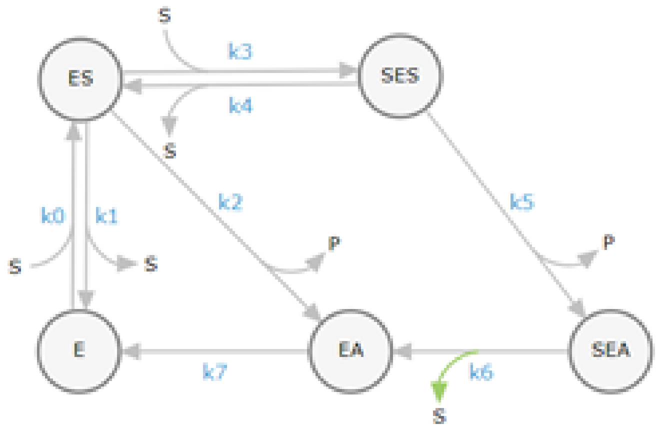
In many biological reactions, enzymes are used to facilitate the synthesis of a product. For the mechanism shown in Figure 1, an enzyme creates a product after binding with one or two substrate molecules.



$$E + S \rightleftharpoons ES \qquad (1)$$

$$ES + S \rightleftharpoons SES \qquad (2)$$

$$ES \rightarrow EA + P \qquad (3)$$

$$SES \rightarrow SEA + P \qquad (4)$$

$$SEA \rightarrow EA + S \qquad (5)$$

$$EA \rightarrow E \qquad (6)$$

In this reaction, *E* represents the enzyme, *S* is the substrate, *ES* and *SES* are enzyme complexes, *EA* and *SEA* are spent enzymes that need to be reduced to *E* to regain catalytic function, and *P* is the product.

Using this reaction scheme, develop the following deliverables:

Deliverable 1: Given that an enzyme's catalytic function is dependent on temperature, the relationship given in Equation 7 can be used to determine the rate constant for the reactions where a product is generated (Equations 3 and 4). Plot how the reaction rates for these two reactions, k_2 and k_5, changes with temperatures increasing from 4°C (refrigerated enzyme) to 65°C (above which proteins denature)

$$ln(k) = ln(T) + \frac{\Delta S}{T} + \frac{\Delta H}{T^2} \qquad (7)$$

where *k* represents the specific reaction rate, *T* is the temperature in K of the reaction and values for ΔH and ΔS are given in Table 1.

Table 1. Values for reaction rate constant and activation energy calculations.

Reaction	ΔH [kJ/mol]	ΔS [kJ/mol K]
Reaction 3, for k_2	9.8×10^3	-52.6
Reaction 4, for k_5	47.4×10^3	-65.1

Associated MATLAB File:

- d_1.m

We first converted temperature from Celsius to kelvin and got a temperature interval between T=277K to T=338K.

Next, solving for k, we got:

