

Hydrogen peroxide decomposition by Baker's yeast

Kinetic studies of a biocatalyst in action!

2021

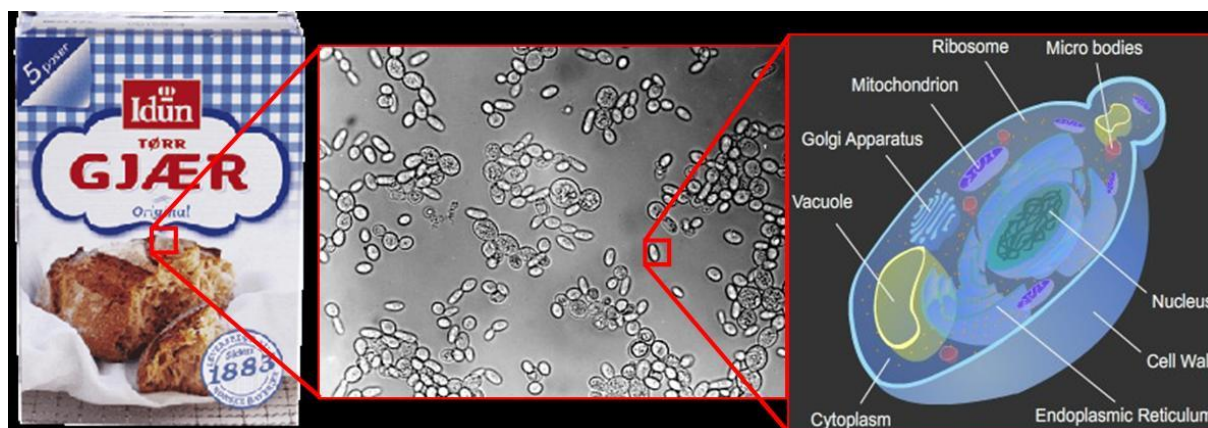
Introduction

Baker's yeast (*Saccharomyces cerevisiae*) is the most well-known member of the yeast family of microorganisms, and has lately also become of great importance in biotechnological research due to its simplicity and large variety of purposes.

Yeast is mostly used for baking and fermentation processes, and according to archaeological findings this has been the case for at least 4000 years now. But yeast has in the last decades also become very important to scientists, not only for understanding and optimizing those long-known processes, but also for obtaining better insight in the biological processes that make yeast to the simple yet extremely useful microfactory as it is today. Baker's yeast was therefore chosen by the Genome Project as the first eukaryotic¹ organism to have its complete genome (approx. 12 million base pairs) unravelled.

The simplicity and importance of yeasts lies in the fact that they are unicellular organisms with a rather high variety of useful enzymes (i.e. biocatalysts). Since they are eukaryotic and thus have a cell nucleus, they are more complex than bacteriae, but at the same time they are not as complex as cells from multicellular organisms like plants, mammals and other fungi, making them extremely suitable as model organisms for studying multicellular organisms.

Yeast has learned us much about, amongst others, how microorganisms reproduce, how they defend themselves to harsh conditions by special combinations of protective enzymes in and on their cell walls and how they handle toxic and waste products formed during the transformation of nutrients.



Scope

One of the most common toxic by-products produced in small quantities in hydrolysis and dehydrolysis (condensation) reactions in organisms is hydrogen peroxide. This simplest of all peroxides can cause serious damage in living organisms when not properly taken care of, and it is most likely therefore that enzymes like catalase are amongst the most active catalysts found in nature. One molecule of catalase is known to convert over 40 million hydrogen peroxide molecules to oxygen and water in just one second, making it a very powerful biocatalyst for the removal of even the smallest traces of H_2O_2 from any kind of watery liquid.

However the major drawback with the use of enzymes for commercial applications is the fact that they only work under very specific conditions and therefore need a precisely controlled environment for optimal activity and selectivity towards the desired products. They are extremely sensitive to changes in temperature, pH and salinity of the surrounding liquid, and they need a constant supply of energy and other biochemical compounds in order to maintain their activity.

