# DEPARTMENT OF BIOTECHNOLOGY

# B.TECH
# BIOPROCESS ENGINEERING LABORATORY MANUAL

# (17BTCC91)

**HOD**

# CONTENTS

## LABORATORY RULES AND REGULATIONS

There are some rules which should be followed for the successful completion of the lab experiments, safety of the student and convenience of others working in the lab

(i) Upon entering the laboratory, place bags, books and other materials in specified locations. Never on bench tops
(ii) Always wear a lab coat before entering the laboratory for protecting clothes from Contamination or accidental discoloration by staining solutions
(iii) Keep the lab windows and doors closed during laboratory sessions to prevent contamination from air currents
(iv) Before and after each lab session wipe your work bench with a disinfectant like Lysol or ethanol
(v) Do not place contaminated instrument such as inoculation loop, needles and pipettes on bench top
(vi) Wash your hands with soap and water upon entering and prior to leaving the lab
(vii) Do not smoke eat or drink in the lab. These activities are absolutely prohibited
(viii) Tie back long hair to minimize its exposure to open flame
(ix) If a live culture is spilled, cover the area with a disinfectant solution for 15 min and then clean it
(x) In the event of personal injury such as cuts or burns, inform the instructor immediately. Open cuts and wounds should be covered
(xi) All microbial culture should be handled as potential pathogen
(xii) Never pipette any broth cultures or chemical reagents by mouth
(xiii) Always keep culture in an upright position in the test tube rack to carry culture when moving around the lab
(xiv) Familize with the exercise to be performed
(xv) Always perform the experiments to be sequence. Handle the apparatus and equipment carefully
(xvi) Label all plates tubes and cultures properly before starting the experiment
(xvii) As you perform the experiment, record your data in the notebook
(xviii) Materials such as stains, reagent bottle test tubes petriplates pipettes must be returned to the original location after use
(xix) Always use gloves while using toxic chemicals and matters.
(xx) In case of accidently splash of chemicals risen with water and inform to instructor
(xxi) Discard waste into appropriate place
(xxii) Speak softly and avoid unnecessary movements around the lab to prevent distraction that may cause accidents
(xxiii) On completion of lab, session, place all the cultures and materials in disposal area as designated by the instructor

# INTRODUCTION TO BIO PROCESS ENGINEERING

Bioprocess Engineering is defined as the process that uses complete living cells or their components (e.g., enzymes, chloroplasts) to effect desired physical or chemical changes. Bioprocess engineers can work in many fields like agriculture research and development, food processing companies, biotechnology firms, waste management sectors, fuel, and pharmaceutical industries. Bioprocess or fermentation technology is an important component of most 'old' and 'new' biotechnology processes and will normally involve complete living cells (microbe, mammalian or plant), organelles or enzymes as the biocatalyst, and will aim to bring about specific chemical and/or physical changes in biochemical..

The most important applications of bioprocess-engineering research and development related to agriculture and food involve production of agricultural chemicals for control of animal and plant diseases, growth-stimulating agents for improved yield, and biological insecticides and herbicides; increasing bioprocess efficiencies for fermented foods, natural food additives, food enzymes as processing aids, and separation and purification of the products; use of plant-cell culture systems to produce secondary metabolites or chemical substances of economic importance; and efficient use of renewable biomass resources for production of liquid fuel and chemical feedstocks and efficient treatment and management of agricultural wastes and wastes from food-processing industries.

## KEY INDUSTRIES THAT USE BIOPROCESS ENGINEERING

Bioprocess technology and its engineers are essential in every industry that relies on biomaterials or biological products. Bioprocess technology is the backbone of the biotechnology industry, translating scientific discoveries to industrial products. The pharmaceutical industry commonly employs bioprocess engineers to develop and organize manufacturing processes for novel drugs, pharmaceuticals, supplements like antibiotics, and vaccines, while the medical industry involves bioprocess development for biopharmaceuticals to generate a safe, effective, and stable product.

**EXP NO:** **DATE:**

## GROWTH KINETICS OF *E.COLI* IN A FERMENTOR VESSEL

**AIM**

To study the growth of *E.coli* in a batch culture in a fermentor vessel.

**PRINCIPLE**

Batch culture is closed culture system which contains an initial, limited amount of nutrition. The inoculated culture will pass through a number of phase;

a. After inoculation there is period during which it appear that no growth takes place, this period is referred to as the lag phase and may be considered as the time of adaptation.
b. Following lag phase there is a period during which the growth rate of the cells gradually increase, the cell grow at a constant, maximum rate and the period is known as Log or exponential phase.

At this phase $dx/dt = \mu x$ – (1)

X = biomass conc.

t = time in hrs.

$\mu$ = specific growth rate $hr^{-1}$

On integrating (1)

$$X_t = X_o . e^{-\mu t}$$

This is the equation for microbial growth in the exponential phase,

Xo = initial biomass concentration

Xt = biomass concentration after time t.

T = time

On taking ln

$$\ln Xt = \ln Xo + \mu t$$

A plot of ln x Vs t gives straight line with slope µ.

(or)

$$\mu = (\ln X_2 - \ln X_1) / (t_2 - t_1)$$

Also, the biomass concentration can be proportional to the OD of the culture at 600nm.

$$\mu = (\ln OD_2 - \ln OD_1) / (t_2 - t_1)$$

Following log phase is the decelerating phase and stationary phase, where the growth is almost constant w.r.t time. On depletion of nutrients this phase is followed by declining growth phase where growth occurs but the death rate is greater.

The yield coefficient: $Y_x/s = \nabla x/\nabla s = Xo - X/S_R - S$

X = concentration of biomass

$S_R$ = initial substrate concentration

S = Residual substrate concentration

**PROCEDURE**

A sterilized fermentor was used with 1.5L media. Known volume of the culture (5%) was inoculated into the vessel through a peristaltic pump. Some sample was taken for analysis at time t = 0. The sample was checked at 600nm using media as blank. Thus OD is the biomass concentration. Then the sample was checked for residual glucose through DNSA method. The analysis was done for different samples taken at different time intervals.

**DNSA Method**

1. 0.5 ml to 3 ml of 0.3 mg/ml of stranded glucose solution was taken in test tubes are labeled as s1,s2,…….s5 to s6.
2. It was made up to 3 ml with distilled water.

3. 3 ml of distilled water was taken as blank and 3 ml of DNSA solution was added to all the test tubes.
4. Then the test tubes were covered with paraffin film.
5. The tubes were placed in water bath at 90 for 10 to 15 minutes until red brown colour develops.
6. 1 ml of 40% potassium sodium tartarate solution was added to sterilize the colour.
7. After cooling to room temperature in a cold water bath. The absorbance was recorded using spectrophotometer.

**OBSERVATION:**

The batch culture in the fermentor vessel was studied**.** The trend of growth was seen with initial log phase followed by the log phase and the stationary phase. The max. sp. Growth rate = ---------- with the doubling time as---------- minutes.

**Tabulation-I**

| Time (Min) | OD at 600 nm | Dry Cell wt (X) | Residual Substrate (S) mg/ml | ln OD | Sp.Growth rate (μ) | Yield (Yx/s) | Doubling time($t_d$) |
|---|---|---|---|---|---|---|---|
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |

**Tabulation-II**

| S.No. | Particulars | B | S1 | S2 | S3 | S4 | S5 | S6 |
|---|---|---|---|---|---|---|---|---|
| 1. | Volume of glucose (ml) | - | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 | 3.0 |
| 2. | Volume of distilled water (ml) | 3.0 | 2.5 | 2.0 | 1.5 | 1.0 | 0.5 | - |
| 3. | Volume of DNS (ml) | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 |
| Incubate in water bath at 90°C | | | | | | | | |
| 4. | Volume of sodium potassium tartarate (ml) | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 5. | Optical density at 600 nm. | | | | | | | |

**RESULT**

The batch fermentation process was studied and the following parameters were found:

Specific growth : μ =…… (by calculation)

μ =……. (by graph)

Doubling time :…….

