Enzyme Function Worksheet

Name

The enzyme peroxidase was isolated from fresh turnips. This enzyme is a slight variation on the catalase you have used in your protein study. In both cases each enzyme breaks down hydrogen peroxide to oxygen gas and water. In this case, however, the oxygen produced reacts with guaiacol, bringing about a color change.

Guaiacol (*colorless*) **+ Oxygen** $\rightarrow$ **Tetraguaiacol** (*brown*)

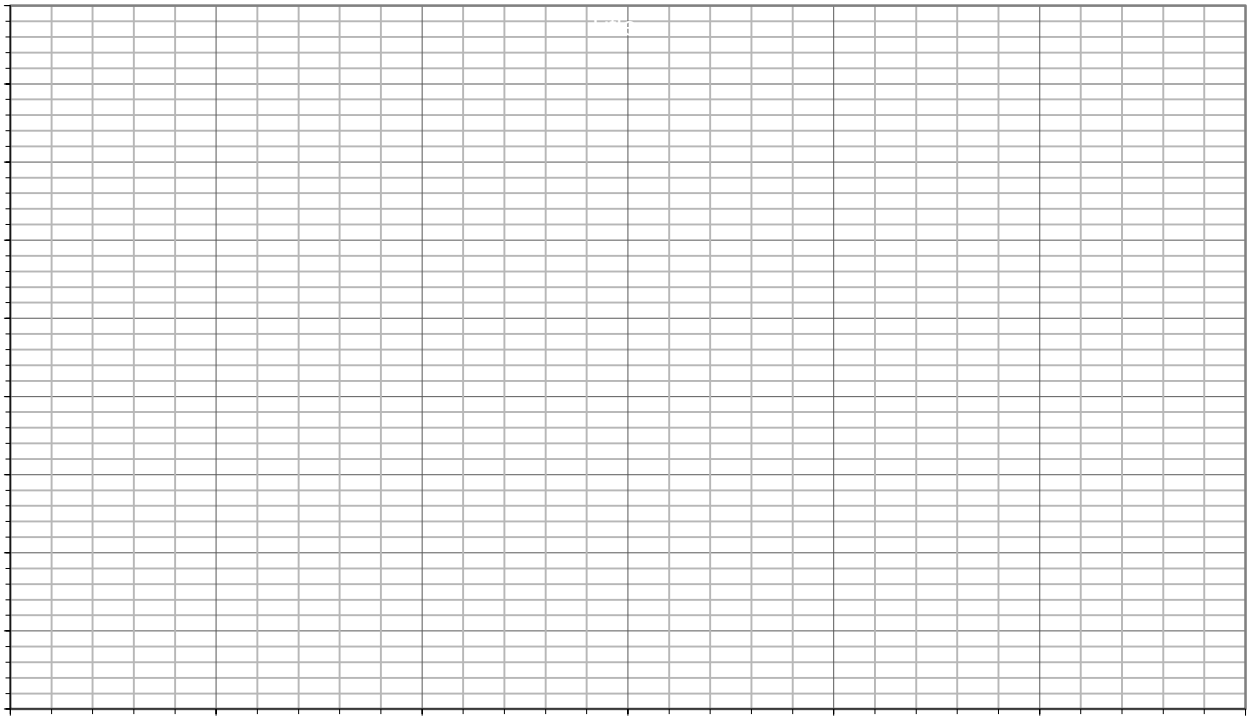
By using a spectrophotometer (or colorimeter), the amount of oxygen produced can be quantified by measuring the increasing amount of brown tetraguaiacol produced. Thus the amount of oxygen produced can be recorded by measuring the absorbance of the brown pigment. Using a standard curve, this can be converted to moles of oxygen. You have done similar measurements using colorimeters in chemistry.

Experiment A

In this initial experiment, 1 ml of a fresh peroxidase-containing solution was mixed with 0.1 % guaiacol and 0.5 % H_2O_2 in a 50 ml beaker containing. Every minute a sample was taken and its absorbance measured. The experiment was stopped after 10 minutes.

Time	0	1	2	3	4	5	6	7	8	9	10
O2 (moles)	0	0.16	0.39	0.48	0.53	0.6	0.6	0.615	0.62	0.612	0.614

Graph these data.