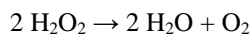
The solution to overcome most of these problems is to use microorganisms as microfactories, using them like a black box in which many other processes can occur simultaneously and the precise conditions are less easily controlled. Two drawbacks are of importance to consider here: The catalytic rate of conversion will be significantly decreased due to (slow) diffusion of reactants into and products out of the cells, and secondly the

¹ Eukaryotic refers to cells in which the DNA is safely stored in a protective cell nucleus.

presence of other active enzymes might affect the overall selectivity towards the desired product due to other conversion reactions.

However, the much lower costs and easier use of microorganisms compared to the use of more selective but expensive well-isolated enzymes, favours the use of microorganisms in many commercially available processes.

This will also be the case in this kinetic experiment in which we will use baker's yeast to convert hydrogen peroxide to water and oxygen.



Nowadays simple chemistry experiments based on baker's yeast and hydrogen peroxide are easily accessible on internet and they clearly demonstrate the power of microorganisms in converting compounds like H_2O_2 . In combination with ordinary dish soap and higher concentrations of hydrogen peroxide (typically 10-30% w/w), a thick, fast-growing foam can be obtained, which is ironically called *elephant toothpaste*². The bubbles that form the soap are filled with pure oxygen generated by the yeast, and water vapour formed by the generated heat of the reaction.

However, under the conditions used in these videos this experiment is simply too fast (and dangerous) to use for any type of kinetic study, so we are forced to reduce the concentrations of all components drastically. Instead of the 30% w/w hydrogen peroxide we will use the much safer 3% w/w solution and instead of a yeast slurry or yeast paste we will use a yeast suspension.

The benefits of reducing the concentrations of both yeast and hydrogen peroxide will not only result in safer experiments, it will also allow us to better control the rate of formation and formed amount of oxygen.

Furthermore, due to the exothermic nature of the reaction, dilution will diminish any temperature increase, thereby making the experiment near-isothermal.

The scope of this kinetic experiment is to gain insight in the catalytic performance of yeast as a catalytic microfactory for the conversion of hydrogen peroxide to water and oxygen. Different questions related to this reaction will be put to the test, like how the performance of yeast changes as function of its concentration, the concentration of H_2O_2 and temperature.

Set-up

As described above the hydrogen peroxide is broken down to water and oxygen. The oxygen is then released as gas, and it is the amount of gas formed per unit of time that will quantify the H_2O_2 conversion rate per gram of yeast.

There are several ways to collect and quantify the amount of oxygen gas released, but not all of them are as accurate or simple to interpret as might be needed here.

Using pressure as the unit for measuring the amount oxygen formed would require knowledge about the exact volume of the system, as well as a very accurate pressure gauge. This in turn would require several calibrations of every piece of glassware as well as knowledge about the difficult mathematic calculations involved in the data analysis.

Another option would be to collect the gas in a water-filled graded cylinder submerged upside-down in a water bath. However, bubbling of the gas up into the cylinder will make it difficult to get accurate readings of the total volume of gas formed since the meniscus will be disturbed continuously.

In order to overcome both the pressure and bubbling problems, we will use low-friction ('friction-free') gas syringes to collect the oxygen gas. When placed in a horizontal position, the piston will slide smoothly and pressure build-up is prevented. Furthermore they are accurately graded and gas tight, so the volume increase observed is coming solely from the oxygen gas formed by the decomposition reaction and can therefore be used directly for further data analysis.

The syringes come in different sizes and absolute accuracies, and the preferred size depends mainly on the maximum amount of oxygen gas that will be formed, which in turn is determined by the amount of hydrogen peroxide used in each of the experiments (more about this can be found in the experimental section).

² A nice example video of the effect of different concentrations of yeast and H_2O_2 can be seen in this Youtube clip: <http://www.youtube.com/watch?v=4N0m95PExHY> (Science Bob's Crazy Foam Experiment)

Apart for the low-friction syringes, the following glassware and equipment is needed:

Set-up

- 1x tripod with clamps
- 1x one-neck round-bottom flask (50 mL)
- Glass tube connector fitting the reaction flask
- Flexible tubing (approx. 50 cm)
- 1x gas syringe (10, 20, 30 or 100 mL)

Preparation (for exp. 1 & 2)

- Dried yeast (packages of approx. 12 gram) or fresh yeast (50 gram)
- Hydrogen peroxide 3 wt% (100 mL - preferably at room temperature)
- Distilled water
- 4x glass beakers (100 or 150 mL)
- 1x graduated cylinder (100 mL)
- 1x (small) spoon
- Weighing cups
- 1x volumetric flask (250 mL) with stopper
- Pasteur's pipettes with balloon