Yield coefficient :…….

**GRAPH**

1.Standard graph, 2.OD vs Time, 3.ln OD vs time, 4.Yeild vs time, 5.OD and RS vs time

## ESTIMATION OF MONOD KINECTIC PARAMETERS

**AIM**

To study the effect of substrate concentration glucose on growth of *E. coli* and to estimate Monod's kinetic parameter.

**THEORY**

The general goal in making a medium is to support growth and high rate of product synthesis. Contrary to intuitive expectation, these doses not necessarily mean that all nutrients should be supplied in great excess. For one thing, excessive concentration of a nutrient can inhibit or even poison cell growth. Moreover, if the cells grow too extensively, their accumulated metabolic end products will often disrupt the normal biochemical processes of the cells. Consequently, it is common practice to limit total growth by limiting the amount of one nutrient in the medium.

If the concentration of one essential medium constituent is varied while the concentrations of all other medium components are kept constant, the growth rate typically changes in a hyperbolic fashion. A functional relationship between specific growth and essential compounds concentration was proposed by Jacques Monod in 1942. Of the same form as the Langmuir adsorption isotherm in 1918, in the standard rate equation for enzyme catalyzed reaction with a single substrate by Michaelis and Menten in 1913, the Monod equation states that

**Monod Equation**

$$\mu = \frac{\mu_{max} \cdot S}{Ks/S}$$

$\mu$ - Specific growth rate, $hr^{-1}$

$\mu_{max}$ - Maximum growth rate, $h^{-1}$

S - Limiting substrate concentration (g/L)

Ks - Saturation constant or Monod constant (g/L)

As S increases, $\mu$ also increases until it attains $\mu_{max}$. Ks indicate the increase in an affinity for an microorganism towards the limiting substrates.

$$1 / \mu = 1/\mu_{max} + Ks / \mu_{max}.1 / S$$

## MATERIALS REQUIRED

(i) A slant of freshly sub cultured *E. coli*

(ii) pH meter

(iii) UV Spectrophotometer

(iv) Orbital shaker

(v) Culture medium

**Luria Broth**

| | |
|---|---|
| Casein enzyme hydrolysate | – 10g / L |
| Yeast extract | – 5g / L |
| Sodium Chloride | – 5g / L |

## PREPARATION OF INOCULUM AND GROWTH OR PRODUCTION MEDIUM

(i) Take 200mL of inoculum media in a 500mL Erlen Meyer flask.

(ii) Take 200mL of five growth media differ in glucose concentration by 1, 2, 5, 10, and 15g / L, while the concentration of other nutrient components are same. Label the flask by their initial glucose concentration.

(iii) Set pH 7 for both inoculums and growth media. Autoclave the media.

(iv) When the medium containing glucose, phosphate and salts are sterilized the following phenomena may occur :

i. The cation of salts forms a precipitate with the phosphates.

ii. Glucose is degraded partially in the presence of other medium ingredients (particularly phosphates) to certain compounds which are toxic to growth of cells.

**Note**

When preparing inoculum and growth medium, the appropriate amount of glucose, phosphates and remaining nutrients, components should be prepared and sterilized separately. After sterilization, they can be mixed together aseptically after cooling.

## INOCULUM PREPARATION

(i) Transfer a loop ful of *E. coli* cells aseptically in laminar air flow into inoculum medium from a freshly subcultured slant.

(ii) Keep in orbital shaker (T – 30°C., Speed – 200rpm, t – 12h).

(iii) After 12h of growth, the inoculum will be ready to inoculate five growth media.

## GROWTH OF *E. COLI* CELLS WITH DIFFERENT INITIAL CONCENTRATIONS

(i) Inoculate each growth medium with 10mL of freshly grown culture, 5% (v/v) aseptically in laminar flow chamber using 10mL sterilized measuring cylinder.

(ii) Place the flask in a orbital shaker (Speed – 200rpm, T – 30°C)

(iii) Run the cultures for 18h.With draw 5mL of samples from 5 flasks in prelabelled tubes at every hour aseptically (including zeroth hour).

After sampling, heat the sample immediately to stop the growth. Dilute the sample 10 times (1mL of sample in 9mL of distilled water).

Analyze then for cell mass by taking O. D. at 620nm.Use distilled water as blank.

**Note**

O. D. gives indirect measurement of cell concentration. To measure the cell mass directly we can measure the dry cell geometrically.

(i) Tabulate your five growth optical data.

(ii) Compare ln (O. D. / O. $D_o$) for the values in the table.

(iii) Plot ln (O. D./ O. $D_o$) against't' for every initial substrate concentration till you get 5 profiles.

Fix exponential growth phase data to linear regression which will yield more accurate result.

(iv) Tabulate the specific growth rate values along with corresponding initial substrate concentration.

(v) Plot $\mu$ against $S_o$ in the linear exponential growth region which is equal to specific growth $\mu$ (h) for that particular growth rate.

(vi) Plot 1 / $\mu$ Vs 1 / $S_o$ for getting more accurate result. Fit the data to linear regression.

Slope = $K_s / \mu_{max}$ Intercept = $1 / \mu_{max}$

**RESULT**

Maximum specific growth rate, $\mu_{max}$ =

Saturation constant $K_s$ =

**EXP NO:** **DATE:**

## MEDIA OPTIMIZATION BY PLACKETT AND BURMAN

## Aim

To optimize the concentration and composition of various nutrients in the media using the Plackett and Burman method

### Theory

Detailed investigation is needed to establish the most suitable medium for an individual fermentation process, but certain basic requirements must be met by any such medium. All micro-organisms require water, sources of energy, carbon, nitrogen, minerals elements and possibly vitamins plus oxygen (if aerobic). On a small scale it is relatively simple to devise a medium containing pure compounds, but the resulting medium, although supporting satisfactorily growth may be unsuitable for use in a large scale process. On a large scale one must normally use sources of nutrients to create a medium which will meet as many possible of the following criteria:

It will produce the maximum yield of product or biomass per gram of substrate used.
It will produce the maximum concentration of product or biomass.
It will permit the maximum rate of product formation.
There will be the minimum yield of undesired products.
It will be of consistent quality and be readily available throughout the year.
It will cause minimal problems during media making and sterilization.
It will cause minimal problem in other aspects of the production process particularly aeration and agitation, extraction, purification and waste treatment.

Medium optimization by the classical method of changing one independent variable (nutrient, antifoam, pH, temperature etc.) while fixing all the other at a certain level can be extremely time

consuming and expensive for a large number of variables. To make a full factorial search which will examine each possible combination of independent variable at appropriate levels will require a large number of experiments $X^n$, where X is the number of levels and n is the number of variables. This may be quite approximate for the three nutrients at two concentrations (2" trails) but not for six nutrients at three concentrations, in this instance 3' (729) trials would be needed. Industrially the aim is to perform minimum number of experiments to determine optimal conditions. Other alternative strategies must be considered which allow more than one variable to be changed at a time.

