The resazurin experiment – Investigating the effect of concentration on rate an enzyme – controlled reaction in yeast

Preliminary experiment and prediction

The experiment we did was to see the colour change in resazurin when it was placed in a warmed water bath in different concentrations of yeast suspensions. We did this to see the rate of reaction in the yeast when it was heated to see how long it would take for the resazurin to change from blue to clear. There were different suspensions of yeast tried out each time.

I predict that the higher the concentration of yeast, the less time it would take to change the colour of resazurin because there would be more of the enzyme dehydrogenase for the resazurin to react with and therefore the colour would change quicker. Dehydrogenase is a catalyst that aids the process of reduction by speeding up the oxidation of the yeast. The reason the resazurin seems to disappear when the reaction is finished is because it changes colour when hydrogen is released through the yeasts process of anaerobic respiration. Glucose is gradually broken down in this process and energy is released. Hydrogen is also released in a process called oxidation, and when hydrogen is released the resazurin gains this hydrogen. The gaining of hydrogen is called "reduction" and molecules which gain this hydrogen are said to be reduced. I think that this will happen faster in my experiment when there is less distilled water in each yeast suspension.

We did two experiments. One to measure the optimum temperature to use the water bath for the yeast to react, and one to determine the best amount of resazurin dye to use.

Experiment one – Finding the optimum temperature of water bath:

Changed: temperature of water in water bath (25, 49 and 72 degrees C)
Kept Constant: volume of yeast (10ml)
Volume of resazurin (2ml)

Results:

25 degrees C: n/a (took longer than 30 min)

49 degrees C: 123 secs
72 degrees C: 30 mins

I found that the best temperature to put the water bath on is at 49 degrees C as it seems to be the optimum temperature at which the yeast reacts.

<u>Experiment 2</u>- Finding the best amount of resazurin to use:

Changed: volume of resazurin (1ml, 2ml & 3ml)
Kept constant: Temperature of water bath (49 degrees), volume of yeast (10ml)

Results:

1ml: 149 secs
2ml: 158 secs
3ml: 169 secs

I found that the best amount of resazurin to use was 1ml because it doesn't take as long and there's less chance of over diluting the yeast with it.

<u>The final equipment to use</u>:
1x boiling tube
2x measuring cylinder
Yeast
1x water bath
1x pipette
1x stopwatch/timer

<u>Final controlled variables</u>:

-49 degrees C in water bath - because 72 degrees C takes too long, and so does 25 degrees C.

-1ml resazurin- because it has the quickest reaction times.

<u>Final method</u>

I will measure out 10 ml of yeast suspension at different concentrations and place in boiling tube
Place in water bath which is at 49 degrees C

Add 1ml of resazurin using a pipette
Time with stopwatch until mixture is clear.

Yeast variables: Suspension of yeast in distilled water (%)

100
80
75
60
50
20

Final results

Yeast suspension (%)	**Time taken to change colour (s)**			
	Exp 1	**Exp 2**	**Exp 3**	**Average**
100	**4.38**	**7.15**	**6.47**	**6.00**
80	**193.94**	**195.76**	**x**	**194.85**
75	**252.56**	**259.12**	**254.78**	**255.49**
60	**268.63**	**266.93**	**x**	**267.78**
50	**336.73**	**337.84**	**x**	**337.29**
25	**n/a**	**n/a**	**n/a**	**n/a**

I did not repeat the experiments for 80, 60 and 50 as they were within 2 seconds of each other therefore I thought my results would be reliable enough.
25% yeast suspension was not carried out as it took too long to react.

(See attached graph for results as graph form)

The graph has a fairly average negative correlation. The plotted data seems to go in a straight line which is why the line I have drawn is a straight line. The pattern in my graph is the higher the percentage of yeast in suspension in distilled water, the less time it takes for the resazurin to lose its colour. This proves that my prediction was correct. As in my prediction, the more yeast in suspension, the more dehydrogenase which therefore the faster the oxidisation of the yeast, and the faster the reduction of the resazurin, which changes to clear when it is fully reduced.

Evaluation

I think that some of my results were a little "out" due to human error and the fact that I only had my eye to judge on whether the substance had become clear or not. This could be resolved if we had an automated device that measure the amount of light let though the boiling tube. Also, other groups were using out water bath, so the average temperature of the water bath would be slightly skewed by the fact there were so many colder boiling tubes being added to it. Also, I think that the measuring equipment we used wasn't accurate enough. If we had more mechanical measuring pipettes, the ones that use vacuum to suck up liquids, then the measurements of yeast and resazurin would be a lot more accurate, and therefore lead to a more reliable experiment.

If I did the experiment again I would also use a lot more variables of yeast suspension, such as ranging from 10% to 100%, going up in 10's. This would provide me with more valid data. Also if I repeated the experiment the results would be a lot more reliable.