Reaction (for exp. 1 & 2)

- 3x graduated pipettes (differing in size from 2 – 25 mL)
- 3x pipette balloons
- Stopwatch

For experiment 3 (still in developing phase)

In addition to the above-mentioned lists, this experiment requires some additional glassware and equipment:

- 3x water baths set at different temperatures (e.g. at 30, 40 and 50°C)
- Several more tripods and clamps (1-2 per bath)
- 9x reaction tubes (approx. volumes 20-25 mL)
- Digital thermometer
- Container with water/ice mixture (approx. similar size to a water bath)

The set-up for each of the experiments is the same. The reaction takes place in a small round-bottom flask which will be closed by a tube connector leading to the gas syringe. This all is supported with clamps onto a tripod. In the case of experiment 3 (the temperature dependence test) the reaction tubes containing the liquids and reagents for heating them to the desired temperature, need to be supported by one or several tripods, and also the round-bottom flask in which the reaction will be performed need to be placed in the water bath in order to minimize temperature changes during the start-up of the experiment.

Preliminary test

Yeast is a natural product, meaning that it is alive and can die at any given moment. Contrary to lifeless catalytic systems this means that a given amount of yeast can behave differently from any other similar amount obtained from a different source. The differences can be as large as a tenfold increase/decrease in activity, even if the batches are coming from the same supplier and have the same expiring date (i.e. when comparing two bags from the same box of yeast).

The same holds when comparing dried yeast with fresh, wet yeast. But as a rule of thumb does 12 grams of dried yeast correspond with 50 grams of fresh, wet yeast.

This rather unpredictable activity means that we need to run a preliminary test run in order to estimate the apparent activity of the yeast. This does not need to be very accurate at this stage, because we only want to get an estimation of the activity, so that we can adapt the yeast concentration to a workable reaction rate window.

In order to get an estimation we need to perform the following the steps:

- Make 100 mL of a yeast suspension in a 100 or 150 mL beaker (dry yeast: 12 g/L ; wet yeast: 50 g/L). It might be difficult to suspend the wet yeast evenly, so it is advised to crumble the wet yeast in tiny pieces before weighing.

NOTE: We make a suspension and not a solution (yeast does not dissolve), so the yeast will start to settle at the bottom of the beaker after some time. It is therefore recommended to homogenize the suspension every time you need to take some out of the stock suspension!

- Prepare the set-up as described above with the 30 mL syringe and make sure you have everything ready before you start adding the reactants to the round-bottom flask!

- Add 8.0 mL of the yeast suspension (homogenize first!) and 18.0 mL of distilled water to the reaction flask.

- Add 4.0 mL of 3 wt% H₂O₂ and close the system immediately with the tube connector! Start the stopwatch!

NOTE: By closing the system you will trap some extra air in the system and you will see that the piston in the syringe will move slightly (< 1 mL). This will not affect the measurement since we don't need to know the absolute volume, but only the volume increase in time!

- Monitor the increase in volume while measuring the time passing, until you reach the 10 mL mark! Stop the time and unplug the tube connector!

IMPORTANT: *In all the experiments we will be using syringes that are smaller than the total volume of gas that will be formed (to improve accuracy), so whenever you are finished with collection the necessary data points, you should open the system immediately! The piston is made out of glass and can brake easily when it drops out of the syringe! And they are not cheap!*

- Check the time needed to form approx. 10 mL of gas. In order to fit nicely in the window we want to work in, this should be close or just over 90 seconds!

If the time measured to form 10 mL of oxygen gas lies outside the 80-120 seconds range, we need to readjust the concentration of the yeast (the same batch as used for this test!) in such an extend that it will fall in this workable time range.

Experiments

This section will be split into three different experiments, and each group will do only one of them. The reaction rate for the conversion of hydrogen peroxide into water and oxygen is dependent on several external parameters and the three most important ones will be tested her:

- 1) Dependence of the rate on the yeast concentration
- 2) Dependence of the rate on the H₂O₂ concentration
- 3) Dependence of the rate on temperature

Independent of the type of experiment there are several important steps and notes that hold for all three tests:

- Since the reaction between yeast and H₂O₂ starts immediately, the peroxide is added *as the last step*, just before closing the system and starting the time.