When more than one variable are to be investigated, the Plackett-Burman design may be used to find the most important variable in the systems, which are then optimized in further studies, (Plackett and Burman, 1946). These authors gave a series of design for up to hundred experiments using an experimental rationale known as balanced incomplete blocks. This technique allows all the evaluation of X – 1 variable by X experiments. X must be a multiple of 4, e.g. 8, 12, 16, 20, 24, etc. Normally one determines how many experimental variables needed to be included in an investigation and then selects the Plackett-Burman design which meets the requirements in most closely in a multiple of 4. Any factors not assigned to variable can be designated as a dummy variable. Alternatively, factors known to have no effect may be included and designated as dummy variables. The incorporation of dummy variables into an experiment makes it possible to estimate the variance of an effect (experimental error).

Table -1 show a Plackett-Burman design for seven variables (A-G) at high and low levels in which two factors, E and G, are designed as dummy variables. These can be used in design to obtain an estimate of error. However, more can be studied if fewer real variables need to be studied in an investigation (Stowe and Mayer, 1966). Each horizontal represents a trail and each vertical column represents an L (low) and H (high) values of one variable in all the trails. This design (Table) requires that the frequency of each level of a variable in a given column should be equal. Consider the variable A; for the trials in which A is high, B is high in two of the trials and low in the other two. Similarly, C will be high in the either two trials and low in the two as will all the remaining variables. For those trials in which A is low B will be high two times and low two times. This will also apply to all the other variables.

**Plackett-Burman design for seven variables**

| Trial | Variables | | | | | | |
|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G |
| 1 | H | H | H | L | H | L | L |
| 2 | L | H | H | H | L | H | L |
| 3 | L | L | H | H | H | L | H |
| 4 | H | L | L | H | H | H | L |
| 5 | L | H | L | L | H | H | H |
| 6 | H | L | H | L | L | H | H |
| 7 | H | H | L | H | L | L | H |
| 8 | L | L | L | L | L | L | L |

H denotes a high level value; L denotes a low level value

Thus, the effects of changing the other variables cancel out when determining the effect of A. The same logic then applies to each variable. However, no changes are made to the high and low values for the E and G columns. Gresham and Inamine (1986) state that although the difference between the levels of each variable must be large enough to ensure that the optimum response will be included, caution must be taken when setting the level differential for sensitive variables, since a differential that is too large could mask the other variables. These trails are carried out in a randomized sequence.

The effects of the dummy variables are calculated in the same way as the effects of the experimental variables. If there are no interactions and no error in measuring the response, the effect shown by a dummy variable should be 0. If the effect is not equal to 0, it is assumed to be a measure of lack of experimental precision plus any analytical error in measuring the response (Stowe and Mayer, 1966).

**The stages in analyzing the data (Table 1 and 2) using Nelson's (1982) example are as follows:**

**Determine the difference between the average of H (high) and L (low) responses for each independent and dummy variable.**

Therefore the difference = ∑ A (H) - ∑ A (L)

The effect of an independent variable on the response is the difference between the average response for the four experiments at the high level and the average value for the four experiments at the low levels.

**Analysis of the yield shown in Plackett-Burman Table**

∑ (H)

∑ (L)

Difference effect

Mean square

Mean square for error

F- Test

Thus the effect of

$$\frac{A}{4} = \frac{\sum \quad A(H) - \sum \quad A(L)}{4} = \frac{2\sum \quad A(H) - \sum \quad A(L)}{8}$$

This value should be near zero for the dummy variables.

**Estimate the mean square of each variable (the variance of effect).**

$$For\ A\ the\ mean\ square\ will\ be\ = \frac{\left(\sum \quad A(H) - \sum \quad A(L)\right)^2}{8}$$

**The experimental error can be calculated by averaging the mean square of the dummy effects of E and G.**

$$mean\ square\ for\ error = \frac{\sum \quad (mean\ square\ of\ dummy\ variables)}{Number\ of\ dummy\ variables}$$

**The final stage is to identify the factors which are showing large effects. This is done using a F-Test which is given by :**

Factor mean square

Error mean square

The factor which is having highest F-Test value is identified as the most important factor.

If dummy variable is zero consider Error mean square value as one

**PROCEDURE**

Prepare the medium according to the Plackett-Burman design for each trial of appropriate high and low concentrations.

| **Medium variables** | **Low (L) g/l** | **High (H) g/l** |
|---|---|---|
| A. Glucose | 0.5 | 4.0 |
| B. Fructose | 0.5 | 4.0 |
| C. Glycine | 0.1 | 1.0 |
| D. Xylose | 0.5 | 4.0 |
| E. Sucrose | 0.5 | 4.0 |
| F. Yeast extract | 0.01 | 1.0 |
| G. Casamino acid | 0.05 | 0.5 |
| Ammonium Chloride | 2.0 | |
| $KH_2PO_4$ | 3.0 | |
| $MgSO_4$ (IM) | 1 ml | |

Medium was inoculated with 1 ml of *E. coli* in each medium.

Zero time optical density reading was noted.

The culture was incubated in 37ºC in a shaker.

Optical density was measured at regular time intervals and recorded.

The highest optical density reading was taken as its response.

**RESULT:**

1. The optimized concentration of the media was found to be in _____

2. The component_____ influences the production of biomass.

**TABLE 2: PLACKETT-BURMAN RESPONSE TABLE**

| Trial | A | B | C | D | E | F | G | Response(OD ) at 540 nm |
|---|---|---|---|---|---|---|---|---|
| 1 | H | H | H | L | H | L | L | |
| 2 | L | H | H | H | L | H | L | |
| 3 | L | L | H | H | H | L | H | |
| 4 | H | L | L | H | H | H | L | |
| 5 | L | H | L | L | H | H | H | |
| 6 | H | L | H | L | L | H | H | |
| 7 | H | H | L | H | L | L | H | |
| 8 | L | L | L | L | L | L | L | |

**TABLE 3: ANALYSIS OF YIELD SHOWN IN TABLE 2.**

| variables / calculated values | A | B | C | D | E | F | G |
|---|---|---|---|---|---|---|---|
| ∑H | | | | | | | |
| ∑L | | | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Difference | | | | | | | |
| Difference effect | | | | | | | |
| Mean square | | | | | | | |
| Mean square for error | | | | | | | |
| F- test | | | | | | | |

**EXP NO.** **DATE:**

## ENZYME IMMOBILIZATION BY ENTRAPMENT IN CROSS LINKED GELATIN GEL

### AIM

To study the cross linked gelatin gel entrapment technique of enzyme immobilization

### THEORY

Enzymes can be immobilized by physical entrapment inside matrix (support) of a water soluble polymer such as polyacrylamide type gels and naturally derived gels e.g. Cellulose triacetate, agar, gelatin, carrageenan, alginate etc.,(Fig ) .The form and nature of matrix vary. Pore size of matrix should be adjusted to prevent the loss of enzyme from the matrix due to excessive diffusion.. Enzyme can be entrapped by several ways: Enzyme inclusion in gels, Enzyme inclusion in fibers (entrapped in fibre format), and Enzyme inclusion in microcapsules. The hydrophobic and hydrophilic forms of the matrix polymerize to form a microcapsule containing enzyme molecules inside.

Gelatin is a heterogonous mixture of water soluble protein of high molecular weight. The molecular weight ranges from 20,000 – 25, 000. Gelatin is derived from collagen and is recovered by hydrolysis. There are several varieties of gelatin, the composition of which depends on the source of collagen and hydrolytic treatment used.

The main feature of gelatin as immobilization media is that the gel formation process is mild and only simple equipment and reagents are needed. The second feature is that it is relatively inexpensive and non-toxic. The retention of enzyme activities for immobilization with a gelatin gel is typically 25 – 50% of the original free enzyme. Gelatin gel has the advantage that the mass transfer resistance is relatively low compared to other entrapment methods, but the rate of enzyme loss due to leakage is high. Highly cross-linked matrices can result in higher mass transfer resistances for both the substrate

## MATERIALS REQUIRED

(i) Gelatin (10%)

(ii) Hardening solution (100mL)

Formaldehyde – 20mL

Ethyl alcohol – 50mL

Water – 30mL

(iii) Storage solution ( 100mL)

50mM Calcium chloride

(iv) Enzyme α - Amylase

(v) Beakers

(vi) Cylinders

(vii) Freezer

(viii) Pipettes.

