Unfortunately the rate has not reached its maximum with a concentration of 20 volumes H_2O_2.
The experiment was only carried out once for each concentration but the uncertainties in the data are small.
The initial temperatures of the reaction mixtures were not all the same. They varied from 21°C to 23°C. This probably did not affect the results a lot, the initial reaction rates could be determined for all the runs over that same time period.

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Suggested improvements
So that the maximum rate of reaction can be determined try the experiment with higher concentrations of H_2O_2 or repeat the experiment with a lower concentration of yeast suspension.

Place the test tubes in a water bath to stabilise the starting temperature.

Try the experiment with peroxidases from different organisms.