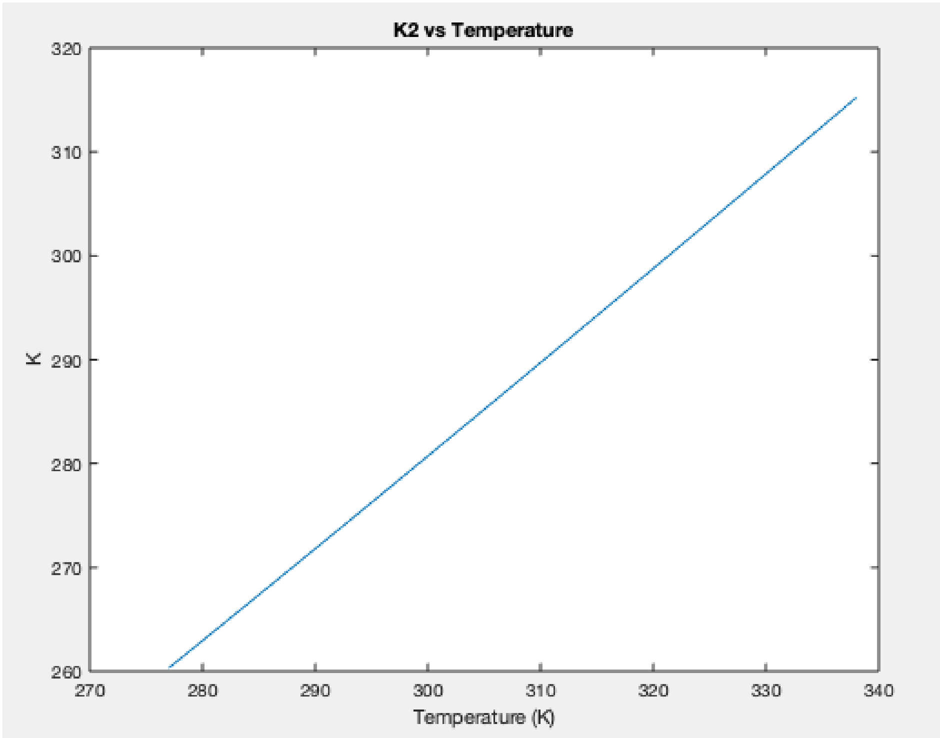
$$k = e^{(ln(T) + \frac{\Delta S}{T} + \frac{\Delta H}{T^2})}$$

Then, we plotted the change in the 2 reaction rate constants over time.

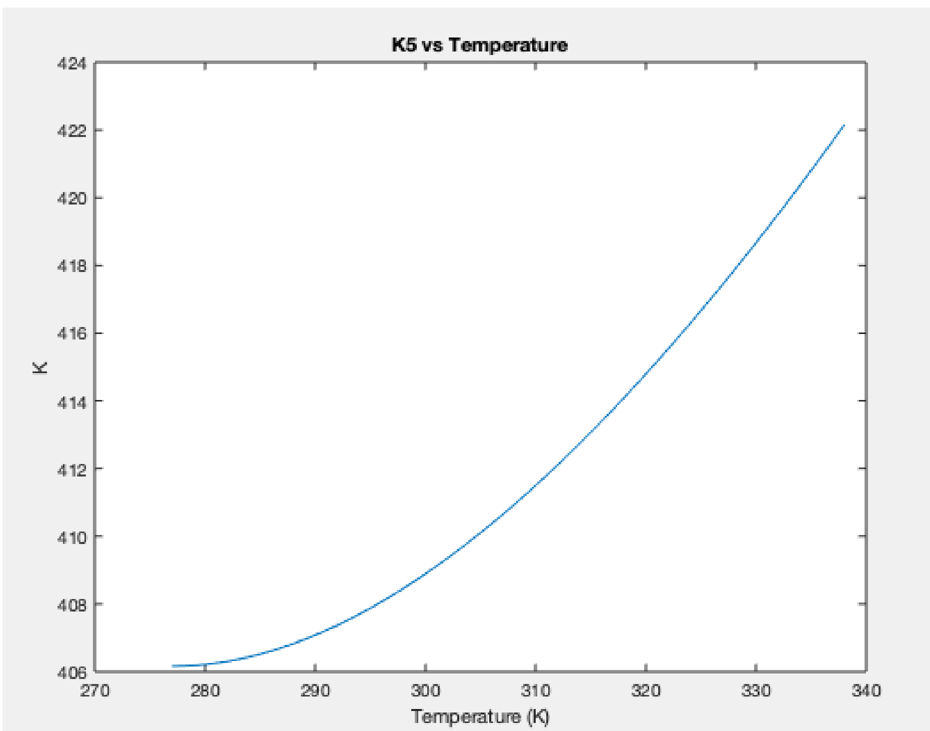
Sample MATLAB Code 1 (d_1):

```
t=277:338; %temp in kelvins

%formula: ln(k)=ln(t)+s/t+h/t^2
%solve for k:
%k=t+e^(s/t)+e^(h/t^2)

h_2=9.8*10^3; %kJ/mol
h_5=47.4*10^3; %kJ/mol
s_2=-52.6; %kJ/molK
s_5=-65.1; %kJ/molK

k_2=exp(log(t)+(s_2./t)+(h_2./(t.^2)));
plot(t,k_2)

figure
k_5=exp(log(t)+(s_5./t)+(h_5./(t.^2)));
plot(t,k_5)
```

Plot 1: Shows the change in the reaction rate (k2) over a temperature range of T=277K to T=338k. Here, we observed a linear relationship between the two variables.