## PROCEDURES

(i) 10% Gelatin was prepared by dissolving 10g of gelatin in 100mL distilled water and heated for complete dissolving.

(ii) 10mL of gelatin was pipette out and when the temperature was approximately 35 – 40 °C. 0.015 g of α - Amylase was added.

Immediately 2mL of hardening solution was added to the above mixture.

After thorough mixing, the contents were poured in to a mold and kept at - 28°C for hours to facilitate gel formation.

When the gel was set, it was left at room temperature for few minutes.

Later, the gel was cut in to small pieces (approximate 3mm).

) The pieces of immobilized enzyme were washed gently with deionized water twice and stored in Calcium chloride solution for further study.

## RESULT

Thus the enzyme amylase was immobilized by entrapping in crosss linked gelatin gel.

**EXP NO:** **DATE:**

## EFFECT OF TEMPERATURE ON ENZYME ACTIVITY

### AIM

To study the effect of temperature on activity of α-amylase enzyme and to find optimum temperature ($T_{opt}$), activation energy (Ea) .

### THEORY

The velocity of enzyme reaction increases when temperature of the medium is increased, reaches a maximum and then decreases due to denaturation. Temperature at which maximum amount of substrate is converted into product is called the optimum reaction time for that enzyme. As temperature is increased, more molecules get activation energy or the molecules at increased rate of motion, so the reaction velocity is enhanced. Denaturation of tertiary structure of protein occur, when temperature is more than 50˚C.so activity of enzyme is decreased.

According to Arrhenius equation, effect of temperature on enzyme activity (reaction rate) is given by

$Ka = Ae^{-Ea/RT}$……..(1)

Where, A is Arrhenius constant; Ea is the activation energy in joule/µmol; Ka is deactivation rate constant in $min^{-}$; R is gas constant ($8.314*10^{-6}$ joule/µmol K); T is temperature in K.

### MATERIALS REQUIRED:

***Equipments***

- Beakers
- Graduated Cylinder
- Balance
- Pipette

- Calorimeter.

***REAGENTS***

- Enzyme alpha amylase solution
- Starch (10gm/l)
- Rochelle's salt (40%)
- DNS Reagent: Dissolve by stirring 1g Dinitosalicylic acid, 200 mg of crystalline Phenol and 50 mg sodium sulphite in 100 ml of 1% NaoH, store at 4 ˚C.

**PROCEDURE*:*** *For Standard*

1. 0.5 ml to 3 ml of 0.3 mg/ml of stranded glucose solution was taken in test tubes are labeled as s1,s2,…….s5 to s6.
2. It was made up to 3 ml with distilled water.
3. 3 ml of distilled water was taken as blank and 3 ml of DNSA solution was added to all the test tubes.
4. Then the test tubes were covered with paraffin film.
5. The tubes were placed in water bath at 90 for 10 to 15 minutes until red brown colour develops.
6. 1 ml of 40% potassium sodium tartarate solution was added to sterilize the colour.
7. After cooling to room temperature in a cold water bath. The absorbance was recorded using spectrophotometer.

**STUDY OF TEMPERATURE EFFECT:**

1. 1% starch solution was prepared using phosphate buffer.
2. 0.5 ml of 1 % substrate solution in test tubes labelled S1-S6 was taken.
3. 1 ml of distilled water was taken in all test tubes.
4. 1.5 ml of distilled water alone serve as blank.
5. 1.5 ml of α-amylase solution was added in all test tubes.
6. The test tubes were incubated at various temperature ranging from 20$^{o}$C- 70$^{o}$C for 10 minutes.
7. 3 ml of DNS was added to all the test tubes and covered with paraffin film.

8. The test tubes were placed in water bath at 90°C for 10 min until red brown colour develops.
9. 1 ml of 40% potassium sodium tartarate solution was added to stabilize the colour.
10. After cooling to room temperature in a cold water bath. Absorbance was recorded at 575 nm.
11. The graph was plotted between temperature and optical density to find out optimum temperature.

**Tabulation-I For standard**

| **S.No.** | **Particulars** | **B** | **S1** | **S2** | **S3** | **S4** | **S5** | **S6** |
|---|---|---|---|---|---|---|---|---|
| 1. | Volume of glucose (ml) | - | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 | 3.0 |
| 2. | Volume of distilled water (ml) | 3.0 | 2.5 | 2.0 | 1.5 | 1.0 | 0.5 | - |
| 3. | Volume of DNS (ml) | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 |
| Test tubes were incubated in water bath at 90°C | | | | | | | | |
| 4. | Volume of sodium potassium tartarate (ml) | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 5. | Optical density at 575 nm. | | | | | | | |

**Tabulation-II For sample**

| **S.No.** | **Particulars** | **B** | **S1** | **S2** | **S3** | **S4** | **S5** | **S6** |
|---|---|---|---|---|---|---|---|---|
| 1. | Volume of starch solution (ml) | - | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 | 3.0 |
| 2. | Volume of distilled water (ml) | 3.0 | 2.5 | 2.0 | 1.5 | 1.0 | 0.5 | - |
| 3. | Volume of α-amylase (ml) | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 |
| Test tubes were incubate at various temperature for 10 min | | | | | | | | |

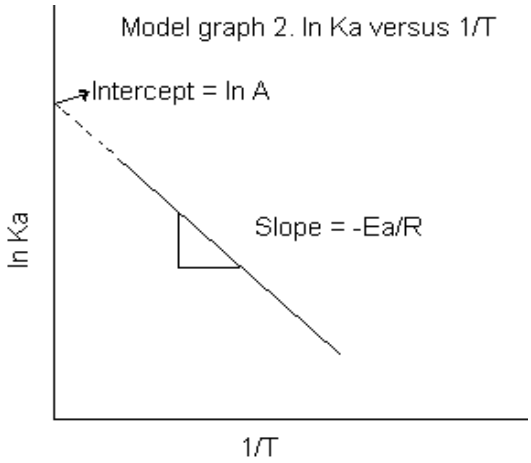
| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 4. | Temperature (°C) | 30 | 20 | 30 | 40 | 50 | 60 | 70 |
| 5. | Volume of DNS (ml) | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 |
| Test tubes were incubated in water bath at 90°C for 15 minutes | | | | | | | | |
| 6. | Volume of sodium potassium tartarate (ml) | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 7. | Optical density at 575 nm. | | | | | | | |

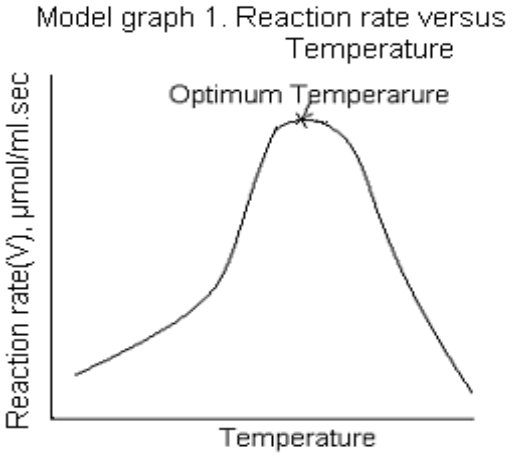
**Tabulation-III**

| Temperature ˚c | Absorbance At 575 nm | Conc. Of Starch (g/l) | Rate of reaction (mol/ml. min) |
|---|---|---|---|
| 30<br>20<br>30<br>40<br>50<br>60<br>70 | | | |

**Tabulation –IV Graph Plot**

| **ln V** | **1/t** |
|---|---|
| | |




**RESULT:**

The effect of temperature on activity of α-amylase enzyme was studied and

Optimum temperature ($T_{opt}$) = ……..……ºC,

Activation energy (Ea) = …….. joule/µmol.

