Concentration vs. Amount

Enzyme studies are typically performed using small volumes of materials. It is thus essential for students to be careful and consistent when pipetting and to be able to document how much of each component was added to the reaction. In a 2 ml reaction, a variation of only 0.2 ml gives a 10% error!

Students become skillful pipettors very quickly in measuring/counting out drops, so their volumes can be considered the same from sample to sample if they have practiced for even just 2-3 minutes counting drops and using the different pipettes.

When adding different components, if final volumes vary, the concentration of each component can be determined only by doing a lot of math, which most of your students will not like … so point out that students should keep total volumes in each reaction constant. This will help to make it easier to detect patterns of activity, like a peak of enzyme activity at one particular pH, with lower activity at more acidic OR more alkaline pH.

Sometimes we care about the concentration (weight per ml, moles per liter) and sometimes we care about the amount (grams, micromoles, milliliters). Note that two units describe a concentration while only one unit describes an amount. In biochemistry in contrast to pure algebra, the numbers represent material. Thus, we need to think carefully about what is happening in an enzymatic or chemical reaction to check on our math!

Setting volumes constant helps us to keep better tabs on concentration. Frozen juice, for instance is actually a 4X concentrate, since the container says to add 3 volumes (cans) of water. So too, we use stock solutions or concentrates for biochemical studies. This helps 3 ways: first, because it's easier to weigh out a larger amount of powder; second, because many enzymes and substrates remain stable in concentrated solutions; and third, because it is easier and more consistent to do the math for dilutions than prepare fresh chemical solutions from scratch.

Remember that water is the (practically) universal volume adjuster. Thus, when working with several different solutions at different concentrations, you can add specified volumes of each component and still come out with a constant volume (by adding water). Generally, we calculate the various volumes needed to generate the concentrations of components and then calculate the volume of water to add to bring the volume to the desired level. Operationally, we add the water first, then the buffer concentrate, and additional small molecules, and lastly enzyme OR substrate, depending on the type of the reaction products we expect.

Molarity is a concentration, "moles" refers to an amount
% is a concentration, "micrograms" refers to an amount
10X is a concentration, 1X is the final concentration

Using hydrogen peroxide as the example, the stock solution from the supermarket is 3%, or 0.88M. We need to have an 0.1M substrate concentration to get a 1 minute reaction at pH 7 using 1 package of yeast per 500 ml. Our 24-well dishes hold 3 ml of liquid, so it will be safe to have a total working (assay reaction) volume of 2 ml. How much peroxide do we need to add to the well to get a 0.1M final concentration?

$C_1V_1=C_2V_2$ or what you need/what you have X final volume = volume of the stock (concentrated) solution to add to get the desired final (1X) concentration.

0.1M / 0.88M X 2 ml= 0.176 ml of the 3% peroxide solution

Hypotheses … ***continued***

Possible statements of a research question:

- What is the effect of varying peroxide concentration on the time it takes the disc to rise?
- What is the relationship between substrate concentration and enzyme activity?

Possible hypotheses:

- If the concentration of hydrogen peroxide is increased, then the time it takes for the disc to rise will decrease.
- As substrate concentration increases, the rate of the catalyzed decomposition of the substrate increases.

Reference: Cothron, M., Giese. and Rezba. R. Students and Research: Practical Strategies for Science Classrooms and Competitions. Kendall/Hunt Publishing Company.
ISBN: 0-8403-7766-5.