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Title
Catalase: Breaking the Bad

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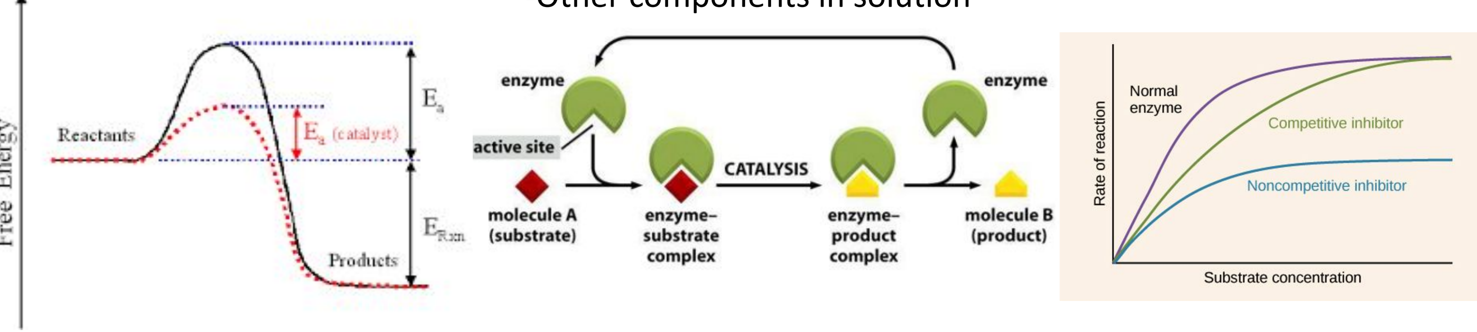
Catalase: Breaking the Bad

University of California, Irvine
Chemical Engineering

Introduction

- Enzymes - a class of proteins which catalyze biochemical reactions
  - -Increase reaction rate -Binding though reversible bond
- Enzyme Kinetics - the study of the rates of catalyzed reactions
  - Enzyme activity depend on: -pH -Temperature -Concentration -Other components in solution

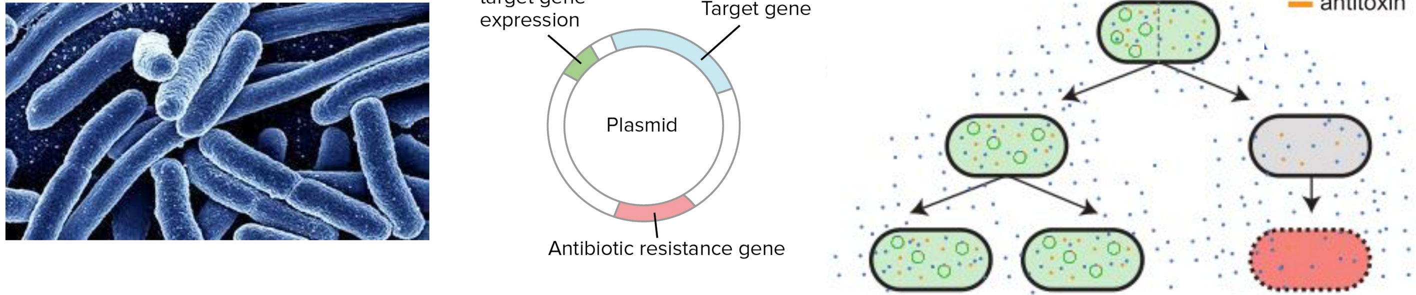


Objective/Ethics

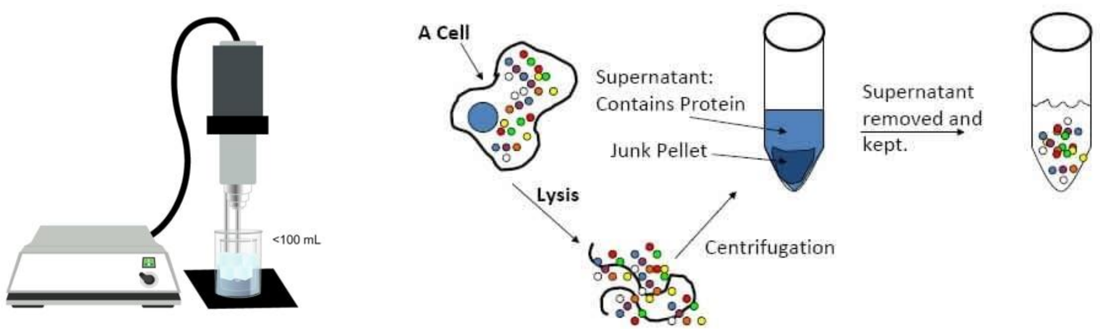
- Objective: Extract enzyme ß–galactosidase from E. Coli cells and perform affinity chromatography in order to characterize reaction kinetics of enzyme with substrate ONPG.
- Question: How does the activity change when ß–gal reacts in clarified lysate rather than a pure enzyme solution
- Ethics: Understanding industrial catalytic processes
  -chemical -petrochemical -oil-refining
  -pharmaceutical organic synthesis -fuel-energy
  Biological understanding