- Since we only know the concentrations accurately at the beginning, but not during the reaction, we will need to find the (initial) reaction rate at the very first points (done by taking the tangent of the volume-time curve – see Data analysis for further details). Therefore there is no need to let the reaction run to the complete end!

- As a rule of thumb we will only need to measure until approx. 40% of the maximal amount of oxygen that theoretically can be formed, is formed!

- We need many more data points at the beginning of the curve than at the end, so that the accuracy of the initial slope will increase (for more details, see Data analysis).

- And in order to increase the accuracy of these initial points even further, we will be using syringes that are approx. 30-50% smaller than the total volume of gas that will be formed. But this means that we need to open the system immediately after we collected our last data point in order to avoid that the glass piston will be pushed out of the syringe and breaks!

- As a last point it should be mentioned that we always will use a constant total amount of liquid in the reaction flask. Apart from the fact that we in that case easily can interchange amounts and concentrations, there is another reason for keeping the total liquid volume constant which is related to the solubility of oxygen in water (i.e. 0.28 mmol/L at 20°C). **Explain!**

Experiment 1: Dependence of the rate on the yeast concentration

In this experiment we will keep the concentration of hydrogen peroxide constant and only vary the amount of yeast, so that we will be able to derive the rate of reaction per mass unit of yeast (dry or wet). Since it is expected to find a linear relation between the initial reaction rate and the amount of yeast, we can use statistical analysis to find the standard deviations in both the slope and the intersection of the final 'rate-yeast' curve (more about this will be explained in the Data analysis chapter).

The experimental procedure is rather similar to the preliminary test, although we will need to change the conditions in order to get different concentrations of yeast.

In this experiment we will always use 4.0 mL 3 wt% H₂O₂! The yeast suspension and water will make up the remaining 26.0 mL, so the total volume will *remain constant*.

- Make a plan for the amounts of yeast suspension you want to use in the different tests. Take at least 6-7 points! The volume of yeast suspension should lie between 2.0 and 20.0 mL and the additional water is adjusted accordingly to keep the total reaction volume constant.

NOTE 1: Higher concentrations of yeast will result in faster reactions and might need some practice first!

NOTE 2: You might find out later that you might measure some of the conditions again to improve statistics, but you might also want to measure some of the points multiple times! This all depends on the time needed to measure the first series of 6-7 points.

- Make a yeast suspension with a concentration based on the preliminary test in a 250 mL volumetric flask! It is advised to weigh the yeast needed for the calculated concentration, place it in the flask and add approx. 50-100 mL of water. It's easier to shake the flask when half full than completely filled! Homogenize as good as possible before adding the rest of the water to just below the mark. Add the last water with a Pasteur's pipette and homogenize the suspension again.

- In order to make things easier during pipetting, fill three small beakers each with one of the three liquids needed (yeast suspension, hydrogen peroxide and water).

- Prepare the set-up with the proper syringe (calculate the maximal amount of oxygen that can be formed!).

- Make on forehand a table with the volumes you want to measure. Note that we are mainly interested in the first part of the total curve!

QUESTION: Why is it better to measure time at regular volume intervals and not the other way round, which would have been more logical?

- Perform the different experiments. Remember to add the yeast suspension and water first! In that way the yeast is suspended even better before the H₂O₂ is added.

See the Data analysis section for more information about retrieving the initial reaction rates and the statistical analysis.

Experiment 2: Dependence of the rate on the H₂O₂ concentration

In this experiment we will investigate the dependence of the reaction rate on the hydrogen peroxide concentration. From the obtained data we will be able to extract several intrinsic kinetic Michaelis-Menten parameters by use of a Lineweaver-Burk plot. More details about the extraction of these parameters can be found in the Data analysis chapter, while more background on Michaelis-Menten kinetics and the use of Lineweaver-Burk plots can be found in most commonly used study books about kinetics.

The experimental procedure is rather similar to the preliminary test, with the difference that we will change the amount (and thus concentration) of the hydrogen peroxide while keeping the amount of yeast and the total reaction volume (i.e. 30 mL) constant. The amount of yeast suspension will be kept at 15.0 mL at the adapted concentration as calculated from the preliminary test.

