Biology of Cells Practical 5: Lysosome Activity

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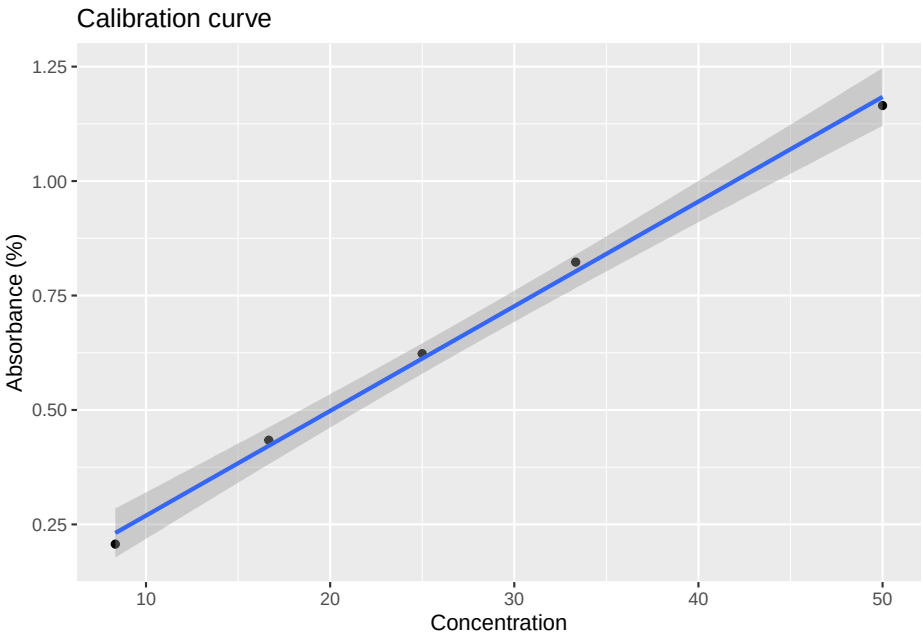
Contents

(B) Calibration curve

```
## # A tibble: 5 x 3
##     vol  conc   abs
##   <dbl> <dbl> <dbl>
## 1   100 33.3  0.823
## 2   150 50    1.16
## 3    50 16.7  0.434
## 4    75 25    0.623
## 5    25  8.33 0.207
```

Curve graph




```
## [1] "The linear model is: 0.0407567567567565 x + 0.0228616216216216"
```

```
## [1] "The r squared value is: 0.99700197536746"
```

Questions

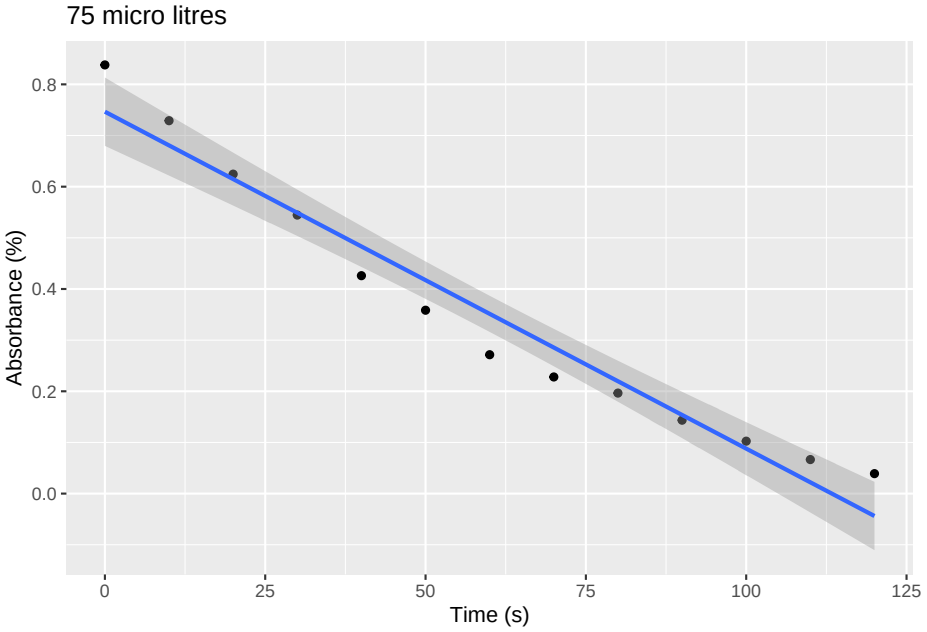
1. Yes, it is linear, with a high R-squared value.