Materials/Methods

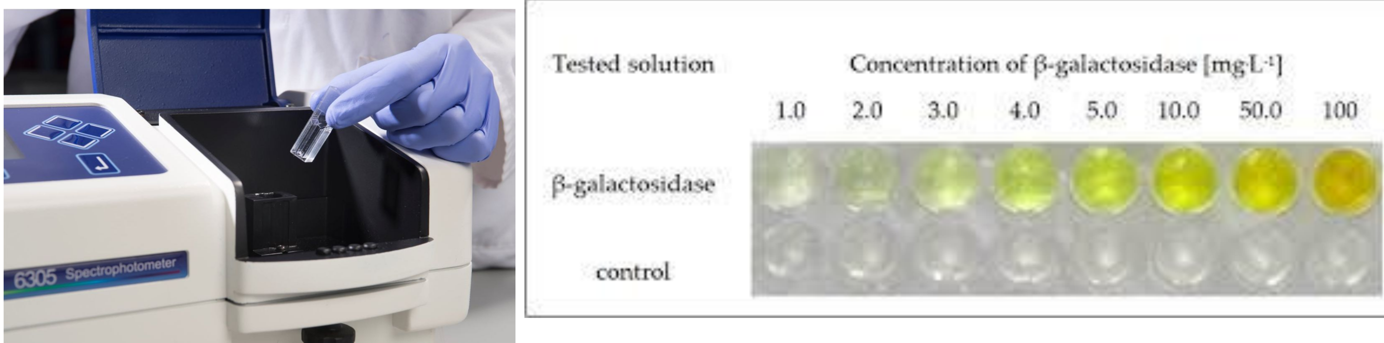
- Grew genetically modified E. Coli cells containing modified DNA gene that mass produces enzyme ß–gal



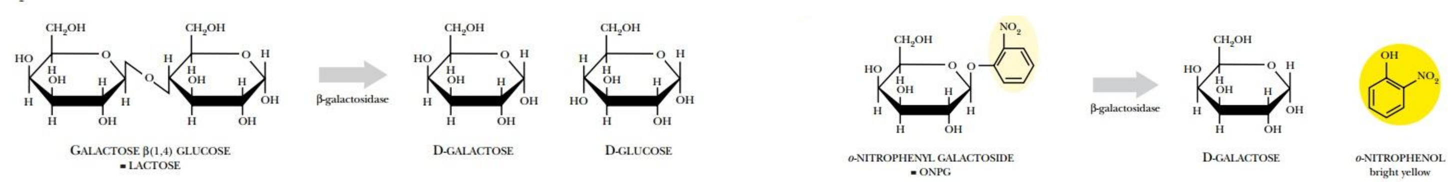
- Cell lysing conducted with sonication
  Pros: Efficiently breaks open cells using high frequency
  Cons: Produces extreme heat
- Separated cell guts with centrifuge collecting the purified lysate



- Reaction conducted in spectrometer to measure amount of product produced per time

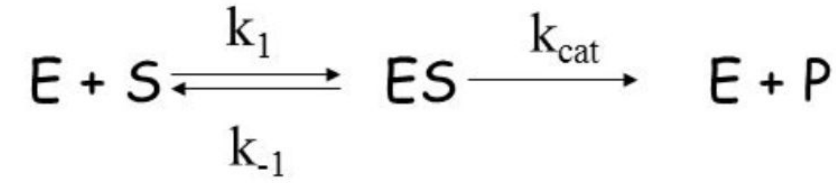


- ß–gal naturally breaks apart lactose but in this lab we are using ONPG as the substrate