- Make a plan for the amounts of 3 wt% H₂O₂ you want to use in the different tests. Take at least 5 points in the range 1.0 – 5.0 mL.

NOTE 1: The points should not be too far apart, since different amounts of H₂O₂ will result in different maximal amounts of oxygen gas and thus require different syringes, which in turn will add to inaccuracies in the final results.

NOTE 2: Higher concentrations of H₂O₂ will result in faster reactions and might need some practice first!

- Make a yeast suspension with a concentration based on the preliminary test in a 250 mL volumetric flask! It is advised to weigh the yeast needed for the calculated concentration, place it in the flask and add approx. 50-100 mL of water. It's easier to shake the flask when half full than completely filled! Homogenize as good as possible before adding the rest of the water to just below the mark. Add the last water with a Pasteur's pipette and homogenize the suspension again.

- In order to make things easier during pipetting, fill three small beakers each with one of the three liquids needed (yeast suspension, hydrogen peroxide and water).

- Prepare the set-up with the proper syringe (calculate the maximal amount of oxygen that can be formed for each of the experiments!).

- Make on forehand a table with the volumes you want to measure. Note that we are mainly interested in the first part of the total curve!

QUESTION: Why is it better to measure time at regular volume intervals and not the other way round, which would have been more logical?

- Perform the different experiments at least twice (this will give you at least 10 data points at the end). Depending on the time needed, you might consider to measure some of the points a third time. This will increase the accuracy even further and it will give you an indication about the statistical error in the measurements when plotted in a Lineweaver-Burk plot.

See the Data analysis section for more information about obtaining the initial reaction rates, making a Lineweaver-Burk plot and retrieving the Michaelis-Menten parameters.

Experiment 3: Dependence of the rate on temperature

(This experiment is still in the development stage and hasn't been tested yet, but it might show the students that experimental work is mostly trial and error and not just a repetition of well-defined experimental steps)

The rate of decomposition of hydrogen peroxide is not only dependent on the initial concentrations of the reactants, but also on temperature. Heat is generally the main driving force for reactions to take place, (unwanted) side reactions included, since it provides the energy needed to overcome intrinsic energy barriers. By measuring the initial reaction rates at different temperatures, it is possible to obtain the overall (apparent) activation energy by means of the Arrhenius equation (more details can be found in the Data analysis chapter).

Since we will measure the temperature dependence of the decomposition reaction, the concentrations of both yeast and hydrogen peroxide will be kept constant: 4.0 mL 3 wt% H₂O₂ and 4.0 mL of a yeast suspension at the adapted concentration obtained from the preliminary test (total volume: 30 mL).

Due to a limited availability of water baths we will not be able to measure at more than 5-6 different temperatures. The temperature intervals will be set for the elevated temperatures, due to the temperature set points of the water baths, but for the lower temperature points there is slightly more freedom, since ice can be used to cool down from room temperature.

NOTE: *It will take time to get your reactants and reaction flask at a given temperature, so make sure that you start in time!*

The following temperature set points or temperature ranges can be used in this experiment:

0°C – 5°C	: This can be obtained by cooling in a water/ice bath
10°C – 15°C	: Water from the tap can be used as coolant in this case
20°C – 25°C	: No cooling or heating is necessary here
30°C / 35°C	: Water bath
40°C / 45°C	: Water bath
50°C / 55°C	: Water bath

The actual set points for the water baths will depend mainly on their limitations (heating/cooling capacities).

- Check that the water baths are working and that you have a supply of ice in case you need it!

- Make the yeast suspension according to the results from the preliminary test in a 250 mL volumetric flask! It is advised to weigh the yeast needed for the calculated concentration, place it in the flask and add approx. 50-100 mL of water. It's easier to shake the flask when half full than completely filled! Homogenize as good as possible before adding the rest of the water to just below the mark. Add the last water with a Pasteur's pipette and homogenize the suspension again.

- Build a set-up with tripods and clamps next to the each of the baths that will be able to support the reaction tubes and the reaction vessel during the preheating (precooling) period.

NOTE: Make sure that you can heat/cool enough liquid (yeast suspension, H₂O₂ and water) for at least two experiments, just in case you need to redo some of them!