(C) Lysozyme activity assay

```
##    time     75     50     30     15
## 1     0 0.8380 0.9740 0.8335 0.7945
## 2    10 0.7290 0.9070 0.7865 0.7435
## 3    20 0.6245 0.8515 0.7505 0.7325
## 4    30 0.5445 0.8190 0.7115 0.7100
## 5    40 0.4260 0.7615 0.6650 0.6685
## 6    50 0.3585 0.6980 0.6170 0.6530
## 7    60 0.2715 0.6440 0.5555 0.6085
## 8    70 0.2280 0.5765 0.5305 0.5875
## 9    80 0.1965 0.5335 0.4845 0.5650
## 10   90 0.1435 0.4910 0.4645 0.5160
## 11  100 0.1025 0.4685 0.3960 0.5090
## 12  110 0.0665 0.4035 0.3965 0.4975
## 13  120 0.0390 0.3840 0.3245 0.4600
```

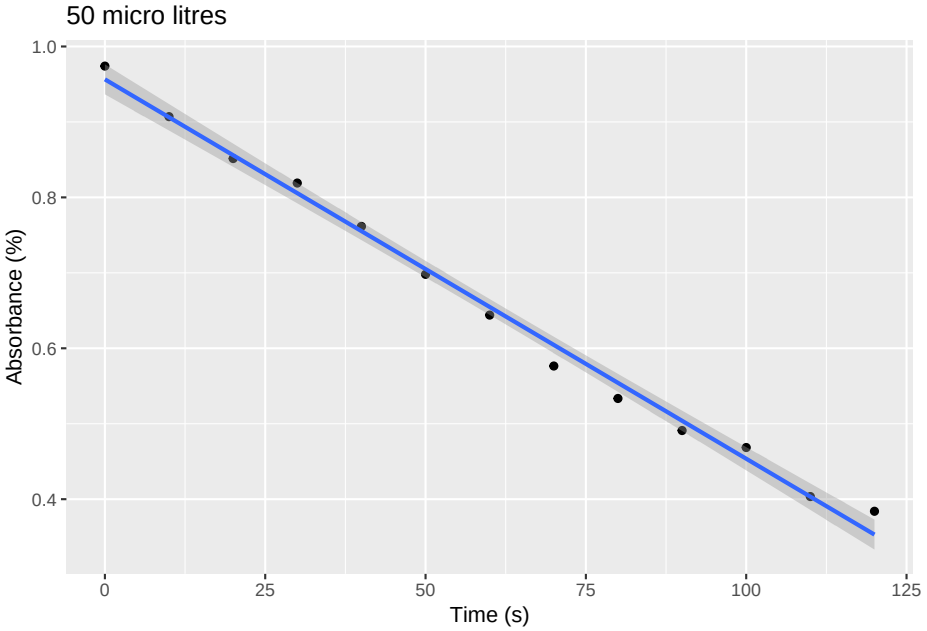
Inital rates of reactions, reaction graphs

For our reaction graphs, these were meant to have a level of logathrimic decay, however due to the lack of time, we were unable to complete this, and thus have done a linear fit for the initial rate of reaction.




```
## [1] "The equation for the initial rate is: 0.83175 x + -0.00985"
```