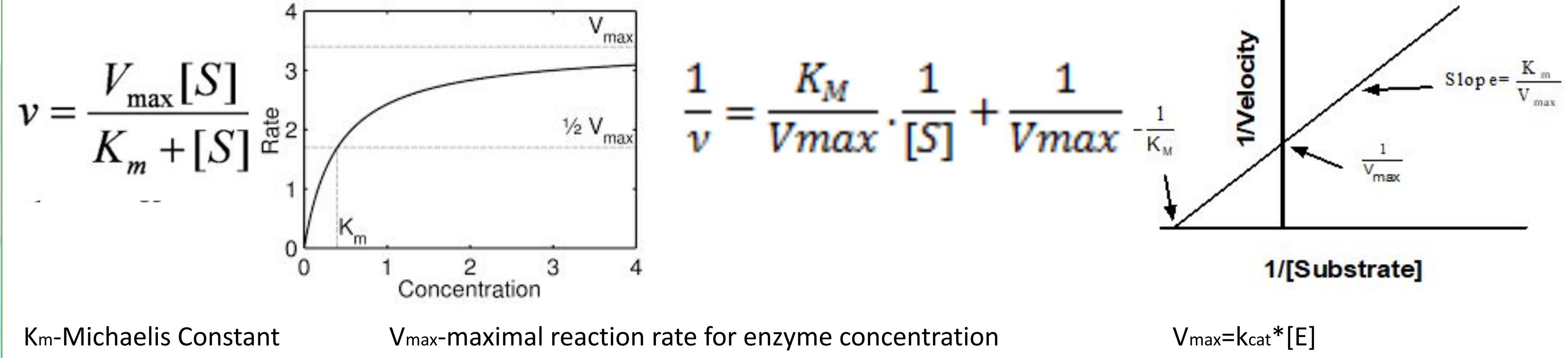
Theory

The reaction in this experiment is based on mass-action kinetics that are dependent on the concentration of enzyme (E), substrate (S), products (P).



$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_{cat}} E + P$$

Michaelis-Menten Kinetics Equation is the enzyme kinetics rate equation for a single substrate. It can be linearized and plotted to form the Lineweaver-Burk plot to easily find Vmax and Km.



$$v = \frac{V_{max}[S]}{K_m + [S]} \qquad \frac{1}{v} = \frac{K_M}{Vmax} \cdot \frac{1}{[S]} + \frac{1}{Vmax}$$

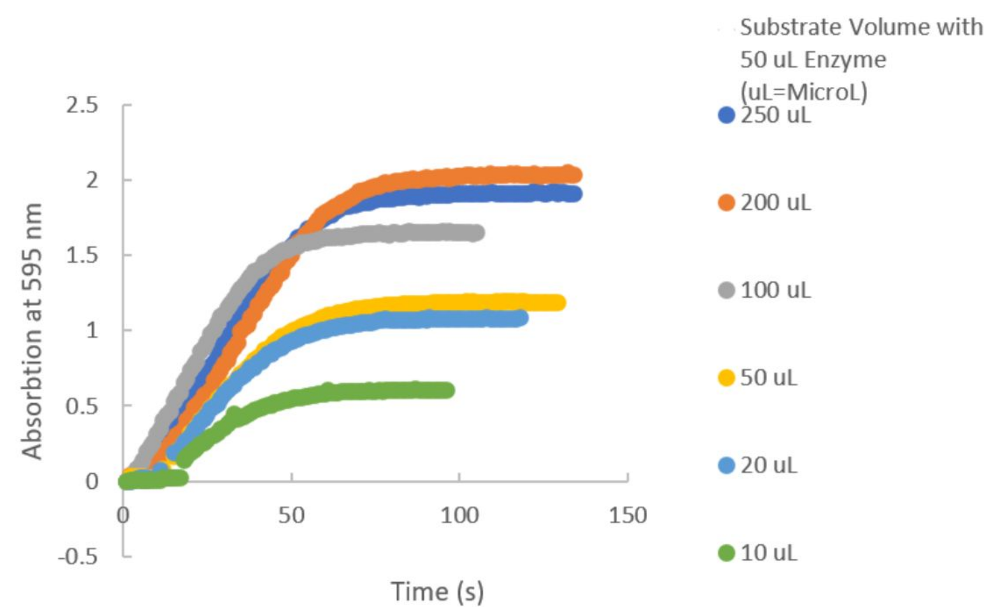
K_m-Michaelis Constant $\quad V_{max}$-maximal reaction rate for enzyme concentration $\quad V_{max}=k_{cat}*[E]$