- Fill the reaction tubes with enough liquid (homogenize the yeast suspension before you transfer it!), and check the temperature regularly. Homogenize the yeast suspensions from time to time in order to get everything heated/cooled evenly. You can start the experiments as soon as the temperature of each of the liquids is close to the wanted reaction temperature!

- Make on forehand a table with the volumes you want to measure. Note that we are mainly interested in the first part of the total curve!

QUESTION: Why is it better to measure time at regular volume intervals and not the other way round, which would have been more logical?

- Perform the different experiments (twice if necessary). Remember to add the yeast suspension and water first!

See the Data analysis section for more information about the use of the Arrhenius equation and the retrieval of the apparent activation energy for the decomposition reaction in the presence of yeast.

Data analysis

Initial reaction rate (all experiments)

The experiment we are performing here is a typical batch reaction, which means that we will run out of hydrogen peroxide after a while. This means that the concentration changes (decreases) in time making it difficult to estimate the rate of reaction at any point in time, except for the very first point where the concentration has hardly changed from the initial, known concentration of hydrogen peroxide.

In order to obtain the initial reaction rate for each individual experiment, we need to plot the formed amount of oxygen as function of time. Any tangent of the obtained curve will then express the reaction rate (dV/dt) as a unit of volume of oxygen formed per unit of time (e.g. $\text{mL}\cdot\text{s}^{-1}$ or $\text{mL}\cdot\text{min}^{-1}$). This can then be converted to moles of H_2O_2 decomposed per unit of time, and since the amount of yeast remains constant during the reaction since it is the catalyst, we can even express the reaction rate per gram of yeast (dry or wet).

But as mentioned before we are not simply interested in any tangent of the obtained curve, but only in the tangent at the initial point of the reaction. This tangent can be obtained in two ways: Either by roughly estimating what the tangent at the first point should be or by approximating it with a straight line through the first 3-4 data points. It is this latter approximation that is the scientific approach to the problem and if one has taken enough data points at the beginning of the measurement, this will give very similar results to the rough estimations based on only the very first point.

- Plot for each of the experiments the formed amount of oxygen as function of time and determine the initial reaction rates.

QUESTION 1: You will notice that most of the reactions show a small delay before the evolution of oxygen gas really starts. Why? Give one or more possible explanations (there are at least three!).

QUESTION 2: As mentioned in the preliminary test section you will trap a small amount of air every time you close the system prior to starting the measurement. Secondly you will never be able to start the timer at precisely the same moment at the start. And thirdly nobody will read off the numbers on the syringe in exactly the same way (the human factor). However the determination of the initial rates will not be affected by these three things at all. Why?

(Hint: Think about the difference between relative and absolute numbers)

Statistical analysis (exp. 1 only)

After we have obtained the initial reaction rates, we can plot them as function of the amount of yeast, and since yeast will not be converted or affected by the reaction and is present in excess, we can expect a linear relationship between the initial rate and the amount of yeast.

However, due to the rather rough approximation in obtaining the initial rates can the accuracy of this linear relation be affected negatively. In order to get some insight in this problem, we will calculate the standard errors in both the slope and the intersection of the obtained linear curve.

- Plot the different initial reaction rates as function of the amount of yeast.

- Determine the slope and intersection. Most graphical computer programmes have no problem with this, but none will give you the statistical errors in the slope and intersection, nor in the individual data points.

The method used for determining the best linear slope and intersection is in nearly all cases the **method of least squares**³. Increasing the number of data points will not influence the slope or intersection very much, but it will in most cases decrease the statistical error.

Before starting the calculations, let us define the different parameters we need for this. The formula for the linear relation can be expressed as $y = ax + b$, with y being the initial reaction rates, x being the amount of yeast used, a being the slope (dy/dx) and b being the intersection with the y -axis.

When using the same symbols, the formulae for the slope and intersection, as most computer programmes will give you automatically, are:

³ A good and short description of how to use this method for calculating the slope, intersection and the errors in both of them can be found in Quantitative Chemical Analysis from D.C. Harris. But many other analytical books will describe the method in a similar way.

$$a = \frac{n \sum (x_i y_i) - \sum x_i \sum y_i}{n \sum (x_i^2) - (\sum x_i)^2}$$

$$b = \frac{\sum (x_i^2) \sum y_i - \sum (x_i y_i) \sum x_i}{n \sum (x_i^2) - (\sum x_i)^2}$$

where n is the total number of points on the linear line.