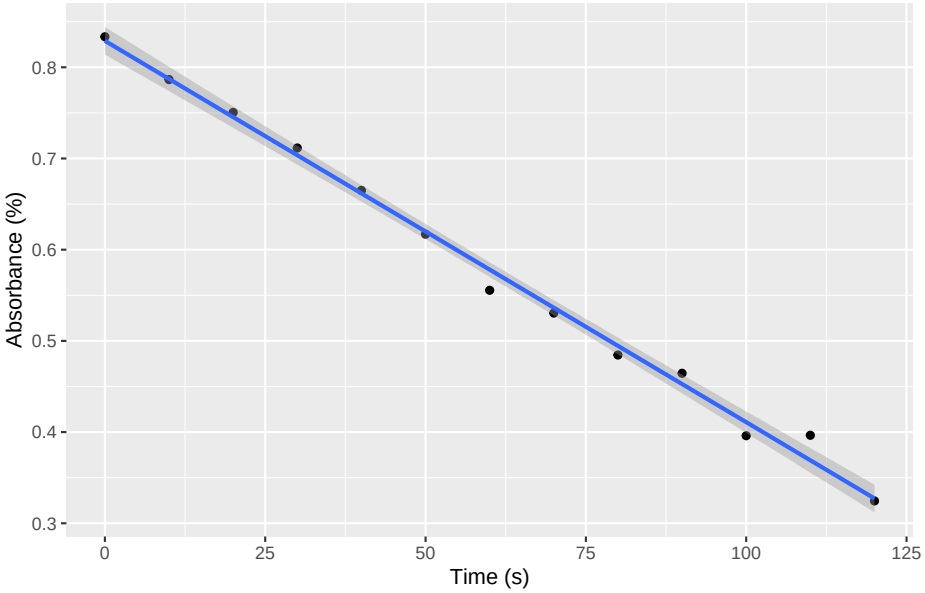
```
## [1] "The r squared value is: 0.995276099422464"
```




```
## [1] "The equation for the initial rate is: 0.96595 x + -0.00520499999999999"
```

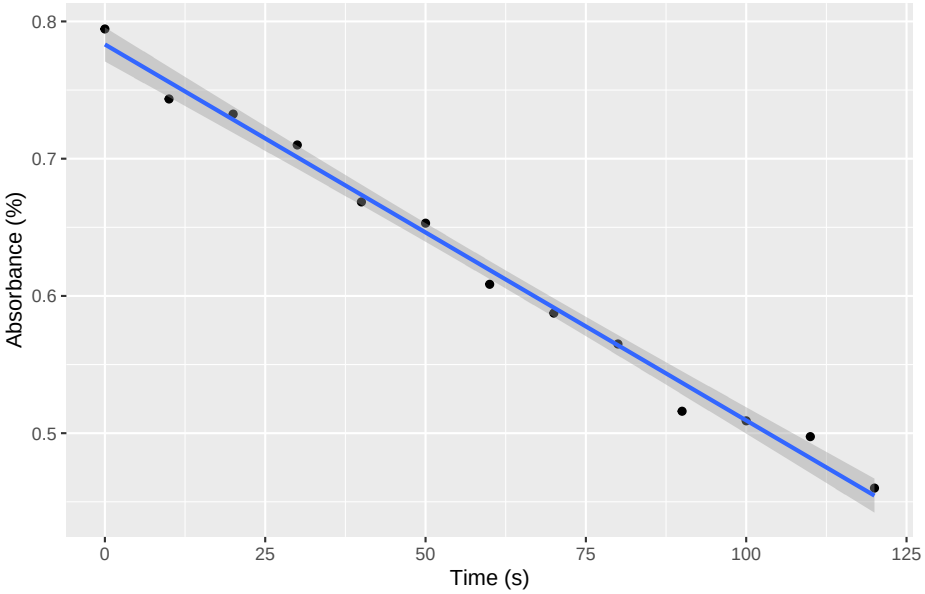
```
## [1] "The r squared value is: 0.978038203453925"
```

30 micro litres




```
## [1] "The equation for the initial rate is: 0.8308 x + -0.00402"
## [1] "The r squared value is: 0.996817172464841"
```

15 micro litres




```
## [1] "The equation for the initial rate is: 0.7848 x + -0.002645"
## [1] "The r squared value is: 0.912440698413734"
```

Rate graph

```
##   volume     rate
## 1     75 0.009850
## 2     50 0.005205
## 3     30 0.004020
## 4     15 0.002645
```

Questions

1. The decrease in OD600 is linear with time, despite expecting a curve that eventually plateaus. Possible reasons for this are that not enough time was allowed for the rate of reaction to plateau and that the solution is at a non-ideal temperature, so there is another limiting factor limiting the rate of reaction other than the substrate.