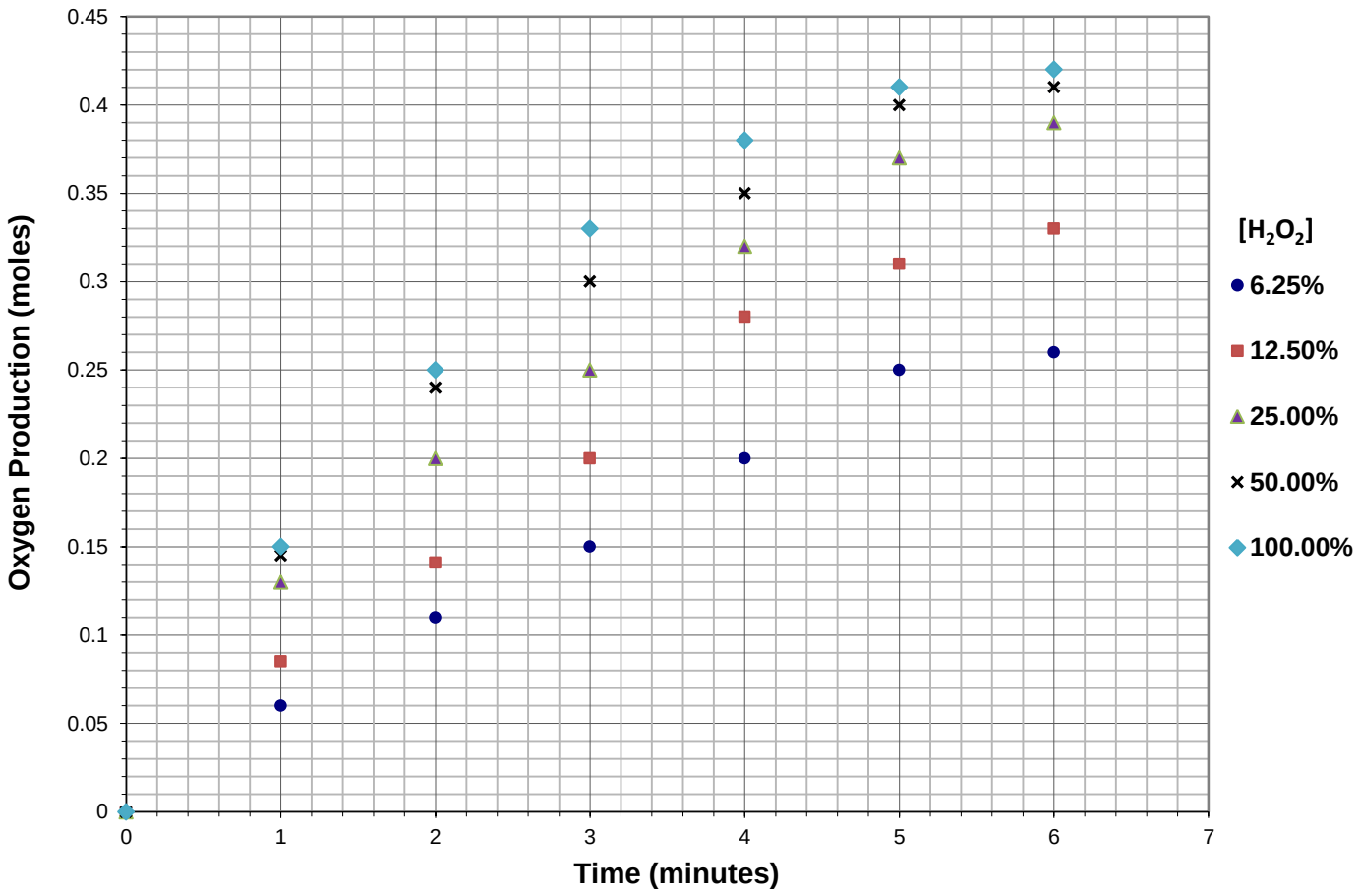


1. Describe the changing shape of the curve.

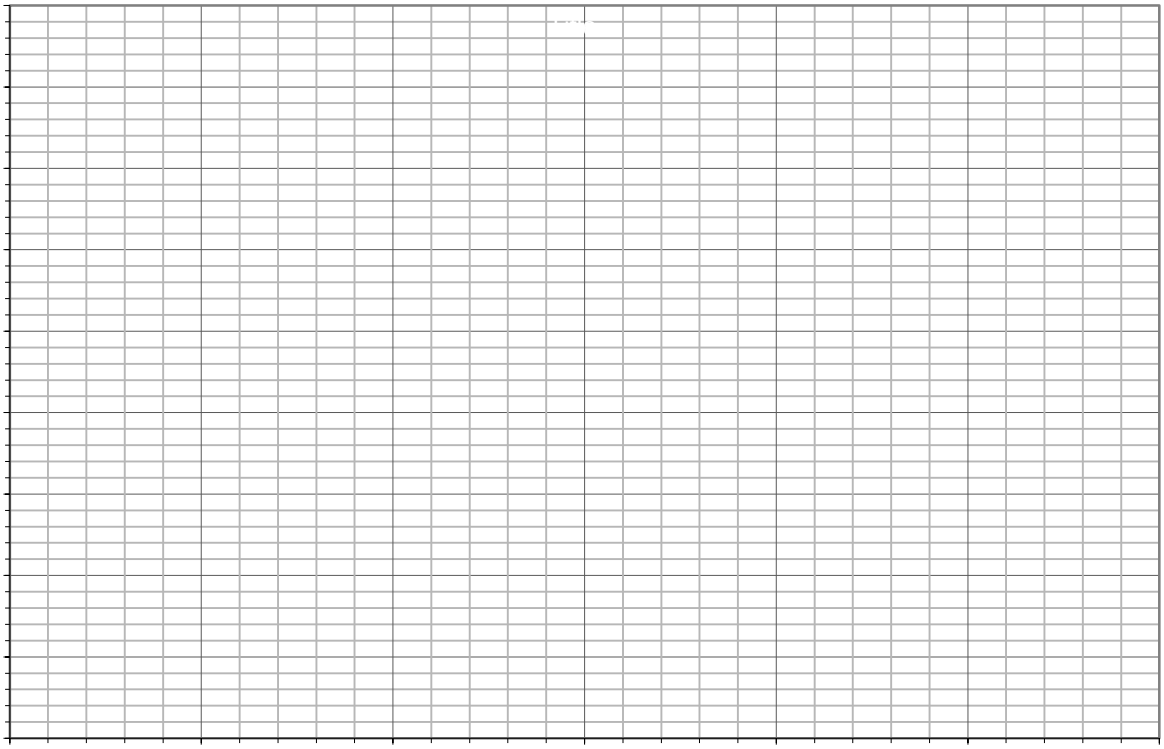
2. Calculate the initial rate of reaction as well as the rate at 7.5 minutes. Explain why these rates are different.

Experiment B

This study was repeated, but this time the amount of substrate was altered. 20 ml of peroxidase solution were mixed with different concentrations of substrate. The oxygen produced was recorded for 6 minutes. The data from these trials are graphed below. Complete the graph by putting in best-fit curves.