Plot 2: Shows the change in the reaction rate (k5) over a temperature range of T=277K to T=338k. Here, we observed an exponential relationship between the two variables.



Deliverable 2: Based on general chemistry concepts, the change in concentration of a reactant or product can be calculated using the reaction rate, *k*, and the concentration of the reaction starting materials (i.e. reactants for a forward reaction, products for a reverse reaction). Set up the system of ordinary differential equations to determine the concentration of each component with time. Using Matlab and an established ode solver, solve your differential equations and plot the change in concentrations of the substrate, product, and enzyme for the reaction run at 37°C in a time span of 0 to 0.5. The initial concentrations of substrate and enzyme are 0.1 and 2.6×10^{-3}, respectively.

Associated MATLAB Files:

- d_1.m
- d_2.m
- q.m

Using the given model and the rate law equation, we can write a system of differential equations to plot the change in concentration of the various components part of the system.

Image 1&2: The Following images are notes / calculations done to show how the 7 different differential equations were derived.

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1. $\frac{dES}{dt} = r_0 - r_1 - r_2 - r_3 + r_4$

2. $\frac{dE}{dt} = r_1 + r_7 - r_0$

3. $\frac{dEA}{dt} = r_2 + r_6 - r_7$

4. $\frac{dSEA}{dt} = r_5 - r_6$

5. $\frac{dSES}{dt} = r_3 - r_4 - r_5$

$r_0 = k_0[E][S]$
$r_1 = k_1[ES]$
$r_2 = k_2[ES]$
$r_3 = k_3[ES][S]$
$r_4 = k_4[SES]$
$r_5 = k_5[SES]$
$r_6 = k_6[SEA]$
$r_7 = k_7[EA]$

Rewritten differential equations:

1. $\frac{dES}{dt} = k_0[E][S] - k_1[ES] - k_2[ES] - k_3[ES][S] + k_4[SES]$

2. $\frac{dE}{dt} = k_1[ES] + k_7[EA] - k_0[E][S]$

3. $\frac{dEA}{dt} = k_2[ES] + k_6[SEA] - k_7[EA]$

4. $\frac{dSEA}{dt} = k_5[SES] - k_6[SEA]$

5. $\frac{dSES}{dt} = k_3[ES][S] - k_4[SES] - k_5[SES]$

6. $\frac{dP}{dt} = r_5 + r_2$

$\frac{dP}{dt} = k_5[SES] + k_2[ES]$

7. $\frac{dS}{dt} = r_1 + r_4 + r_6 - r_0 - r_3$

$\frac{dS}{dt} = k_1[ES] + k_4[SES] + k_6[SEA] - k_0[E][S] - k_3[ES][S]$

Equations 1-7: Below are the seven differential equations used to determine concentrations of substrate, product.

$$1. \frac{dES}{dT} = k_0[E][S] - k_1[ES] - k_2[ES] - k_3[ES][S] + k_4[SES]$$

$$2. \frac{dE}{dT} = k_1[ES] + k_7[EA] - k_0[E][S]$$

$$3. \frac{dEA}{dT} = k_2[ES] + k_6[SEA] - k_7[EA]$$

$$4. \frac{dSEA}{dT} = k_5[SES] - k_6[SEA]$$

$$5. \frac{dSES}{dT} = k_5[ES][S] - k_4[SES] - k_5[SES]$$

$$6. \frac{dP}{dT} = k_5[SES] + k_2[ES]$$

$$7. \frac{dS}{dT} = k_1[ES] + k_4[SES] + k_6[SEA] - k_0[E][S] - k_3[ES][S]$$

Rate constants from all reactions except for k2 and k5 were provided. Therefore, the MATLAB code from deliverable 1 was used to compute values for k2 and k5 at a temperature of 310K.