2. It is linear, despite expecting a curve that plateaus at higher concentrations. This may be because there were not enough data points, and hence are only looking at the linear part of the curve.

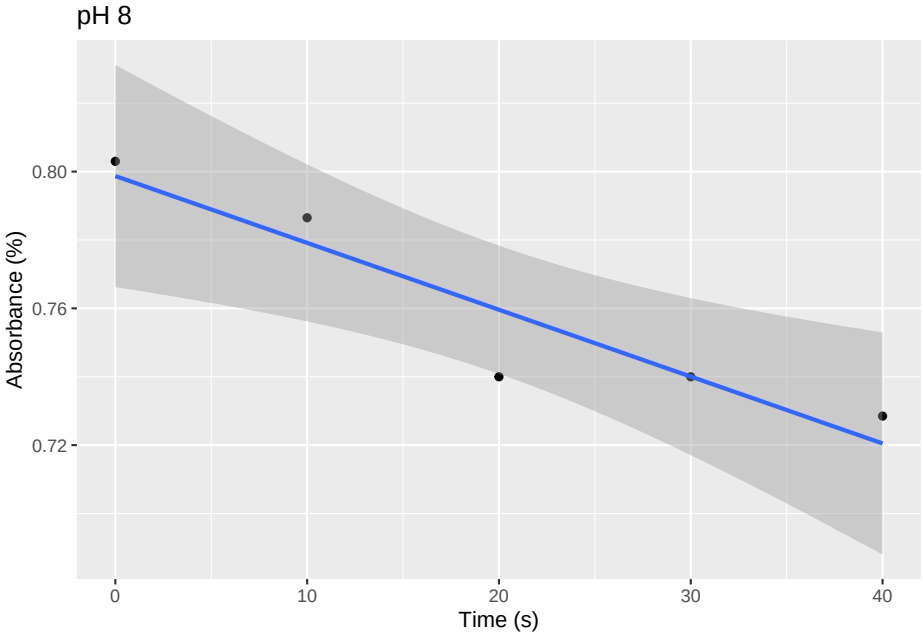
(D) The effect of pH on activity

```
##   time      8   7.2    5.8
## 1    0 0.8030 1.233 0.8735
## 2   10 0.7865 1.198 0.7850
## 3   20 0.7400 1.143 0.7030
## 4   30 0.7400 1.139 0.6295
## 5   40 0.7285 1.091 0.5370
```

Graph of rate against pH

We also included the 50 micro litre reaction in this graph, as it was the same volume of reactants but a pH of 6.4.

```
##    pH        rate
## 1 8.0 0.001955000
## 2 7.2 0.003430000
## 3 5.8 0.008285000
## 4 6.4 0.005028022
```



Questions

1. That the lyzosyme enzyme works best at an acidic pH (of the pHs studied, the one which had the highest enzyme activity was 5.8).
2. It does, as the amino acids glutamine and asparagine that are involved in the catalysis of peptidoglycan require the pH to be slightly acidic so that glutamine is protonated.

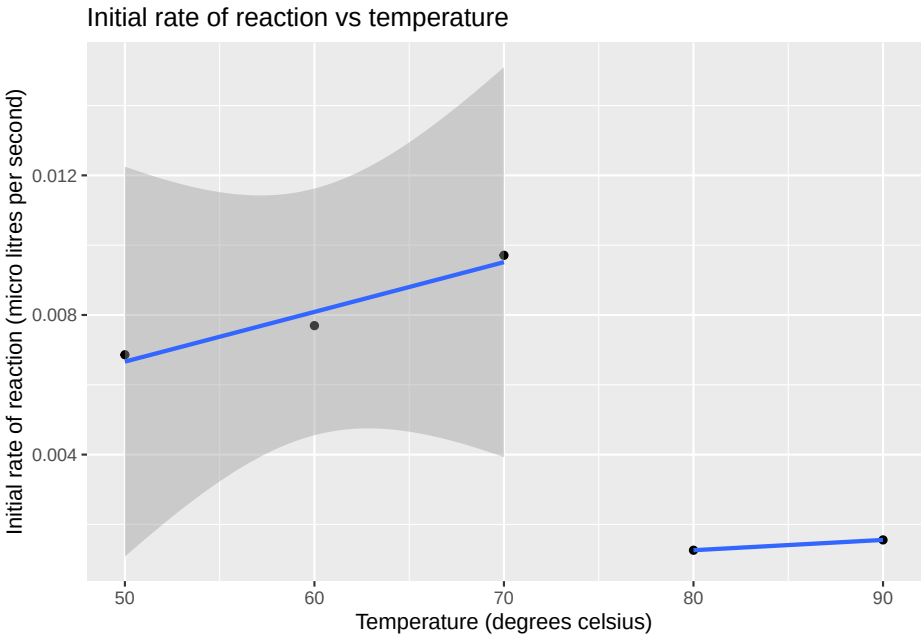
(E) The effect of temperature on activity

Please note this data was collected from other students however the analysis is our own

```
##   time     50     60     70     80     90
## 1    0 0.5155 0.5560 0.6810 0.8140 0.7570
## 2   10 0.4265 0.4550 0.5710 0.8865 0.8405
## 3   20 0.3645 0.3770 0.4625 0.8775 0.8145
## 4   30 0.3075 0.3255 0.3935 0.8590 0.8175
```

Graph of rate against temperature

```
##   temperature     rate
## 1          50 0.006860
## 2          60 0.007695
## 3          70 0.009710
## 4          80 0.001260
## 5          90 0.001555
```




```
##
## Call:
## lm(formula = rate ~ temperature, data = e_rate_table[1:3, ])
##
## Coefficients:
## (Intercept)  temperature
##  -0.0004617    0.0001425
```

Questions

1. There is a small increase in the initial rate of reaction from 60 to 70 degrees, followed by a sudden drop between 70 and 80 degrees to almost 0, and no change between 80 and 90.

2. The lysozyme may have this effect because at lower temperatures, the disulfide bridges are very strong and so minimise the effects of increased temperatures, hence it can still function. An increased temperature may also mean that the substrates have a higher kinetic energy, so there are more collisions between the substrate and the active site, hence explaining the increase in temperature between 60 and 70. This also makes sense because as lysozyme is involved in immune response, it must work well at higher temperatures than the normal body temperature (37 °C). However,

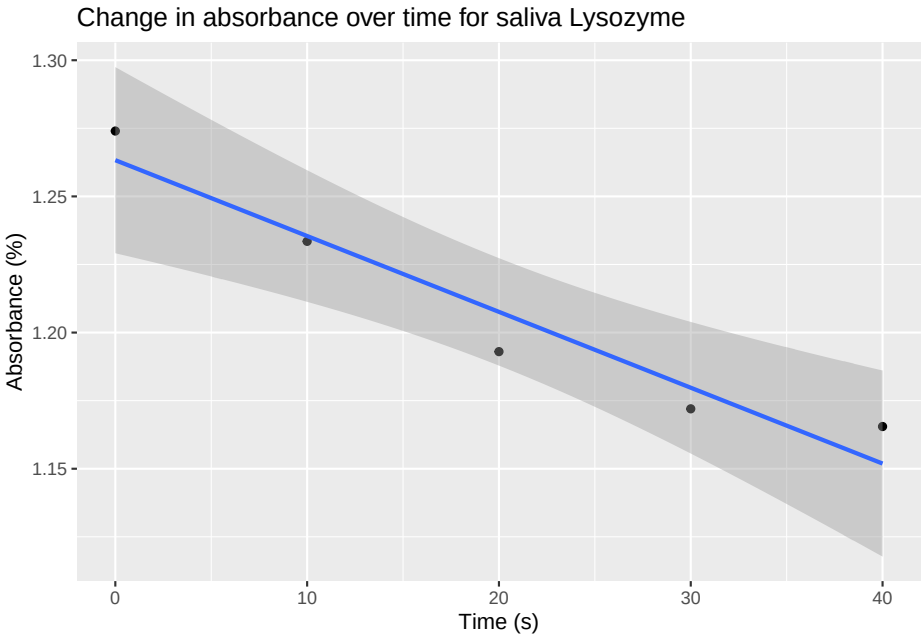
by 80 degrees, the disulfide bridged break, hence it denatured and the enzyme activity decreases.

(F) The search for lysozymes

```
##   time     1     2
## 1    0 1.320 1.228
## 2   10 1.260 1.207
## 3   20 1.170 1.216
## 4   30 1.147 1.197
## 5   40 1.145 1.186
```

Graph of rate against time

```
##   lysozyme     rate
## 1        1 0.002785
## 2        2 3.000000
## 3        1 0.002785
## 4        2 3.000000
```




```
## [1] "linear model using first three values: y =  -0.00405000000000003 x +  1.274"
```

Questions

1. The initial rate of reaction was -0.00405 % absorbance per second.
2. First boil and add a reducing agent to the solution containing the spit, and repeat the experiment (i.e. measure the change in absorbance over 30 seconds). If the breakdown of peptidoglycan is due to an enzyme, no change in absorbance should be seen in the experiment. To prove that

the enzyme is lysozyme, several dilutions of the saliva could be made. If a graph of initial rate over concentration was plotted, the Vmax and Km could be calculated. These could be compared to those of a solution containing only lysozyme, and if the results match up then they should be the same enzyme.

(G) The effect of denaturing the lysozymes on activity

Please note this data was collected from other students however the analysis is our own

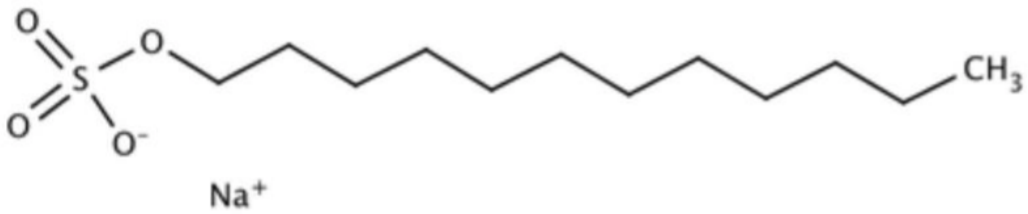
Enzyme treatment	Observation when added to bacteria
Heat	couldy white
DDT then Heat	cloudy white
SDS then Heat	cloudy white
DDT then SDS then HEAT	clear
HEAT then DTT	cloudy white
HEAT then SDS	cloudy white
HEAT then DTT then SDS	cloudy white

Questions

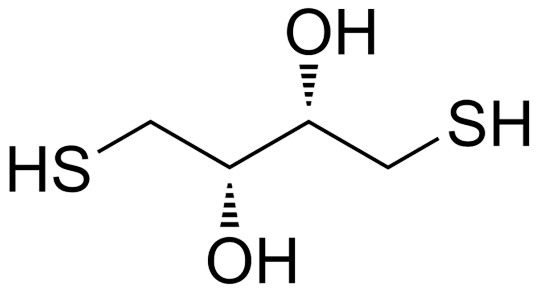
1. The hydrogen bonds, van der waal interactions and disulfide bridges holding the tertiary structure together are disrupted, and forms different bonds to find the most stable arangement, hence the enzyme loses the specific shape of the active site and can no longer catalyse the reaction. The enzyme is said to be denatured.

2. Because when the protein is heated it forms new hydrogen and van der waal bonds without order, which remain when the enzyme is cooled down, so it forms a different tertiary structure, so the active site is no longer complementary to the peptidoglycan, so cannot catalyse the reaction.

3. SDS is an organic sodium salt that is the sodium salt of dodecyl hydrogen sulfate. It has a role as a detergent and a protein denaturant. Image of SDS:



4. Dithiothreitol is the threo-diastereomer of 1,4-dimercaptobutane-2,3-diol. It has a role as a reducing agent. Image of DTT:


OH
SH
HS
OH