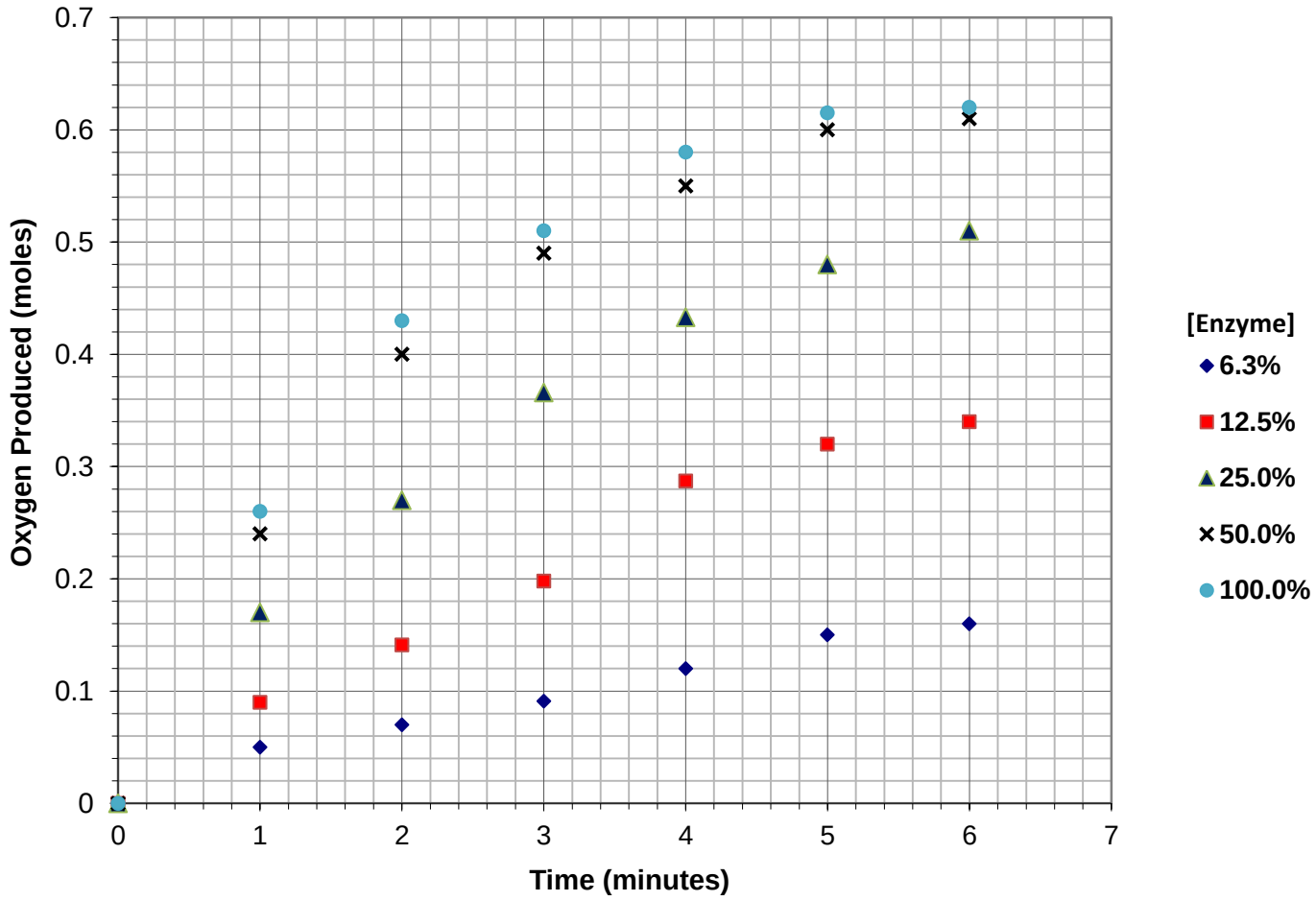
Rate of reaction, Δ product/ Δ time, is typically a more meaningful parameter when discussing and describing enzyme action. Using your best-fit curves, determine the initial rate of reaction for each substrate concentration and record them in an appropriate table on the next page. Using these results, construct a graph showing the relationship between substrate concentration (% H_2O_2) and peroxidase rate of reaction.