Results

Summary of Trials

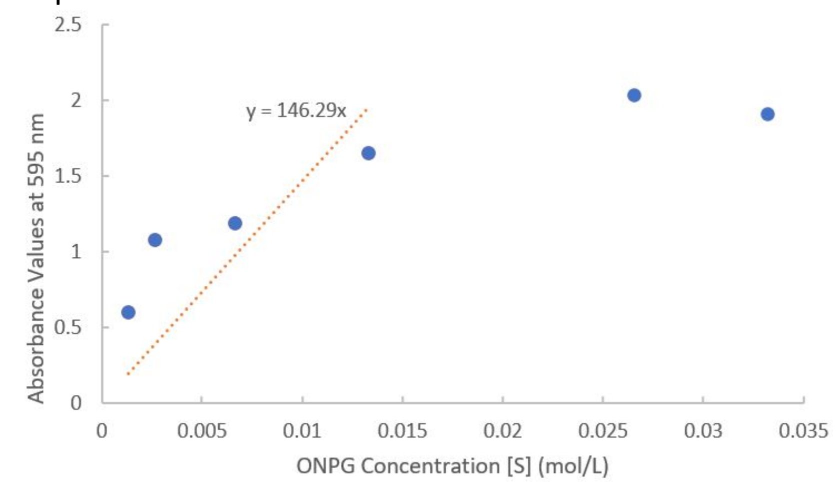
- Volume normalized to 1000 µL using pH 8.0 buffer to keep enzyme concentration constant

Trial	Volume of 50 mM tris, pH 8.0 (µL)	Volume of 4 mg mL^{-1} ONPG (µL)	Volume of purified enzyme (µL)
1	750.	250.	50.
2	800.	200.	50.
3	900.	100.	50.
4	950.	50.	50.
5	980.	20.	50.
6	990.	10.	50.



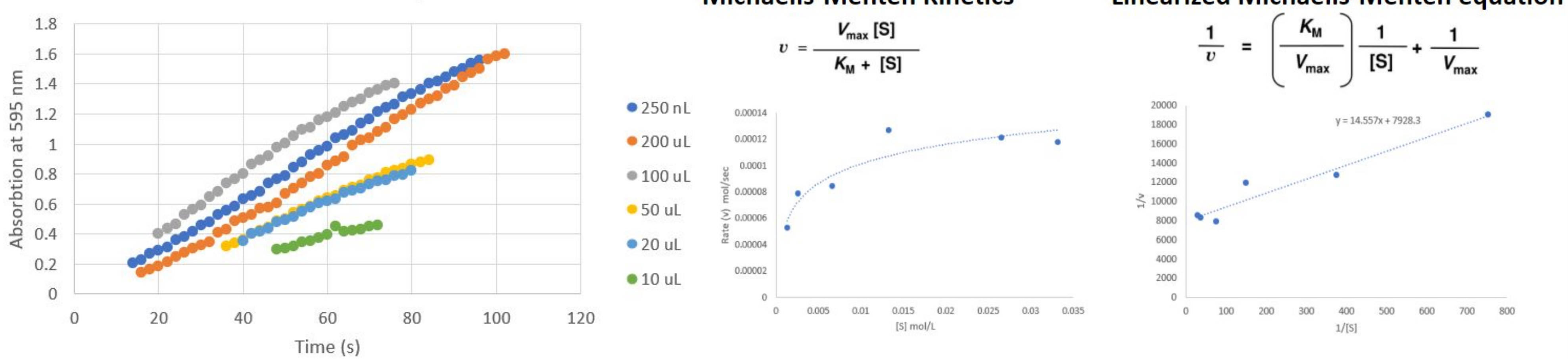
Standard Curve

- Using final absorbance of the amount of substrate used in each trial a relationship between absorbance and concentration of product can be made
- Relationship becomes inaccurate at large concentrations as shown in the theoretical curve here



Reaction Rate Curve

- Using the initial rate of reaction the linearized Michaelis-Menten Kinetics Equation yields the trials K_m and V_{max} values



Conclusion

Results From Lineweaver-Burk Plot

Value	Native β-gal in clarified lysate (Lab results)	Literature Native β-gal
$1/V_{max}$ 1/(mol/sec)	7928.3	
K_m/V_{max}	14.557	
K_m (mol/L)	1.84E-3	0.12E-3
k_{cat} (1/sec)		600
[E] (mol/L)	0.4E-3	