From the above numbers we need to calculate the deviation d_i between each measured y-coordinate and the best linear fit ($ax_i + b$):

$$d_i = y_i - ax_i - b \quad (\text{this number can be either positive or negative})$$

We will use this deviation to calculate the error in the data points (s_y), which in turn can be used to calculate the errors in both the slope (s_a) and the intersection (s_b):

$$s_y = \sqrt{\frac{\sum (d_i^2)}{n-2}}$$

$$s_a = s_y \sqrt{\frac{n}{n \sum (x_i^2) - (\sum x_i)^2}}$$

$$s_b = s_y \sqrt{\frac{\sum (x_i^2)}{n \sum (x_i^2) - (\sum x_i)^2}}$$

When reporting the values and their errors, keep track of the significance! Round off according to the rules, based on the significance of the error values.

Michaelis-Menten kinetics & Lineweaver-Burk plot (exp. 2 only)

Kinetic models are used to determine the relation between the reaction rate, the reactant and product concentrations, and intrinsic parameters like the rate constants. In organic and inorganic chemistry we use Langmuir-Hinshelwood-Hougen-Watson (LHHW) models, but in biocatalysis those models are mostly based on the Michaelis-Menten expression, which is actually identical to the simplest of the LHHW models.

Apart from some exotic ones, all enzymes have a single catalytic centre in which only one reactant molecule at the time can be converted. This makes the kinetic models rather simple, and we can describe the overall reaction mechanism as follows:



with E being the enzyme, S being the reactant (also called substrate in biochemistry) and P being the product or products.

Based on this reaction scheme, the Michaelis-Menten expression becomes:

$$v = k_2 E_0 \frac{[S]}{K_m + [S]}$$

where v is the reaction rate, k_2 is the rate constant for the forward reaction from ES to E + P, E_0 is the total enzyme concentration, $[S]$ is the reactant concentration and K_m is the Michaelis constant (which is the

equilibrium constant for the formation of the ES complex). Furthermore there is defined a parameter V_m , which is the maximum reaction rate at which all enzyme sites are occupied and is equal to the product of the total enzyme concentration and the rate constant k_2 . The expression then becomes:

$$v = V_m \frac{[S]}{K_m + [S]}$$

The conventional way to determine the Michaelis-Menten parameters K_m and V_m from experimental data is to invert this expression to the form:

$$\frac{1}{v} = \frac{1}{V_m} + \frac{K_m}{V_m} \cdot \frac{1}{[S]}$$

When plotting $1/v$ as function of $1/[S]$ this yields the so-called Lineweaver-Burk plot, with K_m/V_m being the slope of the line, $1/V_m$ being the intersection with the y-axis and $-1/K_m$ being the intersection with the x-axis.

- Make a Lineweaver-Burk plot and calculate the Michaelis-Menten parameters. Keep track of the units you have used in your graph!

Arrhenius plot (exp. 3 only)

As mentioned in the experimental section is heat the main driving force for any type of reaction, since the energy can help the system to overcome any of the energy barriers. The overall activation energy needed is in most cases equal or related to the largest barrier in the whole process. If that one can be overcome, the smaller energy barriers will be overcome easily as well.

At a first approximation the overall (apparent) reaction rate is related to the highest energy barrier via the Arrhenius equation:

$$r = A e^{-E_{act}/RT}$$

with r being the reaction rate, A being a temperature-independent rate constant (by first approximation), E_{act} being the activation energy, R being the gas constant ($8.3145 \text{ J}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$) and T being the temperature in Kelvin. We can rewrite this to:

$$\ln r = \ln A - \frac{E_{act}}{R} \cdot \frac{1}{T}$$

When plotting the natural logarithm of the reaction rate as function of the inversed temperature, the slope of the line will be $-E_{act}/R$ from which we can calculate the overall (apparent) activation energy of the decomposition reaction of hydrogen peroxide by baker's yeast.

It should be noted here that we will not be able to get the individual activation energies since we only investigate the overall reaction and not the individual reaction steps.

- Make an Arrhenius plot and calculate the apparent activation energy. Keep track of the unit you have used in your graph!

Optional: Calculate the uncertainty in the activation energy (see the paragraph about statistical analysis for more information about this).