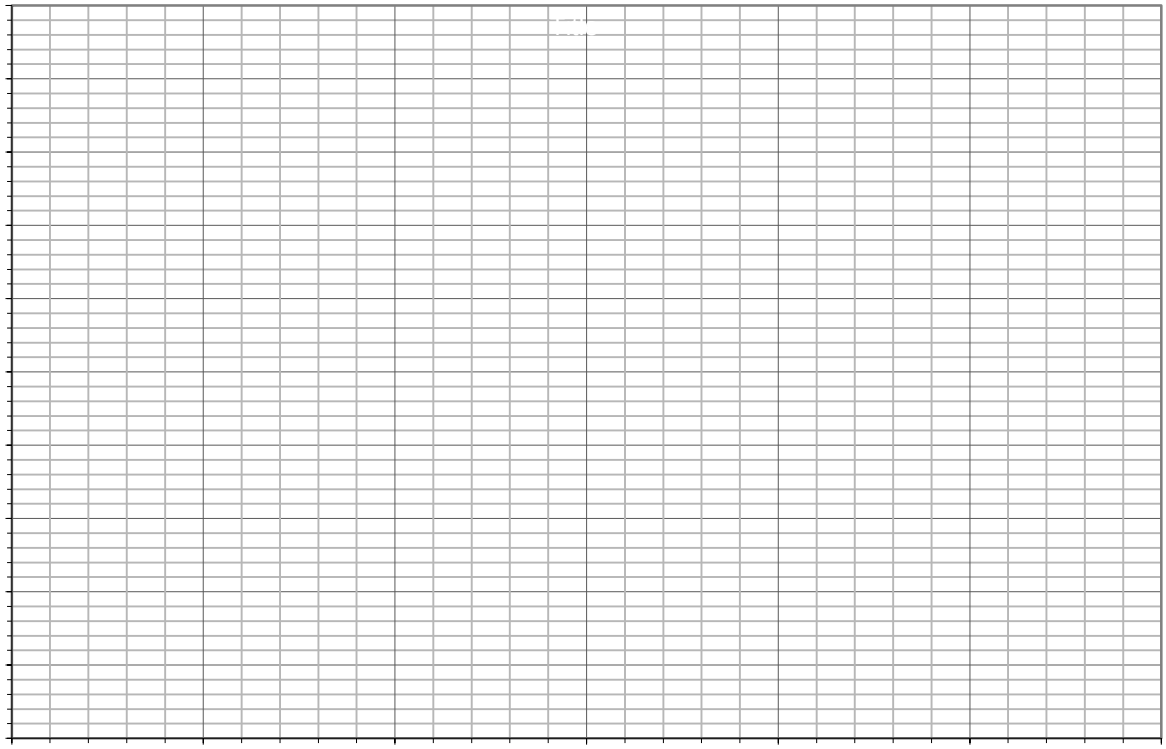
Explain, in terms of the number available of active sites and number of substrate molecules present, why the slope of the curve is different at low, medium, and high substrate concentrations.

Experiment C

This study was repeated, but this time the amount of enzyme was varied by the same dilution process. The oxygen produced was recorded for 6 minutes. The data from these trials are graphed below. Complete the graph by putting in best-fit curves.



Using your best-fit curves, determine the initial rate of reaction for each substrate concentration and record them in an appropriate table on the next page. Using these results, construct a graph showing the relationship between enzyme concentration and peroxidase rate of reaction.



Explain, in terms of the number available of active sites and number of substrate molecules present, why the slope of the curve is different at low, medium, and high enzyme concentrations.