**EXP NO:** **DATE:**

## EFFECT OF pH ON ENZYME ACTIVITY

### AIM

To study the effect of pH on α-amylase activity and to find optimum pH

### THEORY

Enzymes are amphoteric molecules containing a large acid and basic groups, mainly situated on their surface. The charges on this group will vary according to their acid dislocation constants and with the pH of their environment. This will affect the total net charge of the enzyme and their distribution of charge on their surface, in addition to the reactivity of the catalytic active group. These effects are especially important in the neighborhood of the active site. Take together the changes in the charges with pH effect at the activity, structural stability and solubility of the enzyme.

There will be pH characteristics of each enzyme at which the net charge on the molecule is zero. This is called the isoelectric point [PI] at which enzyme generally has minimum (stability) solubility in aqueous solution. In a similar manner to effect on enzyme, the charge and charge distributions on substrates products and coenzymes will also be affected by pH changes. Increasing hydrogen ion concentration will increase the successful competition at hydrogen ion for any metal cationic binding sites on the enzyme reducing the bound metal cation concentration. Decreasing$H_2$ ion concentration, on the other hand, leads to increasing hydroxyl ion concentration, which compete against the enzyme ligands for divalent and invalentcations causing their conversion (to ligands) to hydroxides and at very high hydroxyl ion concentration, their complete removal from free enzyme.

### MATERIALS AND METHODS:

***Equipments***

- Beakers
- Graduated cylinder
- Balance

- Pipe
- Syringe.

***Reagents***

- Enzyme: alpha amylase solution.
- Starch (10g/L) solution.
- Rochelle's salt (40%).
- DNS Reagent: Dissolve by stirring 1g Dinitrosalicylic acid, 200 mg of crystalline Phenol and 50 mg sodium sulphite in 100 ml of 1% NaoH, store at 4 ˚C.

**PROCEDURE: FOR STANDARD**

1. 0.5 ml to 3 ml of 0.3 mg/ml of stranded glucose solution was taken in test tubes are labeled as s1,s2,…….s5 to s6.
2. It was made up to 3 ml with distilled water.
3. 3 ml of distilled water was taken as blank and 3 ml of DNSA solution was added to all the test tubes.
4. Then the test tubes were covered with paraffin film.
5. The tubes were placed in water bath at 90 for 10 to 15 minutes until red brown colour develops.
6. 1 ml of 40% potassium sodium tartarate solution was added to sterilize the colour.
7. After cooling to room temperature in a cold water bath. The absorbance was recorded using spectrophotometer.

**PROCEDURE-SAMPLE**

1. A pH buffer solution ranging from pH 4.9 in increment of pH unit was prepared with starch solution (10 gm/l) was prepared.
2. α-amylase enzyme solution was added in various pH buffers.
3. Reaction of 10 minutes was allowed and then 3 ml of DNSA solution was added to each test tubes.
4. The mixture was kept in a water bath at 90$^{o}$C for 15 min.
5. The reaction mixture was cooled and then colour absorbance was measured at 575 nm after the addition of 1 ml of sodium potassium tartarate.

**Tabulation-I For standard**

| S.No. | Particulars | B | S1 | S2 | S3 | S4 | S5 | S6 |
|---|---|---|---|---|---|---|---|---|
| 6. | Volume of glucose (ml) | - | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 | 3.0 |
| 7. | Volume of distilled water (ml) | 3.0 | 2.5 | 2.0 | 1.5 | 1.0 | 0.5 | - |
| 8. | Volume of DNS (ml) | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 |
| Test tubes were incubated in water bath at 90°C | | | | | | | | |
| 9. | Volume of sodium potassium tartarate (ml) | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 10 | Optical density at 575 nm. | | | | | | | |

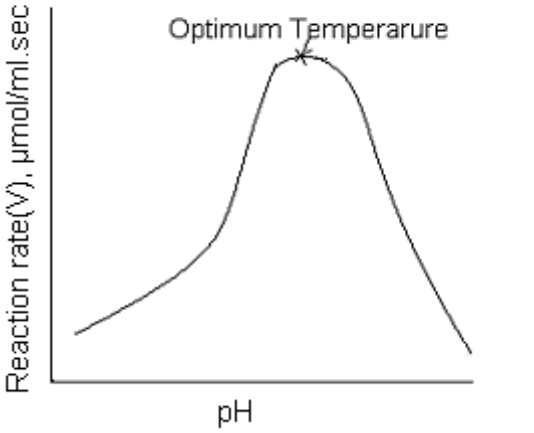
**Tabulation-II For sample**

| S.No. | Particulars | B | S1 | S2 | S3 | S4 | S5 | S6 |
|---|---|---|---|---|---|---|---|---|
| 1. | pH of the solution | 4 | 4 | 5 | 6 | 7 | 8 | 9 |
| 2. | Volume of starch solution 10 gm/l (ml) | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| 3. | Volume of α-amylase 0.5 gm/l (ml) | - | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Test tubes were incubate at room temperature for 10 min | | | | | | | | |
| 4. | Volume of DNS (ml) | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 |
| Test tubes were incubated in water bath at 90°C for 15 minutes | | | | | | | | |
| 5. | Volume of sodium potassium tartarate (ml) | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 6. | Optical density at 575 nm. | | | | | | | |

**Tabulation-III Graph plot**

| pH | V<br>Rate of reaction (mg/ml.min) |
|---|---|
| 4 | |
| 5 | |
| 6 | |
| 7 | |
| 8 | |
| 9 | |

Model graph 1. Reaction rate versus pH



**RESULT:**

The optimum pH that favored α-amylase activity was found to be……

**EXP NO:** **DATE:**

# Enzyme Immobilization Kinetics – Gel Entrapment

## AIM:

To determine the Michael Menton equation Km and Vm from enzyme immobilization technique using entrapment in sodium alginate gel.

## PRINCIPLE

Entrapment is the physical enclosure of enzymes in a small space. Matrix entrapment and membrane entrapment, including micro-encapsulation are the two major methods of entrapment. Matrices used for enzyme entrapment are usually polymeric materials such as calcium alginate, agar, carangeenin, polyacrylamide and collagen. When immobilized in a polymer matrix, enzyme solution is mixed with polymer solution before polymerization takes place. Calcium alginate is just as widely used as polyacrylamide. This is the most commonly used method is entrapment in calcium alginate beads. This method does not alter the chemical nature of enzyme.

Sodium alginate + Enzyme + Calcium chloride

↓

Calcium alginate beads entrapped with enzyme.

Unlike polyacrylamide gels, gelation of calcium alginate does not depend on the formation of more permanent covalent bonds between polymer chains. Rather, polymer molecules are cross-linked by calcium ions. Because of this, calcium alginate beads can be formed in extremely mild conditions, which ensure that enzyme activity yields of over 80% can be routinely achieved. However, just as easily as calcium ions can be exchanged for sodium ions, they can also be displaced by other ions. This property can both be advantageous and disadvantageous. If needed, enzymes or microbial cells can be easily recovered by dissolving the gel in a sodium solution. On the other hand, proper caution must be exercised to ensure that the substrate solution does not contain high concentrations of those ions that can disintegrate the gel.

**MATERIALS REQUIRED:**

Erlenmeyer flasks, Beakers, Pipettes, Test tubes, Temperature bath, Spectrophotometer, alpha-amylase, 0.2 M Calcium chloride, 3 % sodium alginate, DNS and 1% starch solution.

**PROCEDURE: FOR STANDARD**

1. 0.5 ml to 3 ml of 0.3 mg/ml of stranded glucose solution was taken in test tubes are labeled as s1,s2,…….s5 to s6.
2. It was made up to 3 ml with distilled water.
3. 3 ml of distilled water was taken as blank and 3 ml of DNSA solution was added to all the test tubes.
4. Then the test tubes were covered with paraffin film.
5. The tubes were placed in water bath at 90 for 10 to 15 minutes until red brown colour develops.
6. 1 ml of 40% potassium sodium tartarate solution was added to sterilize the colour.
7. After cooling to room temperature in a cold water bath. The absorbance was recorded using spectrophotometer.

**PROCEDURE FOR IMMOBILIZATION KINETICS:**

1. Dissolve 30 gm sodium alginate in 1 l for 3% solution.
2. Mix approximately 0.015 gm of enzyme with 10 ml of 3 % sodium alginate solution.
3. The beads are formed by dipping the polymer solution from s height approximately 20 cm into a 100 ml of 0.2 molarities calcium chloride solution with a syringe and needle at a room temperature.
4. Leave the beads on calcium solution for 0.5 – 3 hrs.
5. 10 test tubes were taken and test tubes were marked 0 to 10.
6. 1 % starch solution was prepared at various concentration of starch solution was taken in all test tubes.
7. The starch solution was not added in the blank.
8. Varying volume of distilled water was added and final volume of reaction mixture was 2 ml.
9. 2 ml of distilled water was added in the blank.
10. 1 gm of immobilizes enzyme was added including blank in all test tubes.
11. The test tubes were incubated at room temperature for 20 mins.
12. 2 ml of DNSA reagent was added to each test tubes and they were kept in boiling water bath for 15 min.
13. 1 ml of Rochelle salt (sodium potassium tartarate) was added.
14. And optical density was recorded at 575 nm.

**Tabulation-I For standard**

| S.No. | Particulars | B | S1 | S2 | S3 | S4 | S5 | S6 |
|---|---|---|---|---|---|---|---|---|
| 1. | Volume of glucose (ml) | - | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 | 3.0 |
| 2. | Volume of distilled water (ml) | 3.0 | 2.5 | 2.0 | 1.5 | 1.0 | 0.5 | - |
| 3. | Volume of DNS (ml) | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 |
| Test tubes were incubated in water bath at 90°C | | | | | | | | |
| 4. | Volume of sodium potassium tartarate (ml) | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 5. | Optical density at 575 nm. | | | | | | | |

**Tabulation-II For sample**

| S.No. | Particulars | B | S1 | S2 | S3 | S4 | S5 | S6 |
|---|---|---|---|---|---|---|---|---|
| 1. | Volume of starch solution 1% (mg/ml) | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| 2. | Volume of α-amylase 0.5 gm/l (ml) | - | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Test tubes were incubate at room temperature for 10 min | | | | | | | | |
| 3. | Volume of DNS (ml) | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 |
| Test tubes were incubated in water bath at 90°C for 15 minutes | | | | | | | | |
| 4. | Volume of sodium potassium tartarate (ml) | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 5. | Optical density at 575 nm. | | | | | | | |

**TABULATION III**

| Substrate [S] mg/ml | Velocity V(mg/ml min) | 1/[S] ml/mg | 1/V ml min/mg. |
|---|---|---|---|
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |

**PLOTS**

1. Standard graph
2. [S] Vs V
3. 1/[S] Vs 1/V.

**RESULT**

Effect of immobilization of α-amylase enzyme using calcium alginate was studied and the

Maximum reaction rate (Vmax) for free enzyme = …… µmol/ml.sec

Maximum reaction rate (Vmax) for Imm'd enzyme = …… µmol/ml.sec

Effectiveness factor (η) = ……..

**EXP NO:** **DATE:**

# ENZYME KINETICS – MICHAELIS-MENTEN PARAMETERS

**AIM:**

To study the Michaelis-Menten kinetics of α-amylase enzyme and hence to determine Vmax and Km using L-B plot, E-H plot, H-W plot.

**THEORY:**

Kinetics of simple enzyme catalyzed reactions is referred to as MichaelisMenten kinetics or saturation kinetics. These models are based on data from batch reaction weight constant volume in which the initial substrate (So) and enzyme (Eo) concentration are known. Saturation kinetics can be obtained from a simple reaction scheme that involves a reversible step for enzyme – substrate complex formation and a dissociation step of the ES complex.

MichaelisMenten equation for steady state kinetics approximation is

$$V = \frac{Vmax\ S}{Km+S} \quad .....(1)$$

Where $V_{max}$ – velocity of enzyme reaction of saturating substrate concentration

S - Substrate concentration

Km -MichelisMenten constant, measure of affinity of enzyme for substrate

Km = [S] at V = $V_{max}$ /2 from the graph V versus [S].

**Lineweaver – Burk plot**:

If is the reciprocal of MichaelisMenten approximation, A plot of 1/V versus 1/[S] gives slope of $Km/V_{max}$; and Y-intercept of $1/V_{max}$ and X intercept of -1/Km.

**Eddie – Hofstee plot**:

A plot of V versus s V/[S] results in a line of slope –Km and y-intercept of $V_{max}$ and X intercept of $V_{max}$ /Km.

**Hanes – Woolf plot**:

A plot of [S]/V versus [S] results in a line of slope 1/ $V_{max}$ ; Y intercept of Km/ $V_{max}$ ; X intercept of – Km.

In this experiment, the glucose formed can be estimated calorimetrically using GOD/POD kit.

## MATERIALS REQUIRED:

Starch Solution (1 %), DNS, Test tubes, pipette, spectrophotometer and α-amylase.

**PROCEDURE-FOR STANDARD**

1. 0.5 ml to 3 ml of 0.3 ml mg/ml of standard glucose solution was taken in test tubes are labeled as s1, s2,……to s6.
2. It was made upto 3 ml with distilled water.
3. 3 ml of distilled water was taken as blank and 3ml of DNSA solution was added to all the test tubes.
4. Then the test tubes were covered with paraffin film.
5. The test tubes were placed in water bath at 90$^{o}$c for 10 to 15 minutes until red brown color develops.
6. 1 ml of 40% potassium sodium tartrate solution was added to sterilize the color.
7. After cooling to room temperature in a cold water bath, the absorbance was recorded at 600 nm using spectrophotometer.

**PROCEDURE-FOR SAMPLE**

- Prepare 10 ml of 1% starch solution (10 mg/ml). Take 7 test tubes and mark them B,S1 to S6. ”B” as blank.
- Add aliquots of 0.5 ml to 3 ml of 1% starch solution to test tubes labeled S1 to S6. Don’t add starch solution to the tube’B’
- Add varying volumes of distilled water and raise the final volume of the reaction mixture to 3 ml and add 3 ml of distilled water to blank (B).
- Add 3 ml of α-amylase enzyme solution (10 mg/ml) in each tube including blank.
- Mix well and keep the test tubes at 35 – 37 ºC for 10 minutes.
- Add 0.5 ml 0.1 N HCl solution to stop the enzymatic digestion process.
- Find the amount of glucose formed by α-amylase using DNS method.
- Find reaction rate in µmol/ml.sec (V) which is equal to d[P]/dt.
- Plot a graph between reaction rate (V) and substrate concentration [S], and (Lineweaver Burk plot) reciprocal of substrate concentration (1/[S]) and reciprocal of reaction rate (1/V).
- Find the values of Vmax and Kmfrom graphs.

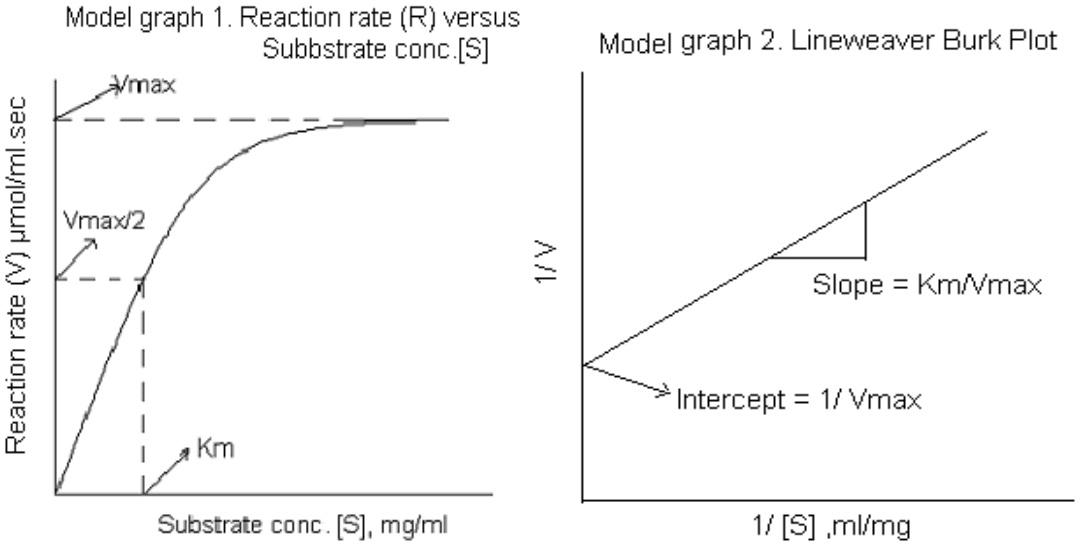
**Tabulation-I (For Standard)**

| S.NO | PARTICULARS | B | S1 | S2 | S3 | S4 | S5 | S6 |
|---|---|---|---|---|---|---|---|---|
| 1 | Volume of glucose (ml) | - | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 | 3.0 |
| 2 | Volume of distilled water(ml) | 3 | 2.5 | 2.0 | 1.5 | 1.0 | 0.5 | - |
| 3 | Volume of DNS | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Incubate in water bath at 90° c for 15 minutes | | | | | | | | |
| 4 | Volume of sodium potassium tartarate(ml) | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 5 | Optical density at 600 nm | | | | | | | |

**Tabulation-I(For Sample)**

| S.NO | PARTICULARS | B | S1 | S2 | S3 | S4 | S5 | S6 |
|---|---|---|---|---|---|---|---|---|
| 1 | Volume of Starch solution (ml) | - | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 | 3.0 |
| 2 | Volume of distilled water(ml) | 3 | 2.5 | 2.0 | 1.5 | 1.0 | 0.5 | - |
| 3 | Volume of α-amylase (ml) | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Incubate the tubes in room temperature for 10 minutes | | | | | | | | |
| 4 | Volume of DNS (ml) | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Test tubes were incubated at 90°C for 15 mins | | | | | | | | |
| 5 | Volumeof sodium potassium tartarate(ml) | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 6 | Optical density at 600 nm | | | | | | | |

## Model Graph




**PLOTS:**

To find Vm and Km,

1. L-B Plot (1/v Vs 1/s)
2. E-H Plot (V Vs V/[s])
3. H-W Plot ([s]/V Vs [s])

**RESULT:**

The Michael's menton parameters Vmax and Km was calculated from the following plots,

1. From L-B Plot

   Km =…… , and Vmax= ……..

2. From E-H Plot,

   Km=……., and Vmax= ……..

3. From H-W Plot

   Km=……., and Vmax= ……..

**EXP NO:** **DATE:**

# ENZYME INHIBITION

## AIM

To study the effects of inhibitor on invertase enzyme and to find the type of inhibition.

## THEORY

### Enzyme Inhibition

Enzyme inhibitors act to decrease the rate of an enzyme reaction. The following are examples of enzyme inhibition:

**Competitive inhibition** occurs when the inhibitor molecule binds to the active site of the enzyme. As a result, the substrate and inhibitor compete for the same spot. Competitive inhibition can be overcome by increasing the substrate concentration so that it is much greater than the inhibitor concentration.

**Uncompetitive inhibition**, however, occurs when the inhibitor molecule binds to a site on the enzyme other than the active site and results in a conformational change of the enzyme resulting in an inability to bind the substrate. Thus, inhibition is not removed by increasing substrate concentration because they are not competing for the same spot.

**Noncompetitive inhibition**occurs when the inhibitor binds both the active site of the enzyme and the enzyme-substrate complex. One way to measure the degree of inhibition is by calculating the inhibitor constant Ki, which is the concentration of inhibitor that causes a 2 fold increase in the slope of the 1/(substrate concentration) versus 1/(velocity) plot, also known as the Lineweaver-Burk plot.

## MATERIALS REQUIRED

1. Yeast solution 1 gm in 100 ml of buffer
2. Copper sulphate solution 0.1 M (stock solution)
3. Sucrose solution 5%
4. Glucose solution 1 mg/ml (for standard).
5. Dinitrosalicylic acid
6. Sodium potassium tartarate

## PROCEDURE

1. Prepare 0.1 M copper sulphate solution and measure the ranges of various concentrations by diluting it with water (ranging from 0.2 mM to 10 mM)
2. The total amount should be 2 ml.th at 90
3. Take two sets of five test tubes.
4. To each test tube of set one add 0.5, 1.0, 1.5, 2.0, 2.5 ml of 5% sucrose solution.
5. Make up the volume to 3 ml using buffer.
6. To each test tube of set two add 0.5, 1.0, 1.5, 2.0, 2.5 ml of 5% sucrose solution followed by the given volume of the inhibitor copper sulphate solution.
7. Make up the volume to 3 ml using buffer
8. To each test tube of both the sets add 3 ml of invertasse.
9. Incubate for 10 min at 37˚ C
10. Then add 3 ml of dinitrosalicylic acid.
11. Place the test tubes in water bath at 90 ˚ C for 10-15 min.
12. Add 1 ml of sodium potassium tartarate to all the test tubes.
13. After cooling to room temperature in a cold water bath read the absorbance in a spectrophotometer at 575 nm.

### FOR STANDARD PLOT

1. 0.5 ml to 3 ml of 0.3 mg/ml of stranded glucose solution was taken in test tubes are labeled as s1,s2,…….s5 to s6.
2. It was made up to 3 ml with distilled water.
3. 3 ml of distilled water was taken as blank and 3 ml of DNSA solution was added to all the test tubes.
4. Then the test tubes were covered with paraffin film.

5. The tubes were placed in water bath at 90 for 10 to 15 minutes until red brown colour develops.
6. 1 ml of 40% potassium sodium tartarate solution was added to sterilize the colour.
7. After cooling to room temperature in a cold water bath. The absorbance was recorded using spectrophotometer.