Image 3: The image below is a screenshot of the command window showing values for k2 and k5 at 310K.

```
k_2 =

  289.7076


k_5 =

  411.4998
```

Next, a function was created in MATLAB that included all the rate constants and differential equations.

Sample MATLAB Code 2 (q.m):

```
function dPdt=q(t,m)

    ES=m(1);
    E=m(2);
    EA=m(3);
    SEA=m(4);
    SES=m(5);
    P=m(6);
    S=m(7);

    k_0=1.3*10^6;
    k_1=17312;
    k_2=289.7076;
    k_3=988;
    k_4=1.3*10^6;
    k_5=411.4998;
    k_6=472;
    k_7=12935;

    dES=(k_0*E*S)-(k_1*ES)-(k_2*ES)-(k_3*ES*S)+(k_4*SES);
    dE=(k_1*ES)+(k_7*EA)-(k_0*E*S);
    dEA=(k_2*ES)+(k_6*SEA)-(k_7*EA);
    dSEA=(k_5*SES)-(k_6*SEA);
    dSES=(k_3*ES*S)-(k_4*SES)-(k_5*SES);
    dP=(k_5*SES)+(k_2*ES);
    dS=(k_1*ES)+(k_4*SES)+(k_6*SEA)-(k_0*E*S)-(k_3*ES*S);
```

```
dPdt(1)=dES;
dPdt(2)=dE;
dPdt(3)=dEA;
dPdt(4)=dSEA;
dPdt(5)=dSES;
dPdt(6)=dP;
dPdt(7)=dS;

dPdt=dPdt';
```

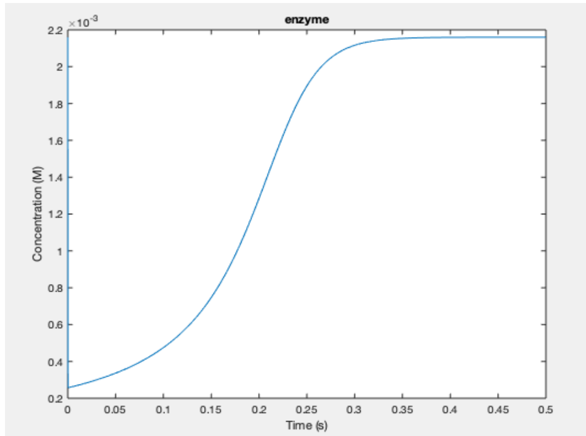
Then, another script in MATLAB was written where the function ode45 was used to reference the MATLAB file q.m with the initial concentrations for all substances entered.

Sample MATLAB Code 3 (d_2):

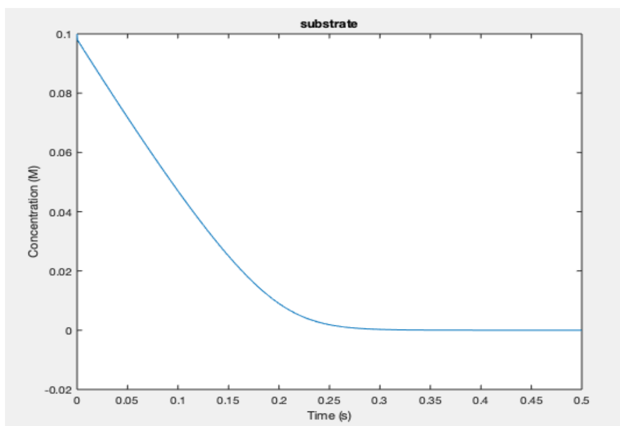
```
[t,ES]=ode45(@q, [0 .5], [0, 2.16*10^(-3), 0, 0, 0, 0, 0.01]);

    ES_conc=ES(:,1);
    E=ES(:,2);
    EA=ES(:,3);
    SEA=ES(:,4);
    SES=ES(:,5);
    P=ES(:,6);
    S=ES(:,7);

    plot(t,S)
    title('substrate')
    figure
    plot(t,P)
    title('product')
    figure
    plot(t,E)
    title('enzyme')
```

Then, the changes in enzyme, substrate, and product were plotted between a time span of 0 and 0.5.

