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ENZYMOLOGY & BIOPROCESSING

Bioprocessing Practical Manual

Course Code: EC-BTP701

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CONTENTS

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1. **Experiment No.: EC-BTP701/01**
2. **Experiment Name:** Exit age distribution curve 'E' for liquid flowing through a vessel (RTD)
3. **Objectives:** To determine exit age distribution curve 'E' for liquid flowing through Continuous Stirred Tank Reactor (CSTR).
4. **Principle:**

   A well-mixed tank is an analogue for many control volumes used in environmental engineering. For example, in our lake example it might be reasonable to assume that pollutants dumped into the lake are rapidly mixed throughout the entire lake. In environmental engineering and chemical engineering, the term *Continuously Stirred Tank Reactor* or *CSTR* is used for such a system. An example of a CSTR is shown in Figure 1.

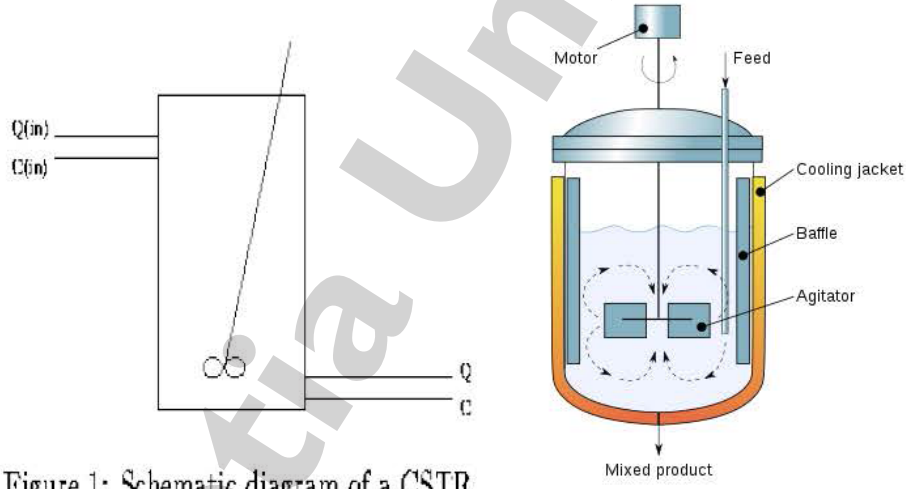
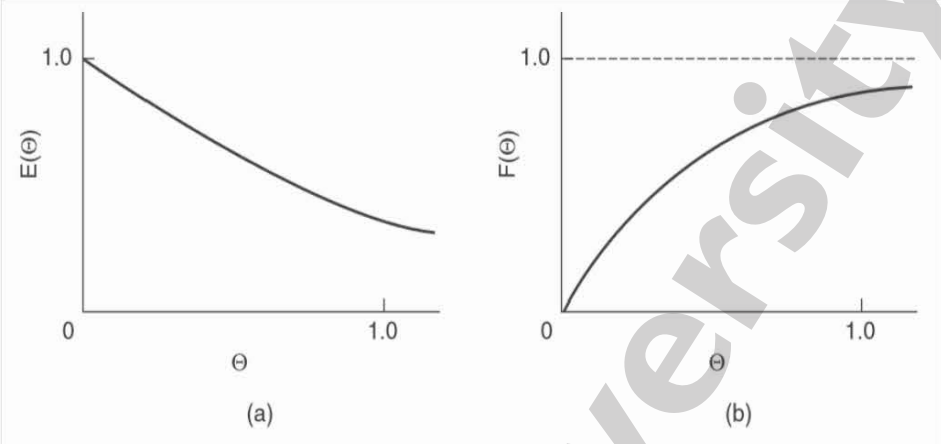


Figure 1: Schematic diagram of a CSTR

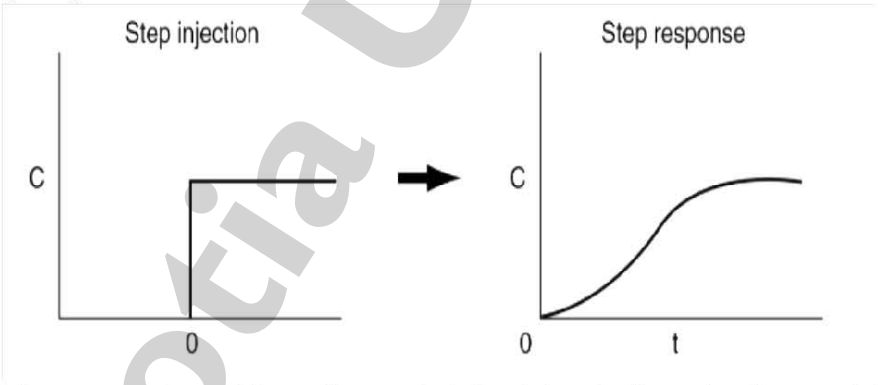
The Residence Time Distribution (RTD) inside the Continuous Stirred Tank Reactor (CSTR) shows the characteristic of the mixing of reactants that occurs inside the reactor. Inside the reactor, the reactants are continually consumed as the reactant flow along the length of the reactor. The residence time distribution function is represent in a plotted graph of E(t) as a function of time. This function shows in a quantitative manner of how much time the mixed fluid stays inside the reactor before leaving the reactor.



(a) E(t) curve and (b) F(t) Curve For Ideal CSTR

RTD is determined experimentally by injecting chemically inert substance known as tracer into the reactor at t = 0 and measuring the concentration of the tracer at the effluent stream as a function of time. The two most common methods of injecting tracer into the reactor are pulse input and step input.

Step Change Input In Continuous Stirred Tank Reactor



Typical Concentration – Time Curve At The Inlet And Outlet Stream For Step Change Input

A constant rate of tracer is added to the feed that is initiated at time t = 0. Thus, the inlet concentration of the tracer, C_o is constant with time. From this experiment, the cumulative distribution can be determined directly, F(t).

$$C_o(t) = \begin{cases} 0 & t < 0 \\ (C_o)\ \text{constant} & t \leq 0 \end{cases}$$

The cumulative distribution, F(t) represents the fraction of effluent that has been in reactor for time t = 0 until t = t.

$$\text{Cumulative Distribution, } F(t) = \left[\frac{C_{out}}{C_0}\right]_{step}$$

Differentiation of the cumulative distribution function yield to RTD function,

$$\text{Residence Time Distribution Function, } E(t) = \frac{d}{dt}\left[\frac{C(t)}{C_0}\right]_{step}$$

The mean residence time, tm shows the average time the fluids stay inside the reactor

$$\text{First Moment, Mean Residence Time, } t_m = \int_0^\infty t\,E(t)\,dt$$

The spread of the distribution which is the magnitude of the variance, σ^2. The greater the magnitude, the greater the distribution's spread will be

$$\text{Second Moment, Variance, } \sigma^2 = \int_0^\infty (t - t_m)^2 E(t)\,dt$$

The extent that a distribution is skewed in one direction is measured by the sleekness's magnitude which also means how differs the distribution is compared to the normal distribution

$$\text{Third Moment, Skewness, } s^3 = \frac{1}{\sigma^3}\int_0^\infty (t - t_m)^3 E(t)\,dt$$

5. Materials

a) Deionized Water.

b) Oxalic acid

c) Sodium Hydroxide (NaOH)

d) Sulfuric acid (H_2SO_4)

e) Sodium Chloride, NaCl.

f) Phenolphthalein Indicator

6. Procedure:

a) Standardized solution of 0.05(N) oxalic acid is used for back titration.

b) **General Start-Up Procedures**

i) The 20-L feed tank is filled with deionized water.

ii) Take 20 ml of 1(N) H_2SO_4 in a syringe and inject all of it at one instant in the reactor and start the stopwatch simultaneously.

(Sometimes add tracer chloride is dissolved in tank. The salt is made sure to dissolve completely and the solution is ensure to be homogenous).

iii) The power for the control panel is turned on.

iv) The stirrer assembly is made sure to secure properly to avoid damage to the mechanical seal.

v) The way valve is set to position, so that the deionised water from tank will flow into reactor.

vi) Pump is switched on to fill up the reactors with deionised water.

vii) After 5 minutes, pump is then switched off. The way valve is set to position.

vii) The needle valve is adjusted as to set the flow rate, flow rate to 150mL/min. Stirrers is then switched on at ~180 rpm.

c) Start the flow of water in the CSTR and maintain it at a constant value also start the stirrer and keep the speed of agitation sufficient enough for proper mixing and at a constant value.

d) Take 20 ml of solution in a syringe and inject all of it at one instant in the reactor and start the stopwatch simultaneously.

e) Take known volume of sample at the outlet of the reactor every ten seconds and titrate this using 0.05N NaOH

f) Take at least 10 readings.

7. Observations:

a) Normality of NaOH = 0.05N

b) Flow rate of water = F_w (ml/sec)

c) Normality of Oxalic Acid = 0.05N

d) Mean residence time in the reactor = t = V/ Fw (sec)

7.1 Observation Table:

Sl No	Volume of sample V_2 (ml	Volume of NaOH (ml)	Outlet concentration of H_2SO_4 C(t)	Time (t) seconds	E(t)	t E(t)	$(t-tm)^2$ E(t)

7.2 Calculations:

Plot concentration of NaOH (Ca) versus time and determine the area under the curve

Find $E(t) = C(t) / \int C(t)\, dt$

t_m = Mean residence time = $\int t.\, E(t)\, dt$ (min)

σ^2 (Variance) = $\int (t-tm)^2 . t .dt$ $(min)^2$

σ_θ^2 (skewness) = σ^2 / t_m^2 (dimensionless)

$\sigma_\theta^2 = 2/Pe - 2/Pe^2 (1-e^{-Pe})$

By trial & error

Pe = ----------

7.3 Graphs:

Plot the graph of C(t) v/s time

Plot the graph of E(t) v/s time

Plot the graph of t E(t) v/s time

Plot the graph of $(t-tm)^2$ E(t) v/s time

8. Inference/discussion

9. Safety precautions

General Shut-Down Procedures

i) The pumps and stirrer is switched off. The needle valve is closed.

ii) All liquids in the reactors are drained by opening valves.

iii) The power for the control panel is switched off.

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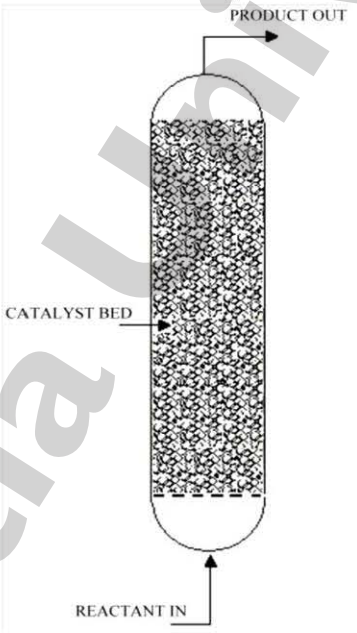
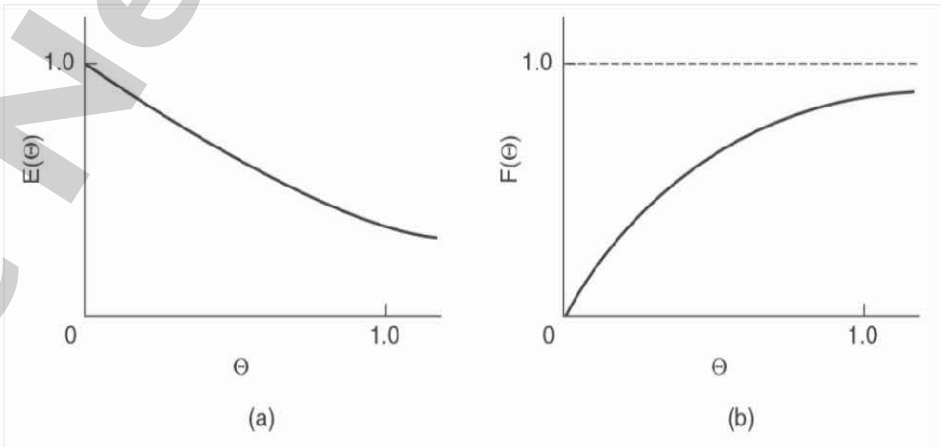
1. **Experiment No.: EC-BTP701/02**
2. **Experiment Name:** Transforming an experimental C_{pulse} curve in to an E curve.
3. **Objective:** To determine an experimental C_{pulse} curve in to an E curve for liquid flowing through packed bed reactor (PBR).
4. **Principle:**

The Residence Time Distribution (RTD) inside the Packed Bed Reactor (PBR) shows the characteristic of the mixing of reactants that occurs inside the reactor. Inside the reactor, the reactants are continually consumed as the reactant flow along the length of the reactor. The residence time distribution function is represent in a plotted graph of E(t) as a function of time. This function shows in a quantitative manner of how much time the mixed fluid stays inside the reactor before leaving the reactor.



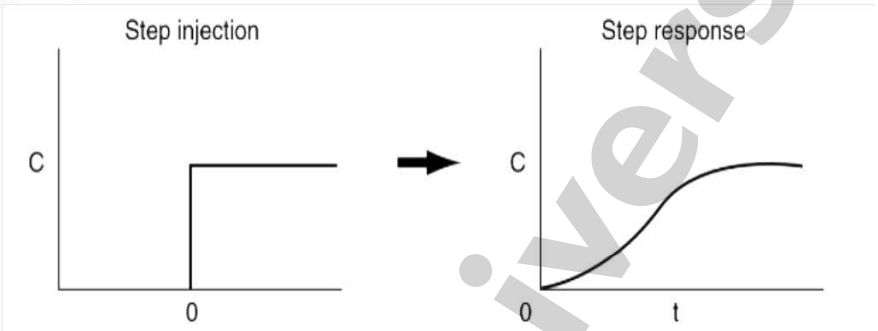
Packed Bed Reactor



(b) E(t) curve and (b) F(t) Curve For Ideal CSTR

RTD is determined experimentally by injecting chemically inert substance known as tracer into the reactor at t = 0 and measuring the concentration of the tracer at the effluent stream as a function of time. The two most common methods of injecting tracer into the reactor are pulse input and step input.

Step Change Input In Continuous Stirred Tank Reactor



Typical Concentration – Time Curve at the Inlet and Outlet Stream for Step Change Input

A constant rate of tracer is added to the feed that is initiated at time t = 0. Thus, the inlet concentration of the tracer, C_o is constant with time. From this experiment, the cumulative distribution can be determined directly, F(t).

$$C_o(t) = \begin{cases} 0 & t < 0 \\ (C_o)\ \text{constant} & t \leq 0 \end{cases}$$

The cumulative distribution, F(t) represents the fraction of effluent that has been in reactor for time t = 0 until t = t.

$$\text{Cumulative Distribution, } F(t) = \left[\frac{C_{out}}{C_0}\right]_{step}$$

Differentiation of the cumulative distribution function yield to RTD function,

$$\text{Residence Time Distribution Function, } E(t) = \frac{d}{dt}\left[\frac{C(t)}{C_0}\right]_{step}$$

The mean residence time, tm shows the average time the fluids stay inside the reactor

$$\text{First Moment, Mean Residence Time, } t_m = \int_0^\infty t\,E(t)\,dt$$

The spread of the distribution which is the magnitude of the variance, σ^2. The greater the magnitude, the greater the distribution's spread will be

$$\text{Second Moment, Variance, } \sigma^2 = \int_0^\infty (t - t_m)^2 E(t)\,dt$$

The extent that a distribution is skewed in one direction is measured by the sleekness's magnitude which also means how differs the distribution is compared to the normal distribution

$$\text{Third Moment, Skewness, } s^3 = \frac{1}{\sigma^3} \int_0^\infty (t - t_m)^3 E(t)\, dt$$

5. Materials

a) Deionized Water

b) Oxalic acid

c) Sodium Hydroxide (NaOH)

d) Sulfuric acid (H_2SO_4)

e) Sodium Chloride, NaCl

f) Phenolphthalein Indicator

6. Procedure:

i. Standardized solution of 0.05(N) oxalic acid is used for back titration.

ii. **General Start-Up Procedures**

i) The 20-L feed tank is filled with deionized water.

ii) Take 20 ml of 1(N) H_2SO_4 in a syringe and inject all of it at one instant in the reactor and start the stopwatch simultaneously.

(Sometimes add tracer chloride is dissolved in tank. The salt is made sure to dissolve completely and the solution is ensure to be homogenous).

iii) The power for the control panel is turned on.

iv) The stirrer assembly is made sure to secure properly to avoid damage to the mechanical seal.

v) The way valve is set to position, so that the deionised water from tank will flow into reactor.

vi) Pump is switched on to fill up the reactors with deionised water.

vii) After 5 minutes, pump is then switched off. The way valve is set to position.

vii) The needle valve is adjusted as to set the flow rate, flow rate to 150mL/min. Stirrers is then switched on at ~180 rpm.

iii. Start the flow of water in the Packed bed reactor to build up the liquid level and to achieve steady state.

iv. Measure the steady state flow rate.

v. Withdraw the sample at regular interval of time and titrate it against std. 0.05N oxalic acid.

vi. Take 18 to 20 such readings.

vii. Plot graph of C(t) Vs time, E(t) Vs time , t.E(t) Vs time and (t^2). E(t) vs time using the readings of observation table and calculate area under the curve of each graph.

6. Observations:

1) Normality of Oxalic acid = 0.05N

2) Flow rate = _____ml/sec

3) Normality of NaOH = 0.05N

6.1 Observation Table:

Sl no	Volume of sample V_2 (ml	Volume of NaOH (ml)	Outlet concentration of H_2SO_4 C(t)	Time (t) seconds	E(t)	t E(t)	$(t-tm)^2$ E(t)

6.2 Calculations:

From graph calculate the values of

$\int C(t)\, dt$ = _____

t_m = Mean residence time =

$\int t.\, E(t)\, dt$ (min) 0

$\sigma^2 = \int (t-tm)^2 .E(t) .dt\ (min)^2$

$\sigma_\theta^2 = \sigma^2 / t_m^2$ (dimensionless)

$\sigma_\theta^2 = 2/Pe - 2/Pe^2 (1 - e^{-Pe})$

Hence calculate peclet no (by trial and error) from above equation.

7. **Inference/discussion**

8. **Safety precautions**

General Shut-Down Procedures

i) The pumps and stirrer is switched off. The needle valve is closed.

ii) All liquids in the reactors are drained by opening valves.

iii) The power for the control panel is switched off.

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1. **Experiment No.: EC-BTP701/03**
2. **Experiment Name:** To determine the relation between F and E curves.
3. **Objective:** To determine the relation between F and E curves for liquid flowing through Plug Flow Reactor (PFR).
4. **Principle:**

The Plug Flow Reactor (PFR) is used to model the chemical transformation of compounds as they are transported in ``pipes.'' The ``pipe'' may represent a river, a region between two mountain ranges through which air flows, or a variety of other conduits through which liquids or gases flow. Of course, it can even represent a pipe. As fluid flows down the PFR, the fluid is mixed in the radial direction, but mixing does not occur in the axial direction---each plug of fluid is considered a separate entity as it flows down the pipe. However, as the plug of fluid flows downstream, time passes. Therefore, there is implicit time dependence even in steady-state PFR problems. However, because the velocity of the fluid in the PFR is constant, time and downstream distance are interchangeable: $t = x/v$. We will use this observation together with the mass balance formulations we have worked with already to determine how pollutant concentrations vary during flow down a PFR.

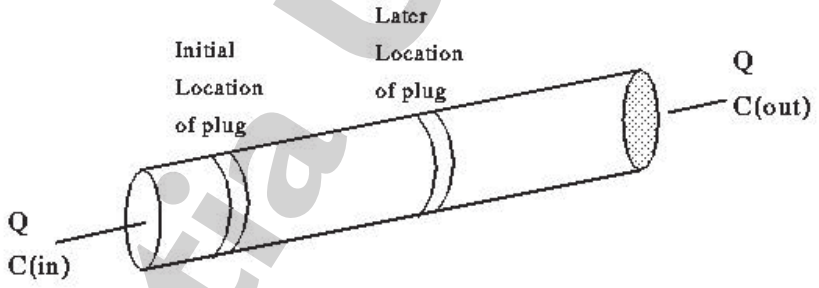
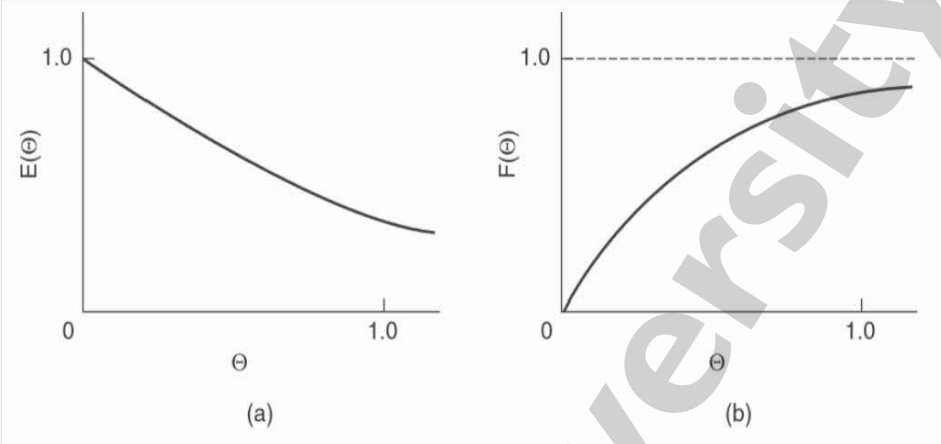


Figure 3: The Plug Flow Reactor

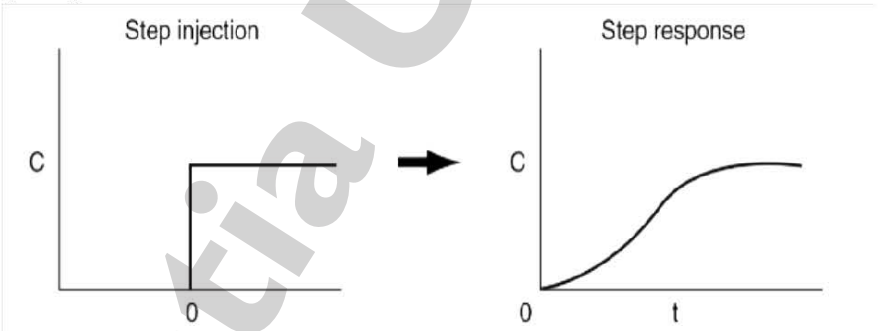
The Residence Time Distribution (RTD) inside the Plug Flow Reactor (PFR) shows the characteristic of the mixing of reactants that occurs inside the reactor. Inside the reactor, the reactants are continually consumed as the reactant flow along the length of the reactor. The residence time distribution function is represent in a plotted graph of E(t) as a function of time. This function shows in a quantitative manner of how much time the mixed fluid stays inside the reactor before leaving the reactor.



(c) E(t) curve and (b) F(t) Curve For Ideal CSTR

RTD is determined experimentally by injecting chemically inert substance known as tracer into the reactor at t = 0 and measuring the concentration of the tracer at the effluent stream as a function of time. The two most common methods of injecting tracer into the reactor are pulse input and step input.

Step Change Input In Continuous Stirred Tank Reactor



Typical Concentration – Time Curve At The Inlet And Outlet Stream For Step Change Input

A constant rate of tracer is added to the feed that is initiated at time t = 0. Thus, the inlet concentration of the tracer, C_o is constant with time. From this experiment, the cumulative distribution can be determined directly, F(t).

$$C_o(t) = \begin{cases} 0 & t < 0 \\ (C_o)\ \text{constant} & t \leq 0 \end{cases}$$

The cumulative distribution, F(t) represents the fraction of effluent that has been in reactor for time t = 0 until t = t.

$$\text{Cumulative Distribution, } F(t) = \left[\frac{C_{out}}{C_0}\right]_{step}$$

Differentiation of the cumulative distribution function yield to RTD function,

$$\text{Residence Time Distribution Function, } E(t) = \frac{d}{dt}\left[\frac{C(t)}{C_0}\right]_{step}$$

The mean residence time, tm shows the average time the fluids stay inside the reactor

$$\text{First Moment, Mean Residence Time, } t_m = \int_0^\infty t\,E(t)\,dt$$

The spread of the distribution which is the magnitude of the variance, σ^2. The greater the magnitude, the greater the distribution's spread will be

$$\text{Second Moment, Variance, } \sigma^2 = \int_0^\infty (t - t_m)^2 E(t)\,dt$$

The extent that a distribution is skewed in one direction is measured by the sleekness's magnitude which also means how differs the distribution is compared to the normal distribution

$$\text{Third Moment, Skewness, } s^3 = \frac{1}{\sigma^{\frac{3}{2}}}\int_0^\infty (t - t_m)^3 E(t)\,dt$$

5. **Materials**
   a) Deionized Water
   b) Oxalic acid
   c) Sodium Hydroxide (NaOH)
   d) Sulfuric acid (H_2SO_4)
   e) Sodium Chloride, NaCl
   f) Phenolphthalein Indicator

6. **Procedure:**
   i. Standardized solution of 0.05(N) oxalic acid is used for back titration.
   ii. **General Start-Up Procedures**

   i) The two 20L feed tanks (T1 and T2) are filled with deionized water.

   ii) Take 20 ml of 1(N) H_2SO_4 in a syringe and inject all of it at one instant in the tank. The acid is made sure to dissolve completely and the solution is ensuring to be homogenous.

   (Sometimes add tracer chloride is dissolved in tank. The salt is made sure to dissolve completely and the solution is ensure to be homogenous).

   iii) The power for the control panel is turned on.

iv) The stirrer assembly is made sure to secure properly to avoid damage to the mechanical seal.

v) The way valve 1 & 2 are set to position, so that the deionised water and acid solution from tank 1 & 2 will flow into axial pipe of the reactor.

vi) Pump is switched on to fill up the reactors with deionised water.

vii) After 5 minutes, pump is then switched off. The way valve is set to position.

vii) The needle valve is adjusted as to set the flow rate, flow rate to 150mL/min.

iii. Start the flow of water in the Packed bed reactor to build up the liquid level and to achieve steady state.

iv. Measure the steady state flow rate.

v. Withdraw the sample at regular interval of time and titrate it against std. 0.05N oxalic acid.

vi. Take 18 to 20 such readings.

vii. Plot graph of C(t) Vs time, E(t) Vs time , t.E(t) Vs time and (t^2). E(t) vs time using the readings of observation table and calculate area under the curve of each graph.

7. **Observations:**

1) Normality of NaOH = 0.05N

2) Flow rate of water = (ml/sec)

3) Normality of Oxalic Acid = 0.05N

4) Mean residense time in the reactor = t = V/ Fw (sec)

7.1 Observation Table:

Sl no	**Volume of sample V_2 (ml**	**Volume of NaOH (ml)**	**Outlet concentration of H_2SO_4 C(t)**	**Time (t) seconds**	**E(t)**	**t E(t)**	**$(t-tm)^2$ E(t)**

7.2 Calculations:

Plot concentration of H_2SO_4 (C_a) versus time and determine the area under the curve

Find $E(t) = C(t) / \int C(t)\, dt$

t_m = Mean residence time = $\int t.\, E(t)\, dt$ (min)

σ^2 (Variance) = $\int (t\text{-}tm)^2 . t .dt$ $(min)^2$

σ_θ^2 (skewness) = σ^2 / t_m^2 (dimensionless)

$\sigma_\theta^2 = 2/Pe - 2/Pe^2 (1\text{-}e^{-Pe})$

By trial & error

Pe = ----------

Graphs:

Plot the graph of C(t) v/s time

Plot the graph of E(t) v/s time

Plot the graph of t E(t) v/s time

Plot the graph of $(t\text{-}tm)^2$ E(t) v/s time

8. Inference/discussion

9. Safety precautions

a) The pumps and stirrer is switched off. The needle valve is closed.

b) All liquids in the reactors are drained by opening valves.

c) The power for the control panel is switched off.

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1. **Experiment No.: EC-BTP701/04**
2. **Experiment Name:** To develop a flow model to represent the vessel from the tracer output data presented graphically.
3. **Objective:**
   To develop a flow model to represent the vessel from the tracer output data presented graphically.
4. **Principle:**

   The overall goal is to use the following equation

   RTD Data + Kinetics + Model = Prediction

   The choice of the particular model to be used depends largely on the engineering judgment of the person carrying out the analysis. It is this person's job to choose the model that best combines the conflicting goals of mathematical simplicity and physical realism. There is a certain amount of art in the development of a model for a particular reactor, and the examples presented here can only point toward a direction that an engineer's thinking might follow. For a given real reactor, it is not uncommon to use all the models discussed previously to predict conversion and then make a comparison. Usually, the real conversion will be bounded by the model calculations.

   One-Parameter Models

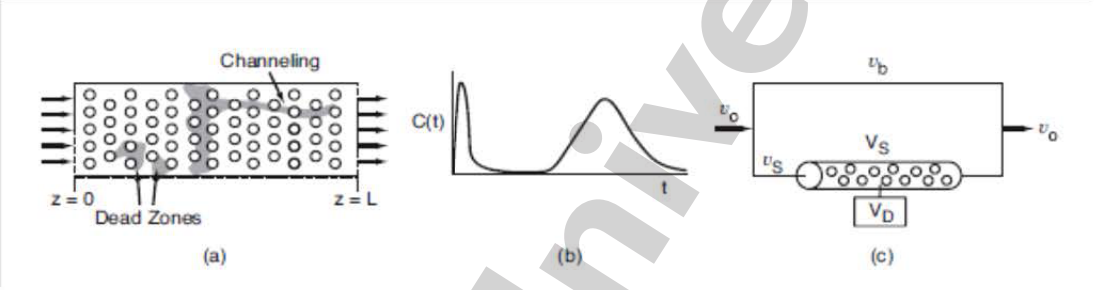
   Here we use a single parameter to account for the nonideality of our reactor. This parameter is most always evaluated by analyzing the RTD determined from a tracer test. Examples of one-parameter models for nonideal CSTRs include a reactor dead volume VD, where no reaction takes place, or a fraction f of fluid bypassing the reactor, thereby exiting unreacted. Examples of one-parameter models for tubular reactors include the tanks-in-series model and the dispersion model. For the tanks-in-series model, the parameter is the number of tanks, n, and for the dispersion model, it is the dispersion coefficient, Da. Knowing the parameter values, we then proceed to determine the conversion and/or effluent concentrations for the reactor.

   We first consider nonideal tubular reactors. Tubular reactors may be empty, or they may be packed with some material that acts as a catalyst, heat-transfer medium, or means of promoting interphase contact. Until now when analyzing ideal tubular reactors, it usually has been assumed that the fluid moved through the reactor in piston-like flow (PFR), and every atom spends an identical length of time in the reaction environment. Here, the velocity profile is flat, and there is no axial mixing. Both of these assumptions are false to some extent in every tubular reactor; frequently, they are sufficiently false to warrant some modification. Most popular tubular reactor models need to have means to allow for failure of the plug-flow model and insignificant axial mixing assumptions; examples include the unpacked laminar flow tubular reactor, the unpacked turbulent flow, and packed-bed reactors. One of two approaches is usually taken to compensate for failure of either or both of the ideal assumptions. One approach involves modeling the nonideal

tubular reactor as a series of identically sized CSTRs. The other approach (the dispersion model) involves a modification of the ideal reactor by imposing axial dispersion on plug flow.

Two-Parameter Models

The premise for the two-parameter model is that we can use a combination of ideal reactors to model the real reactor. For example, consider a packed bed reactor with channeling. Here the response to a pulse tracer input would show two dispersed pulses in the output as shown in Figures below.



(a) Real system; (b) outlet for a pulse input; (c) model system.

Here we could model the real reactor as two ideal PBRs in parallel with the two parameters being the fluid that channels, v_b, and the reactor dead volume, V_D. The real reactor volume is $V = V_D + V_S$ with $v_0 = v_b + v_S$.

5. **Materials**
   - i) Nutrient broth
   - ii) Sterile petriplates
   - iii) Micropipettes
   - iv) Cuvette
   - v) Conical flask
   - vi) Sterile tips
   - vii) Culture – Overnight culture of *S.cerevisiae*.
   - viii) UV Spectrophotometer

6. **Procedure:**

The following guidelines are suggested when developing models for nonideal reactors:

a) *The model must be mathematically tractable*. The equations used to describe a chemical reactor should be able to be solved without an inordinate expenditure of human or computer time.

b) The model must realistically describe the characteristics of the nonideal reactor. The phenomena occurring in the nonideal reactor must be reasonably described physically, chemically, and mathematically.

7. *The model must not have more than two adjustable parameters*. This constraint is used because an expression with more than two adjustable parameters can be fitted to a great variety of experimental data, and the modeling process in this circumstance is nothing more than an exercise in curve fitting. The statement "Give me four adjustable

parameters and I can fit an elephant; give me five and I can include his tail!" is one that I have heard from many colleagues. Unless one is into modern art, a substantially larger number of adjustable parameters are necessary to draw a reasonable-looking elephant. A one-parameter model is, of course, superior to a two-parameter model if the one-parameter model is sufficiently realistic. To be fair, however, in complex systems (e.g., internal diffusion and conduction, mass transfer limitations) where other parameters may be measured *independently*, then more than two parameters are quite acceptable.

The Guidelines

Look at the reactor.

i. Where are the inlet and outlet streams to and from the reactors? (Is by-passing a possibility?)
ii. Look at the mixing system. How many impellers are there? (Could there be multiple mixing zones in the reactor?)
iii. Look at the configuration. (Is internal recirculation possible? Is the packing of the catalyst particles loose so channeling could occur?).

Look at the tracer data.

i. Plot the E(t) and F(t) curves.
ii. Plot and analyze the shapes of the $E(\Theta)$ and $F(\Theta)$ curves. Is the shape of the curve such that the curve or parts of the curve can be fit by an ideal reactor model? Does the curve have a long tail suggesting a stagnant zone? Does the curve have an early spike indicating bypassing?
iii. Calculate the mean residence time, t_m, and variance, σ^2. How does the t_m determined from the RTD data compare with τ as measured with a yardstick and flow meter? How large is the variance; is it larger or smaller than τ^2?
iv. *Choose a model or perhaps two or three models.*
v. *Use the tracer data to determine the model parameters* (e.g., n, D_a, ν_b).
vi. *Use the CRE algorithm in Chapter 4.*

7. **Observations:**
   Calculate the exit concentrations and conversion for the model system you have selected.
8. **Inference/discussion**
9. **Safety precautions**

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1. **Experiment No.: EC-BTP701/05**
2. **Experiment Name:** K_m and V_{max} determination of a amylase
3. **Objective:** Determination of K_m and V_{max} determination of a amylase
4. **Principle:**

Of any biological system enzymes are the catalyst and they are very efficient as well as specific like that of catalyst. The most important function of an enzyme is to accelerate the reaction rate by million factors as compared to the rate of the reaction when enzymes are not present.

According to Michaelis-Menten equation

$$V = \frac{V_{max}[S]}{K_m + [S]_k}$$

V= Initial reaction velocity

V_{max}= Maximum Velocity

K_m= Michaelis costant

$[S]$ = Substrate concentration

K_m is enzyme characteristics and is particular substrate that reflects the affinity of the enzymes for the substrate. It is numerically equal to the concentration of the substrate at which the reaction velocity is same as half value of V_{max}.

It is not dependent on enzyme concentration.

Small K_m indicates the enzymes high affinity for the substrate low concentration of the substrate is required for saturating enzymes. Large K_m is the low affinity of the enzymes for substrate.

5. **Materials**
   i. Enzymes
   ii. Starch Solution- 2% stock and 0.1-2% working solution.
   iii. 0.1(N) HCl
   iv. Iodine Solution - 5g KI in 100ml of water. Then KI solution is added to 1g of iodine and then allows to completely dissolving.
   v. Amalase solution.
   vi. Potassium phosphate buffer having pH-7.
   vii. UV Spectrophotometer
6. **Procedure:**
   a) Prepare different concentration of soluble starch from the stock solution. The different concentration is 0.01, 0.025, 0.05, 0.1, 0.3, 0.5, 0.7 and 1.0 mg/ml.
   b) Add 1ml amylase solution to every test tube to initiate the reaction. Then kept it for incubation at 37°C for about 10 mins.
   c) Stop this reaction by adding 0.1(N) HCl. Then add 0.1ml of iodine solution.
   d) Take absorbance at 546 nm.

e) Draw graph with absorbance with V in Y-axis and substrate concentration in X-axis and then determine the value of V_{max} and K_m.

7. **Observations:**

Absorbance measurement

Substrate concentration	Absorbance

10. **Inference/discussion**

It is vital to have

11. **Safety precautions**

a) The performance of enzyme characteristics is to be utilized efficiently.
b) Wear eye protection for all but the most-dilute solutions.
c) Handle iodine solid using forceps or wear protective gloves especially for larger amounts.
d) Avoid breathing iodine vapor, eg, by using a fume cupboard.

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1. **Experiment No.: EC-BTP701/06**
2. **Experiment Name:** Determination of yield factor $Y_{x/s}$ for yeast growth.
3. **Objective:** To determine yield factor $Y_{x/s}$ for yeast growth.
4. **Principle:**

   One way to track the fermentation is to calculate yield factors, written as a capital Y with two subscripts denoting product and substrate. For example, $Y_{X/S}$ is the yield factor for amount of cell mass (g of yeast, X) per amount of substrate (g of glucose consumed, S), and is equal to:

   $$Y_{X/S} = -\Delta X / \Delta S$$

   The yield factor can be calculated at the end of the fermentation to evaluate overall performance, but also for a specific time period during the fermentation to compare the rates of greatest growth. Typical yield factors observed for S. cerevisiae grown aerobically on glucose are around 0.5 g/g, with significantly lower yields under anaerobic conditions.

   $Y_{P/S}$ stands for the amount of product (g of ethanol, P) produced per amount of substrate (g of glucose, S) consumed and is calculated the same way:

   $$Y_{P/S} = -\Delta P / \Delta S$$

   When working with any microorganism, avoiding contamination is vitally important. This prevents other organisms from depleting necessary nutrients and/or producing inhibitory metabolites. Among the aseptic techniques that you will use in this lab are: disinfecting surfaces with 70% ethanol; sterilizing media and equipment under steam pressure in an autoclave; using disposable, radiation-sterilized syringes; and preventing airborne contaminants from entering the fermentor environment by clamping off tubing and attaching 0.2 µm filters, which is smaller than the size of all microorganisms, onto exhaust and inlet vents.
5. **Materials**
   i) Nutrient broth
   ii) Sterile petriplates
   iii) Micropipettes
   iv) Cuvette
   v) Conical flask
   vi) Sterile tips
   vii) Culture – Overnight culture of Yeast strain *S.cerevisiae*
   viii) UV Spectrophotometer
6. **Procedure:**

a) Yeast culture

i) An isolated colony of the organism (*S.cerevisiae.*) was inoculated into 15 ml nutrient broth and kept for overnight incubation.

ii) Following day, the OD of this culture was measured and confirmed.

iii) In order to adjust the OD of the inoculums to the standard value (0.05) the following dilution formula was used

$$OD1V1 = OD2V2$$

Where,

OD1 = OD of the broth culture, inoculated the previous day.

V1 = volume of this broth culture to be added to the inoculums

OD2 = OD of the inoculum (as a standard, this value was adjusted to 0.05)

V2 = volume of the inoculums (in this experiment, 50 ml)

iv) Substitute the values in the equation and V1 was calculated.

v) That much amount (V1) of the inoculums was pipetted out before adding an equivalent amount of the broth to it, so that the net volume remains constant.

vi) The OD was checked at every 30 minutes interval and recorded.

vii) Using this OD value, a standardized growth curve of the organism was plotted. (Absorbance verses time).

viii) Generation time was calculated.

b) Bioethanol Production

i) 100 ml of this media was then poured in each of 250 ml of flask.

ii) 15g of each sample (biomass) are added and then allowed to incubate for next 72 h in an incubator at 30°C.

iii) Fermented broths will be removed after 72 hours of interval.

iv) Then the broth will be analyzed by distillation and filtration.

c) Distillation and Filtration

i) The mixture of ethanol and hot water were separated by simple distillation unit at a temperature of 78-96℃.

ii) Determination of ethanol content was done by spectrophotometric method.

7. Observations:

This experiment will track three parameters throughout the fermentation: glucose concentration, ethanol concentration and Yeast cells. These will be measured using the spectrophotometer to collect OD (optical density) readings.

Then calculate- i)$Y_{X/S}$ is the yield factor for amount of cell mass and ii) $Y_{P/S}$ stands for the amount of product.

12. Inference/discussion

13. Safety precautions

a) After inoculation into a particular sugar, sterilize the loop in order to avoid cross contamination of the tube with other sugars.
b) Keep uninoculated sugar tubes as control tubes.
c) Do not use the tubes with Durham tubes that are partially filled or with bubbles.
d) Over incubation will help the bacteria to degrade proteins and will result give false positive results.

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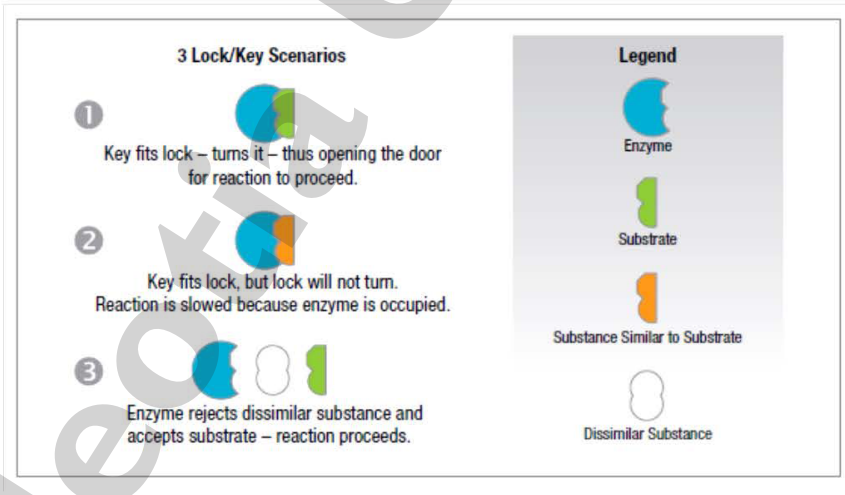
1. **Experiment No.: EC-BTP701/07 & EC-BTP701/08**
2. **Experiment Name:** Enzyme activity.
3. **Objective:**
   i) To study the effect of inhibitors and activators on enzyme activity.
   ii) Effect of different substrate concentration on enzyme activity
4. **Principle:**

Enzyme inhibitors are substances which alter the catalytic action of the enzyme and consequently slow down, or in some cases, stop catalysis. There are three common types of enzyme inhibition - competitive, non-competitive and substrate inhibition.

Most theories concerning inhibition mechanisms are based on the existence of the enzyme-substrate complex ES. As mentioned earlier, the existence of temporary ES structures has been verified in the laboratory.

Competitive inhibition occurs when the substrate and a substance resembling the substrate are both added to the enzyme. A theory called the "lock-key theory" of enzyme catalysts can be used to explain why inhibition occurs.

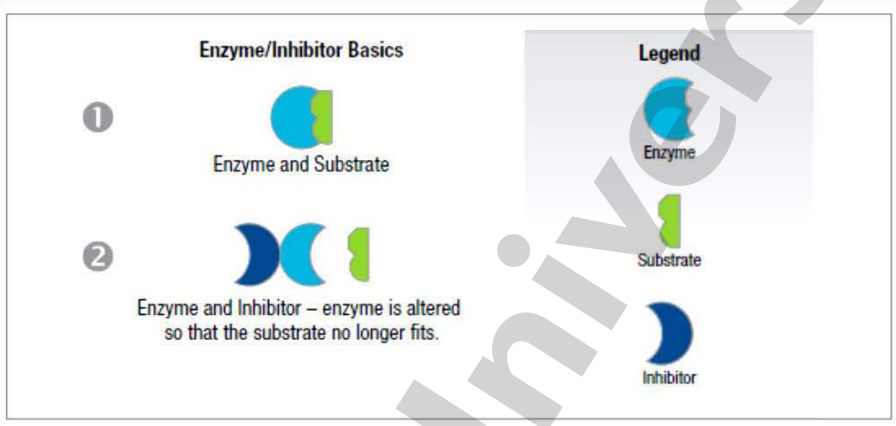


Lock and key theory – competitive analysis.

The lock and key theory utilizes the concept of an "active site." The concept holds that one particular portion of the enzyme surface has a strong affinity for the substrate. The substrate is held in such a way that its conversion to the reaction products is more favorable. If we consider the enzyme as the lock and the substrate the key (Figure above)- the key is inserted in the lock, is turned, and the door is opened and the reaction proceeds. However, when an inhibitor which resembles the substrate is present, it will compete with the substrate for the position in the enzyme lock. When the inhibitor wins, it gains the lock position but is unable to open the lock. Hence, the observed reaction is slowed down because some of the available enzyme sites are occupied by the inhibitor. If a

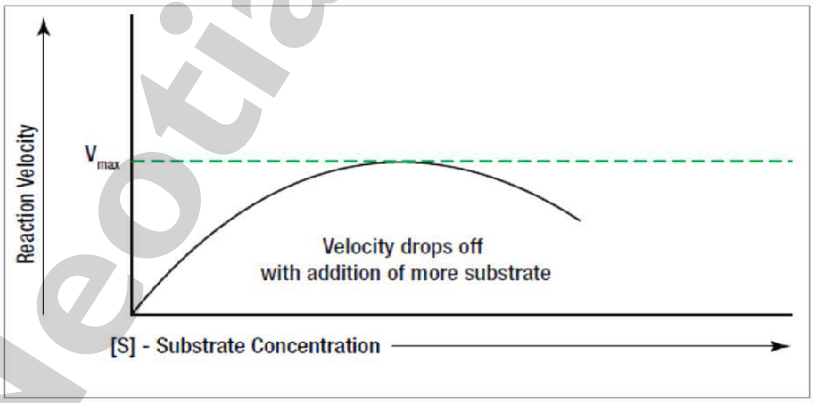
dissimilar substance which does not fit the site is present, the enzyme rejects it, accepts the substrate, and the reaction proceeds normally.

Non-competitive inhibitors are considered to be substances which when added to the enzyme alter the enzyme in a way that it cannot accept the substrate (Figure below).



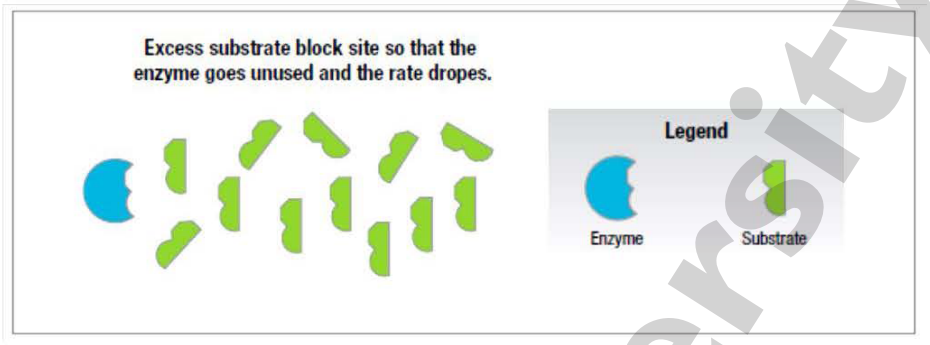
Noncompetitive inhibition

Substrate inhibition will sometimes occur when excessive amounts of substrate are present. Figure below shows the reaction velocity decreasing after the maximum velocity has been reached.



Substrate becoming rate inhibiting

Additional amounts of substrate added to the reaction mixture after this point actually decrease the reaction rate. This is thought to be due to the fact that there are so many substrate molecules competing for the active sites on the enzyme surfaces that they block the sites and prevent any other substrate molecules from occupying them.

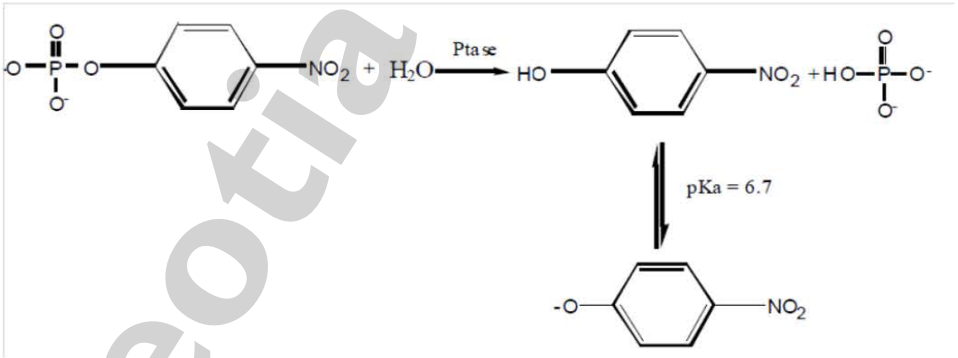


Substrate inhibition.

This causes the reaction rate to drop since all of the enzyme present is not being used.

Alkaline phosphatase Assay and Kinetics

Alkaline phosphatase is an enzyme that is found in the blood, intestine, liver, and bone cells of the human body. Like all enzymes, it is needed in small amounts to trigger specific chemical reactions. However, high levels of this enzyme in the body may be due to a variety of factors, which include bone fracture healing, liver diseases, bone diseases, hepatitis, or skeletal diseases; low levels may be indicative of poor nutrition, magnesium deficiency, or protein deficiency. Thus, alkaline phosphatase plays a crucial role in the body.



5. Materials

a) 1 bottle Enzyme Assay Buffer (pH 8.0)
b) 1 bottle Enzyme Assay Buffer (pH 9.0)
c) 1 bottle Enzyme Assay Buffer (pH 7.2)
d) 1 bottle Enzyme Assay Buffer (pH 5.0)
e) 1 vial Enzyme Substrate (pNPP)
f) 1 vial Enzyme (AP)
g) 1 bottle Enzyme Stop Solution
h) 1 vial Enzyme Inhibitor
i) 1.5ml Tubes
j) Stopwatch or timer
k) Pipettes and tips

l) Cuvettes (1ml) and Spectrophotometer

Additional Equipment Required

- Buffers and Enzyme Inhibitor can be stored at room temperature
- Enzyme should be stored at -20°C
- Enzyme Substrate should be stored at -20°C, prepare solutions 30 minutes before lab.
- Briefly centrifuge all small vials before opening to prevent waste of reagents.

6. Procedure

a) Prepare Enzyme Solution

i) Transfer 2ml of Assay Buffer (pH 8.0) to the vial of Enzyme (AP). Mix by vortexing or inverting the tube.

ii) When completely dissolved aliquot 300µl to each group

b) Prepare Substrate Solution

Prepare the enzyme substrate the morning of the experiment, no more than two hours before the start of the experiment.

i. To prepare 5mM Enzyme Substrate (pNPP) solution: Add 10ml distilled water to a 15ml tube.

ii. Remove 1ml water and add to the vial of Enzyme Substrate (pNPP). Mix until the substrate in completely dissolved. Transfer the 1ml of dissolved substrate back into the 15ml tube.

iii. Mix by inverting 4-6 times and supply each group with 1.5ml. Label vials Substrate Solution.

c) Miscellaneous

i. Aliquot 9ml of Enzyme Assay Buffer pH 8.0 and 1ml each of Enzyme Assay Buffers pH 9.0, 7.2, and 5.0 to each group.

ii. Aliquot 200µl Enzyme Inhibitor (10mM) into six tubes and supply each group with one tube.

iii. Aliquot 13ml Enzyme Stop Solution to each group.

d) ***Alkaline phosphatase Assay & Kinetics.***

The aim is to follow the kinetics of alkaline phosphatase enzyme using three different substrate concentrations over a 20 minute period.

i) ***Prepare Enzyme Stop Solution***: Label three sets of twelve 1.5ml centrifuge tubes for each assay of different substrate concentrations. Mark each set with a blank and time course intervals from 0-20 minutes with 2 minute intervals, as follows:

Assay A: Blank A, A-0m, A-2m, A-4m, A-6m, …, A-20m

Assay B: Blank B, B-0m, B-2m, B-4m, B-6m, …, B-20m

Assay C: Blank C, C-0m, C-2m, C-4m, C-6m, …, C-20m

ii) Add 0.1ml Enzyme Stop Solution and 0.8ml distilled water (or pure lab water) to the entire Assay A, B and C tubes.

iii) *Prepare enzyme reaction tubes*: Label three 1.5ml centrifuge tubes "Assay A", "Assay B", and "Assay C". Set up the following reactions as shown in the table and mix the contents by pipetting up and down several times.

Assay #	Substrate Solution (5mM)		Enzyme Assay Buffer (pH8.0)
	Volume (µl)	*Final Concentration (mM)*	*Volume (µl)*
Assay A	60	0.25	1160
Assay B	120	0.50	1100
Assay C	240	1.00	980

iv) *Prepare reaction blanks*: Transfer 0.1ml solution from the above enzyme reaction tubes to the appropriate tubes marked Blank and mix. They are your reaction controls or blanks.
*0.1ml solution from tube **Assay A** to tube Blank A; 0.1ml solution from tube **Assay B** to tube Blank B; and 0.1ml solution from tube **Assay C** to tube Blank C.*

v) *Initiate the enzyme reaction*: Add 25µl Enzyme (AP) to only the Assay A enzyme reaction tube and quickly mix by inverting the tube twice. ***Immediately***, remove 0.1ml reaction solution and mix with Enzyme Stop Solution in tube A-0m, this is your zero time point. Start the timer or stop watch immediately.

vi) Transfer 0.1ml reaction solution every 2 minutes from the assay tube to the corresponding Assay A Enzyme Stop Solution.

vii) On completion of Assay A, start Assay B by repeating steps 5 and 6 using the Assay B enzyme reaction tube. Once completed, carry out Assay C by repeating steps 5 and 6.

viii) Set the spectrophotometer at 410-415nm. Blank the spectrophotometer with Blank A when measuring the absorbance of Assay A tubes. Followed by the Assay B and Assay C. Record the absorbances in the table in the results section.

ix) Save Blank B for experiments II and III. Save all remaining assay tubes and rinse them with water for the following experiments.

e) ***Effect of pH on alkaline phosphatase activity***

i) *Prepare Enzyme Stop Solution*: Label 4 sets of eleven 1.5ml tubes: Mark each set with time course intervals from 0-20 minutes with 2 minute intervals, as follows:
Assay A: A-0m, A-2m, A-4m, A-6m, …, A-20m
Assay B: B-0m, B-2m, B-4m, B-6m, …, B-20m
Assay C: C-0m, C-2m, C-4m, C-6m, …, C-20m
Assay D: D-0m, D-2m, D-4m, D-6m, …, D-20m

ii) Add 0.1ml Enzyme Stop Solution and 0.8ml distilled water (or pure lab water) to all the Assay A, B, C and D tubes.

iii) *Prepare enzyme reaction tubes*: Label four 1.5ml centrifuge tubes "Assay A", "Assay B", "Assay C" and "Assay D". Set up the following reactions as shown in the table and mix the contents by pipetting up and down several times:

Assay#.	Substrate	Solution (5mM)	Enzyme Assay Buffer
		(pH)	***Volume (µl)***
Assay A	110µl	pH 5.0	1000
Assay B	110µl	pH 7.2	1000
Assay C	110µl	pH 8.0	1000
Assay D	110µl	pH 9.0	1000

iv) *Initiate the enzyme reaction*: Add 25µl Enzyme (AP) to only the Assay A enzyme reaction tube and quickly mix by inverting the tube twice. ***Immediately***, remove 0.1ml reaction solution and mix with Enzyme Stop Solution in tube A-0m, this is your zero time point. Start the timer or stop watch immediately.

v) Transfer 0.1ml reaction solution every 2 minutes from the assay tube to the corresponding Assay A Enzyme Stop Solution.

vi) On completion of Assay A, start Assay B by repeating steps 4 and 5 using the Assay B enzyme reaction tube. Once completed, carry out Assay C by repeating steps 5 and 6, then Assay D.

vii) Set the spectrophotometer at 410-415nm. Blank the spectrophotometer with Blank 2 saved from the first experiment. Measure the absorbance of all tubes and record the values in the table in the results section.

viii) Save the blank 2 for following experiment III. Save all assay tubes and rinse them with water for following experiment III.

f) Alkaline phosphatase and competitive inhibition.

This experiment tests the effects of different concentrations of an inhibitor on enzyme activity.

i) Prepare 1 to 10 serial dilutions of the Enzyme Inhibitor: The provided 10mM Enzyme Inhibitor needs to be diluted to two additional dilutions (1mM and 0.1mM), as follows:

a. Label two 1.5ml tubes with 1.0mM and 0.1mM.

b. To each tube, add 180µl Assay Buffer pH 8.0.

c. Transfer 20µl Enzyme Inhibitor (10mM) to the 1mM labeled tube and pipette up and down to mix.

d. Transfer 20µl from the 1mM Enzyme Inhibitor tube to the 0.1mM labeled tube and pipette up and down to mix.

ii) Prepare Enzyme Stop Solution: Label 4 sets of eleven 1.5ml tubes: Mark each set with time course intervals from 0-20 minutes with 2 minute intervals, as follows:

Assay A: A-0m, A-2m, A-4m, A-6m, …, A-20m

Assay B: B-0m, B-2m, B-4m, B-6m, …, B-20m

Assay C: C-0m, C-2m, C-4m, C-6m, …, C-20m

Assay D: D-0m, D-2m, D-4m, D-6m, …, D-20m

iii) Add 0.1ml Enzyme Stop Solution and 0.8ml distilled water (or pure lab water) to the entire Assay A, B, C and D tubes.

iv) *Prepare enzyme reaction tubes*: Label four 1.5ml centrifuge tubes "Assay A", "Assay B", "Assay C" and "Assay D". Set up the following reactions as shown in the table and mix the contents by pipetting up and down several times:

Assay #.	Substrate Solution (5mM)	Enzyme Assay Buffer pH 8.0	Enzyme Inhibitor	
			Concentration (mM)	*Volume*
Assay A	110µl	101µl	0	0µl
Assay B	110µl	900µl	0.1	110µl
Assay C	110µl	900µl	1.0	110µl
Assay D	110µl	900µl	10	110µl

v) *Initiate the enzyme reaction*: Add 25µl Enzyme (AP) to only the Assay A enzyme reaction tube and quickly mix by inverting the tube twice. ***Immediately***, remove 0.1ml reaction solution and mix with Enzyme Stop Solution in tube A-0m, this is your zero time point. Start the timer or stop watch immediately.

vi) Transfer 0.1ml reaction solution every 2 minutes from the assay tube to the corresponding Assay A Enzyme Stop Solution.

vii) On completion of Assay A, start Assay B by repeating steps 4 and 5 using the Assay B enzyme reaction tube. Once completed, carry out Assay C by repeating steps 5 and 6, then Assay D.

viii) Set the spectrophotometer at 410-415nm. Blank the spectrophotometer with Blank 2 saved from the first experiment. Measure the absorbance of all tubes and record the values in the table in the results section.

7. Observations

Alkaline phosphatase Assay & Kinetics

Substrate Conc.	Absorbance										
	0	**2**	**4**	**6**	**8**	**10**	**12**	**14**	**16**	**18**	**20**
0.25mM											
0.5mM											
1.0mM											

Plot Absorbance vs. Time for each concentration on a graph. Include all concentrations on the same graph for easy comparison.

Effect of pH on alkaline phosphatase activity

pH	Absorbance										
	0	2	4	6	8	10	12	14	16	18	20
5.0											
7.2											
8.0											
9.0											

Plot Absorbance vs. Time for each concentration on a graph. Include all curves on the same graph for easy comparison.

Alkaline phosphatase and competitive inhibition

Inhibitor Concentration (mM)	Absorbance										
	0	2	4	6	8	10	12	14	16	18	20
0											
0.1											
1.0											
10											

Make a graph of Absorbance vs. Time for each concentration of substrate with the different inhibitor concentrations.

8. Inference/discussion

9. Safety precautions

a) Any waste enzyme powder should be dissolved in water before disposal into the sewage system.

b) Enzyme on the skin or inhaled should be washed with plenty of water.

c) Liquid preparations are inherently safer but it is important that any spilt enzyme is not allowed to dry as dust formation can then occur.

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