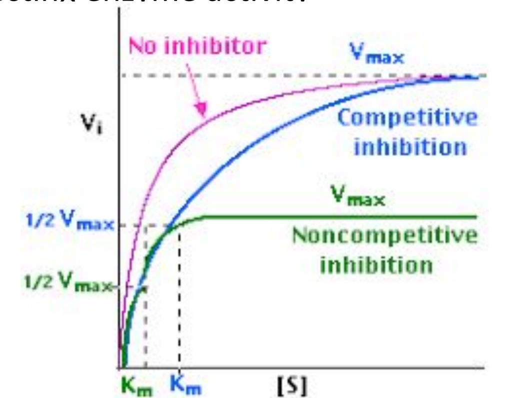
The goal of this lab is to see how the activation of β-galactosidase is affected when the reaction is taking place in clarified lysate (mixture of all light weight material from cells) rather than in a pure solution of enzyme. Our experimental value for Km is much larger than that of the literature value. Because Km is in the denominator of the Michaelis-Menten equation, it has an inverse relationship to reaction velocity. The findings of this lab shows Km from the enzyme reaction in the clarified lysate is larger concluding that the reaction velocity was slower than that of pure enzyme conditions.

Possible causes leading to a slower reaction rate:

•Km depends on enzyme structure, therefore factors affecting enzyme structure:
- Purified lysate might contain inhibitors/inhibiting properties slowing reaction velocity
- pH and temperature might not be in optimal range affecting enzyme activity

Noncompetitive inhibition causes the Km value of the enzyme to be larger than its non inhibited state

It can be concluded that enzyme taken from the cell lysate reduces enzyme reaction activity through competitive inhibition characteristics.



Future experiments can be conducted varying the levels of pH or temperature of the enzyme and substrate solution.. Finding the optimal pH and temperature where maximal Vmax is found can optimize the reaction velocity to its fastest rate and optimize the production of the desired product.

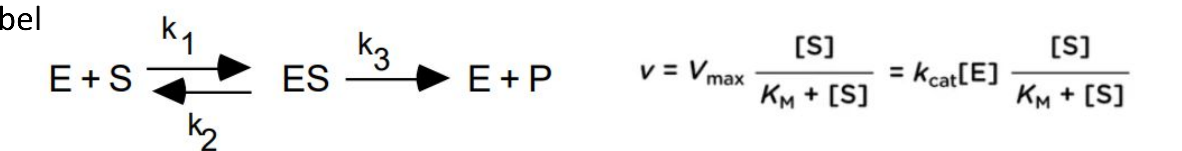
Design Extension

Suppose a process requires lactose levels to be reduced in a gallon of milk for intolerant customers. Typically, a cup of milk contains approximately 12-13 grams of lactose. The goals of this process would be to first calculate the amount of lactose to be removed from the gallon of milk. Then, to add the required amount of lactase to the milk in order to achieve this goal based on theoretical values.

A gallon of milk contains 16 cups, with an average of 12.5 g of lactose per cup:

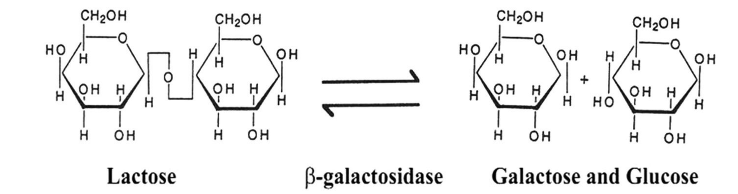
(12.5 g lactose/cup) x (16 cup/gal) = 200 g lactose gal^{-1}

Using the Michaelis-Menten Kinetics equation, the amount of lactase enzyme can be calculated as shown bel



$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P \qquad v = V_{max}\frac{[S]}{K_M + [S]} = k_{cat}[E]\frac{[S]}{K_M + [S]}$$

Enzyme concentration from experiment is .4E-3 M, V_{max}=.96 mol sec^{-1}, and using a substrate concentration of .15 M, the rate of substrate (Lactose) removal over time, v=.023 mol sec^{-1}. Initially the rate of removal will be high and then as the lactose concentration decreases, the rate of reaction will decrease as well as seen in the results section. Roughly 95% of lactose can be removed from milk within the first 24 hours using β-galactosidase enzyme [4].



Acknowledgments

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