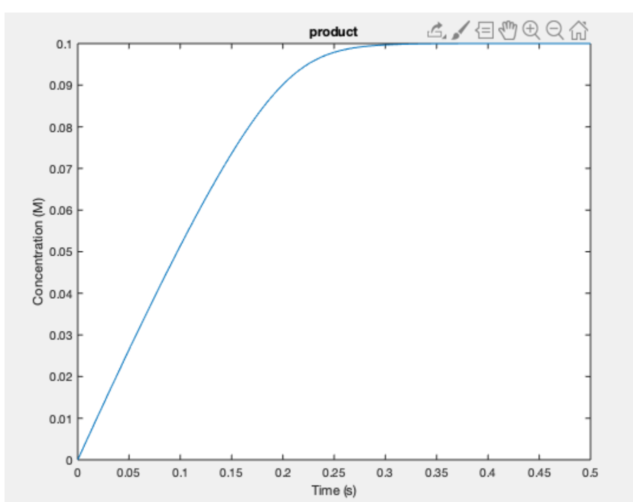
Plot 3: Shows the change in enzyme concentration over time. We observed that the enzyme was rapidly used up initially and then slowly reached the initial concentration as time went on.



Plot 4: Shows the change in substrate concentration over time. We observed that the substrate decreased in concentration as the reactions proceeded.



Plot 5: Shows the change in product concentration over time. We observed that the product increased in concentration as the reactions proceeded.



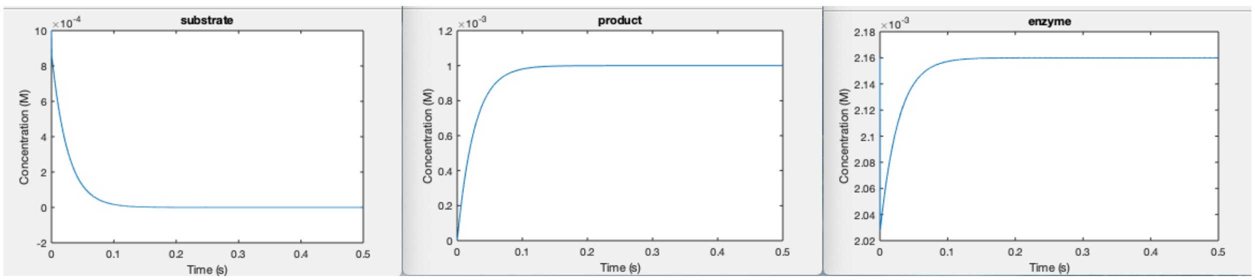
Deliverable 3: Discuss the impact of changing the initial substrate concentration on the overall reaction. Support your discussion using calculations and graphs for concentrations ranging from 0.001 to 0.25.

Associated MATLAB Files:

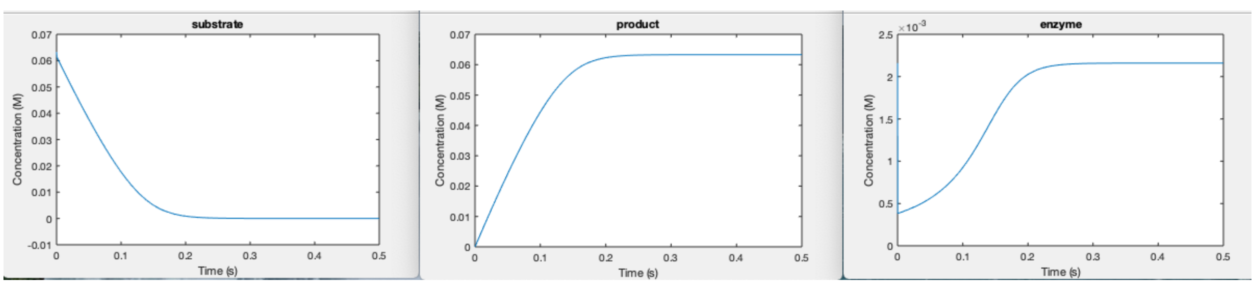
- d_2.m
- q.m

We split the concentration range from 0.001M to 0.25M into 5 evenly spaced segments and plotted the changes in the concentrations of all 3 substances of importance (product, enzyme, and substrate). We used the same MATLAB files, Project_deliverable_2.m and q.m, but simply changed the initial substrate concentration each time and ran the code 5 times.

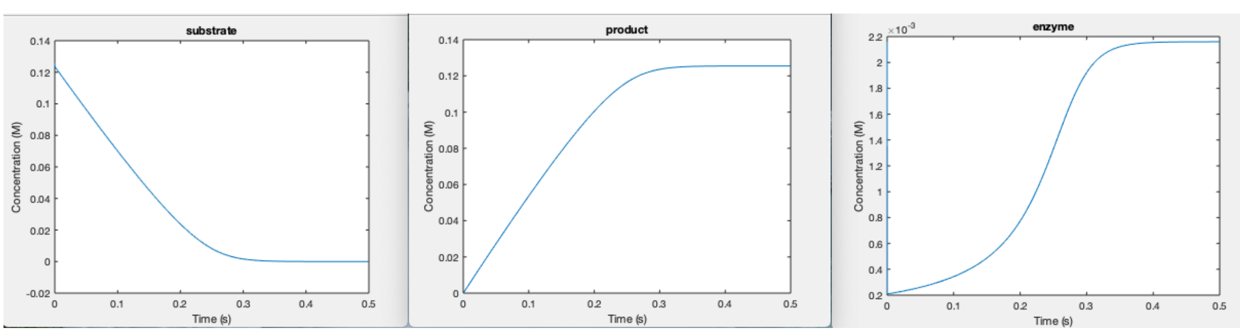
When the initial substrate concentration is equal to 0.001M:



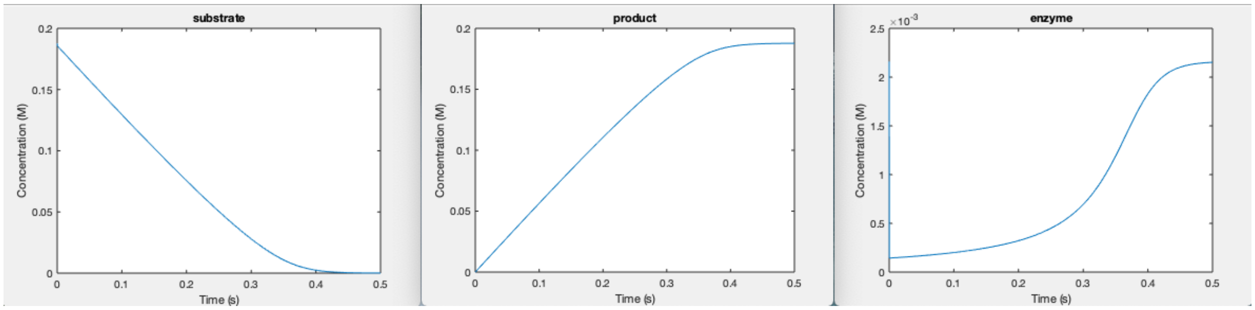
When the initial substrate concentration is equal to 0.0633M:



When the initial substrate concentration is equal to 0.1255M:



When the initial substrate concentration is equal to 0.1878M:



And lastly, when the initial substrate concentration is equal to 0.25M: