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Determination of the Kinetic constants, Km, Kcat and Vmax of yeast alcohol dehydrogenase (ADH) with three alcohol substrates (ethanol, propan ol and butan-1-ol).   
Introduction   
The objective of this experiment is to find the Km, kcat and Vmax values of yeast alcohol dehydrogenase. We used ethanol, propan-1-ol and butan-1-ol to carry out this experiment.   
With a molecular weight of 150. 000, yeast dehydrogenase is classified as an NAD linked enzyme. There are four subunits within NAD which consist of Zn2+ ions, the purpose of which is to aid in transfereing a hydride ion from the alcohol to NAD+ .   
The overall reaction equation is :   
RCH2OH + NAD+  RCHO + NADH + H+   
Method   
As written in the lab protocol sheet   
Results   
Table 1   
Ethanol   
Time   
Absorbance values for Substrate concentrations   
Seconds   
Minutes   
0. 080   
0. 040   
0. 020   
0. 010   
0. 005   
0. 000   
0. 000   
0. 000   
0. 000   
0. 000   
0. 000   
0. 000   
10. 000   
0. 167   
0. 267   
0. 120   
0. 081   
0. 073   
0. 035   
20. 000   
0. 333   
0. 369   
0. 165   
0. 126   
0. 133   
0. 061   
30. 000   
0. 500   
0. 439   
0. 264   
0. 169   
0. 178   
0. 098   
40. 000   
0. 667   
0. 550   
0. 318   
0. 217   
0. 216   
0. 127   
50. 000   
0. 833   
0. 600   
0. 384   
0. 258   
0. 252   
0. 155   
60. 000   
1. 000   
0. 725   
0. 439   
0. 308   
0. 287   
0. 178   
Fig. 1   
Fig 1. 1   
Fig 1. 2   
Fig 1. 3   
Fig 1. 4   
Table 2   
Propan-1-ol   
Time   
Absorbance values for Substrate concentrations   
Seconds   
Minutes   
0. 08   
0. 04   
0. 02   
0. 01   
0. 005   
0   
0. 000   
0. 000   
0. 000   
0. 000   
0. 000   
0. 000   
10   
0. 167   
0. 018   
0. 041   
0. 036   
0. 033   
0. 038   
20   
0. 333   
0. 053   
0. 061   
0. 044   
0. 039   
0. 040   
30   
0. 500   
0. 085   
0. 085   
0. 062   
0. 052   
0. 044   
40   
0. 667   
0. 115   
0. 111   
0. 079   
0. 035   
0. 052   
50   
0. 833   
0. 143   
0. 137   
0. 097   
0. 076   
0. 058   
60   
1. 000   
0. 168   
0. 159   
0. 112   
0. 087   
0. 063   
70   
1. 167   
0. 196   
0. 184   
0. 128   
0. 099   
0. 071   
Fig 1. 5   
Fig 1. 6   
Fig 1. 7   
Fig 1. 8   
Fig 1. 9   
Table of absorbance values at 340nm using different substrate concentration for Butan-1-ol and the graphs to show this data.   
Table 3   
Butan-1-ol   
Time   
Absorbance values for Substrate concentrations   
Seconds   
Minutes   
0. 08   
0. 04   
0. 02   
0. 01   
0. 005   
0   
0. 000   
0. 000   
0. 000   
0. 000   
0. 000   
0. 000   
10   
0. 167   
0. 043   
0. 03   
0. 029   
0. 027   
0. 024   
20   
0. 333   
0. 078   
0. 05   
0. 048   
0. 038   
0. 032   
30   
0. 500   
0. 119   
0. 082   
0. 074   
0. 048   
0. 036   
40   
0. 667   
0. 161   
0. 108   
0. 083   
0. 057   
0. 041   
50   
0. 833   
0. 193   
0. 134   
0. 098   
0. 066   
0. 047   
60   
1. 000   
0. 23   
0. 16   
0. 116   
0. 076   
0. 051   
70   
1. 167   
0. 264   
0. 183   
0. 13   
0. 083   
0. 054   
Fig 2. 0   
Fig 2. 1   
Fig 2. 2   
Fig 2. 3   
Fig 2. 4   
The graphs depicted above have been plotted for for 0. 080, 0. 040, 0. 020, 0. 010 and 0. 005M substrate concentrations. All of these graphs have absorbance (340nm) on the y-axis and Time (min) on the x-axis. The graphs were drawn individually however due to a great deal of overlap in the data, it was better to combine it into a single graph for each alcohol as it made the interpretation of the date considerably easier.   
Michaelis Menten curves   
Velocity calculation:   
1) The linear line equations of the absorbance vs. Time plot. provided the gradient.   
2) The gradient was divided by the Enzyme co-efficient which in this case was   
6220 l/mol/cm   
3) The above answer was multiplied by the volume of the cuvette which was 0. 003 (3ml÷1000)   
4) The above answer was multiplied with 106 to convert the volume into micro mol.   
5) The answer from step 4 in µmol was divided by 0. 1 which was the amount of enzyme used.   
Example for Ethanol:   
1) 0. 851 ÷ 6220 = 1. 37 x   
2) 1. 37 x10-4 × 0. 003 = 4. 10 x10-7   
3) 4. 10 x10-7 × 106 = 0. 41   
4) 0. 41 ÷ 0. 1 = 4. 1µmol min-1 ml-1   
The same method was used for the velocity calculation of others. The results have been tabulated above.   
Ethanol:   
Table 4   
Ethanol   
Gradient   
Enzyme co-efficient (l/mol/cm)   
Cuvette (L)   
Convert to micromoles (µmol)   
Enzyme used (µmol min-1 ml   
0. 851   
6220   
0. 003   
1000000   
0. 1   
0. 502   
6220   
0. 003   
1000000   
0. 1   
0. 331   
6220   
0. 003   
1000000   
0. 1   
0. 356   
6220   
0. 003   
1000000   
0. 1   
0. 192   
6220   
0. 003   
1000000   
0. 1   
Table 5   
Substrate Concentration (M)   
Velocity (V)   
0. 080   
4. 105   
0. 040   
2. 421   
0. 020   
1. 596   
0. 010   
1. 717   
0. 005   
0. 926   
Propan-1-ol:   
Table 6   
Propan-1-ol   
Gradient   
Enzyme co-efficient (l/mol/cm )   
Cuvette (L)   
Convert to micromoles   
enzyme used   
0. 174   
6220   
0. 003   
1000000   
0. 1   
0. 165   
6220   
0. 003   
1000000   
0. 1   
0. 116   
6220   
0. 003   
1000000   
0. 1   
0. 097   
6220   
0. 003   
1000000   
0. 1   
0. 080   
6220   
0. 003   
1000000   
0. 1   
Table 7   
Substrate Concentration (M)   
Velocity (V)   
0. 080   
0. 839   
0. 040   
0. 796   
0. 020   
0. 559   
0. 010   
0. 468   
0. 005   
0. 386   
Butan-1-ol:   
Table 8   
Butan-1-ol   
Gradient   
Enzyme co-efficient (l/mol/cm )   
Cuvette (L)   
Convert to micromoles   
enzyme used   
0. 235   
6220   
0. 003   
1000000   
0. 1   
0. 159   
6220   
0. 003   
1000000   
0. 1   
0. 144   
6220   
0. 003   
1000000   
0. 1   
0. 093   
6220   
0. 003   
1000000   
0. 1   
0. 069   
6220   
0. 003   
1000000   
0. 1   
Table 9   
Substrate Concentration (M)   
Velocity (V)   
0. 080   
1. 133   
0. 040   
0. 767   
0. 020   
0. 695   
0. 010   
0. 449   
0. 005   
0. 333   
The Michaelis Menten graphs are attached, fig 2. 5, 2. 6 and 2. 7.   
Lineweaver Burk plots   
. Since the velocity had already been calculated from the gradient values of the previous absorbance plots, they could be used in plotting the above mentioned plots.   
The Graphs of were plotted by dividing the values of velocity and substrate concentrations both by 1 Now that all the info was here, it was possible to plot the Lineweaver Burk plots.   
Ethanol:   
Table 10   
Ethanol   
Substrate Concentration (M)   
Velocity (V)   
1/S   
1/V   
0. 080   
4. 105   
12. 500   
0. 244   
0. 040   
2. 421   
25. 000   
0. 413   
0. 020   
1. 596   
50. 000   
0. 627   
0. 010   
1. 717   
100. 000   
0. 582   
0. 005   
0. 926   
200. 000   
1. 080   
Fig 2. 8   
Propan-1-ol:   
Table 11   
Propan-1-ol   
Substrate Concentration (M)   
Velocity (V)   
1/[S]   
1/V   
0. 080   
0. 008   
12. 500   
125. 000   
0. 040   
0. 008   
25. 000   
125. 000   
0. 020   
0. 006   
50. 000   
166. 667   
0. 010   
0. 005   
100. 000   
200. 000   
0. 005   
0. 004   
200. 000   
250. 000   
Fig 2. 9   
Butan-1-ol:   
Table12   
Butan-1-ol   
Substrate Concentration (M)   
Velocity (V)   
1/S   
1/V   
0. 080   
1. 133   
12. 500   
0. 883   
0. 040   
0. 767   
25. 000   
1. 304   
0. 020   
0. 695   
50. 000   
1. 439   
0. 010   
0. 449   
100. 000   
2. 227   
0. 005   
0. 333   
200. 000   
3. 003   
Fig 3. 0   
Eadie Hofstee   
The final set of graphs that needed to be plotted were the Eadie Hofstee plots. These are plots of V/[S] against velocity. All graphs for each alcohol had a negative gradient because of the inverse relationship between the V/S and velocity.   
Ethanol:   
Table 13   
Ethanol   
Substrate Concentration (M)   
Velocity (V)   
V/S   
0. 080   
4. 105   
51. 313   
0. 040   
2. 421   
60. 525   
0. 020   
1. 596   
79. 800   
0. 010   
1. 717   
171. 700   
0. 005   
0. 926   
185. 200   
Fig 3. 1   
Propan-1-ol:   
Table 14   
Propan-1-ol   
Substrate Concentration (M)   
Velocity (V)   
V/S   
0. 080   
0. 839   
10. 488   
0. 040   
0. 796   
19. 900   
0. 020   
0. 559   
27. 950   
0. 010   
0. 468   
46. 800   
0. 005   
0. 386   
77. 200   
Fig 3. 2   
Butan-1-ol:   
Table 15   
Butan-1-ol   
Substrate Concentration (M)   
Velocity (V)   
V/S   
0. 080   
1. 133   
14. 163   
0. 040   
0. 767   
19. 175   
0. 020   
0. 695   
34. 750   
0. 010   
0. 449   
44. 900   
0. 005   
0. 333   
66. 600   
Fig 3. 3   
Kcat Calculations   
1) Step one is: 0. 1÷1000= 0. 001g/ml   
2) The molecular weight of the enzyme was 150, 000g/mol   
3) The 0. 001 g/ml is divided by 150, 0000. 001÷150000= 6. 67×mol/ml   
4) Convert mol to µmol by 6. 67×mol/ml × = 6. 67µmol/ml   
5) Kcat= Vmax÷[E]t   
Kcat = 3. 48 ÷ 6. 67 = 5217min-1   
1/60 5217 = 86. 95 s-1   
Summary table   
Vmax, Km and Kcat values for each substrate and each analytical method used have been summarized in the tables below.   
Table 16   
  
Ethanol   
Propan-1-ol   
Butan-1-ol   
Velocity   
Vmax (V)   
4. 2   
0. 85   
1. 145   
Km (M)   
0. 031   
0. 07   
0. 015   
Kcat   
  
  
  
Lineweaver Burk   
Vmax (V)   
3. 48   
0. 82   
1. 076   
Km (M)   
0. 01   
0. 01   
0. 011   
Kcat   
86. 95   
20. 48   
26. 88   
Eadie Hofstee   
Vmax (V)   
4. 96   
0. 913   
1. 25   
Km (M)   
0. 026   
0. 01   
0. 016   
Kcat   
123. 93   
22. 81   
31. 23   
Lineweaver Burk   
For the Lineweaver Burk method the Vmax and Km values were worked out using the following method:   
Example for Propan-1-ol:   
Equation of the linear line: y= 0. 007x + 1. 216   
Step 1   
The value 1. 216 has been taken from the linear line equation, which in this case is C in the equation.   
Step 2   
Km = Slope Vmax   
The value for the slop was taken from the linear line equation which was the gradient. The Vmax in the above equation has already been calculated in step 1.   
Eadie Hofstee   
Example for Butan-1-ol:   
Linear line equation: y=-62. 20x + 77. 92   
Y= 0   
To work out the Vmax we want “ x” to be the subject therefore:   
To work out the Km the following equation was used:   
The -62. 20 value was taken from the gradient of the linear line equation. This value of gradient was then by -1 because this value was part of the Eadie Hofstee equation.   
Discussion   
Only the first 4 plots were used in making the Absorbance vs. Time because using all the absorbance values would give a curvy orientation to the graph. The reason for this is that the enzyme sites were occupied by the substrates because of which there were no more enzymes sites where the substrate could bind. This is an indication of the Vmax meaning the all the catalytic sites have been occupied and the enzyme has become saturated with the substrate.