**TABULATION-I FOR STANDARD**

| S.No. | Particulars | B | S1 | S2 | S3 | S4 | S5 | S6 |
|---|---|---|---|---|---|---|---|---|
| 1. | Volume of glucose (ml) | - | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 | 3.0 |
| 2. | Volume of distilled water (ml) | 3.0 | 2.5 | 2.0 | 1.5 | 1.0 | 0.5 | - |
| 3. | Volume of DNS (ml) | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 |
| Test tubes were incubated in water bath at 90°C | | | | | | | | |
| 4. | Volume of sodium potassium tartarate (ml) | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 5. | Optical density at 575 nm. | | | | | | | |

**TABULATION-II WITHOUT INHIBITOR**

| S.No. | Particulars | B | S1 | S2 | S3 | S4 | S5 | S6 |
|---|---|---|---|---|---|---|---|---|
| 1. | Volume of 5 % sucrose solution (ml) | - | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 | 3.0 |
| 2. | Volume Buffer (ml) | 3.0 | 2.5 | 2.0 | 1.5 | 1.0 | 0.5 | 0.25 |
| 3. | Volume of Invertase (ml) | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 |
| Incubate at room temperature for 10 mins | | | | | | | | |
| 4. | Volume of DNS (ml) | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 |
| Test tubes were incubated in water bath at 90°C for 15 minutes | | | | | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 5. | Volume of sodium potassium tartarate (ml) | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 6. | Optical density at 575 nm. | | | | | | | |

**TABULATION-II WITH INHIBITOR**

| **S.No.** | **Particulars** | **B** | **S1** | **S2** | **S3** | **S4** | **S5** | **S6** |
|---|---|---|---|---|---|---|---|---|
| 1. | Volume of 5 % sucrose solution (ml) | - | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 | 3.0 |
| 2. | Volume of $CuSO_4$ (ml) | 3.0 | 2.5 | 2.0 | 1.5 | 1.0 | 0.5 | 0.25 |
| 3. | Volume of buffer (ml) | 3.0 | 2.5 | 2.0 | 1.5 | 1.0 | 0.5 | 0.25 |
| 4. | Volume of Invertase (ml) | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 |
| Incubate at room temperature for 10 mins | | | | | | | | |
| 5. | Volume of DNS (ml) | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 |
| Test tubes were incubated in water bath at 90°C for 15 minutes | | | | | | | | |
| 6. | Volume of sodium potassium tartarate (ml) | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 7. | Optical density at 575 nm. | | | | | | | |

**CALCULATION TABLE**

| S.No | Sample | [S] mg/ml | 1/[S] ml/mg | Concentration from standard curve mg/ml | V $mg.ml^{-1}.min^{-1}$ | 1/V $ml.min.mg^{-1}$ |
|---|---|---|---|---|---|---|
| **Without inhibitor** | | | | | | |
| **With inhibitor** | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | |

**RESULT**

The effects of enzyme inhibition was studied and the results are

1. The Km value of the enzyme ……...
    I. With inhibitor
    II. Without inhibitor
2. The Vm value of the enzyme
    I. With inhibitor
    II. Without inhibitor
3. Type of inhibition

**EXP NO:** **DATE:**

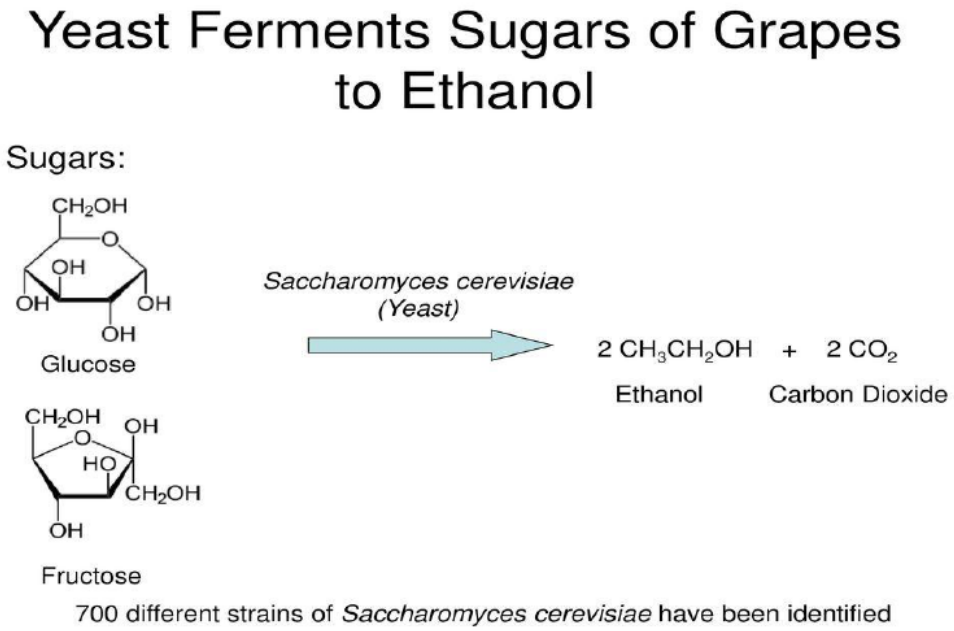
## PRODUCTION OF ETHANOL BY YEAST

### Aim

To produce ethanol from grape fruit juice by the yeast fermentation process

### Principle

The most popular and best-known baker's yeast – *Saccharomyces cerevisiae* is used for alcohol production through anaerobic fermentation. The yeast is used for brewing beer, making bread, making wine, ethanol and distilled beverages. The yeasts appear to be more tolerant of ethanol than other strains of yeasts so that they can produce the wine that contains 20% v/v of alcohol whereas brewer's yeasts yield only 9% v/v of ethanol. Yeasts are grown on grapes for making wine anaerobically and the yield of alcohol from the fermentation depends on the amount of substrate (sugars) that is being utilized during the fermentation process.



**PROCEDURE:**

Add fresh and healthy black grapes in a glass beaker and squeeze them to collect the juice

Filter the collected juice

Transfer the filtered juice to a sterile Erlenmeyer flask and close tightly

Add 1.5 g of brewer's yeast and 200 g of sugar per kg of grapes

Incubate the flask at 15 – 20°C for 8-15 days

## REAGENT PREPARATION:

**Potassium dichromate solution ($K_2 Cr_2O_7$):**

Weigh 34 g of Potassium dichromate and dissolve in 500 mL of distilled water in a one-liter standard measuring flask.

**Concentric sulphuric acid (Conc. $H_2SO_4$ – 5 M):**

Measure 325 mL of concentric sulphuric acid and slowly add to the potassium dichromate solution by keeping it in an ice bucket.

**DNS Solution**

Dinitrosalicylic Acid Reagent Solution

Dinitrosalicylic acid: 10 g

Sodium sulfite: 0.5 g

Sodium hydroxide: 10 g

Deionized water : 1 liter

Potassium sodium tartrate solution, 40%

**QUANTIFICATION OF BIOMASS:**

1 mL of the fermented culture was collected at regular time intervals and centrifuged at 8000 rpm for 10 minutes

Supernatant was transferred to another eppendrof tube and air dried cell pellet weight was noted down

$$Weight\ of\ the\ biomass\ (cell\ pellet) = ((Weight\ of\ the\ eppendrof\ with\ pellet) - (Weight\ of\ the\ empty\ eppendrof))$$

**PREPARATION OF STANDARD CURVE FOR ETHANOL ESTIMATION:**

Prepare 2 % (v/v) Ethyl alcohol as a stock solution

Take 0.1, 0.2, 0.3, 0.4 and 0.5 ml of this 2 % alcohol and add it to the test tubes and makeup to 10.0 ml using distilled water

Take 0.5 mL of wine sample collected at regular time intervals and make it up to 10.0 mL using distilled water

Add 1.0 mL of potassium dichromate solution to all the test tubes

Incubate the test tubes at 90 ˚C for 15 minutes

Measure optical density at 590 nm

Plot the standard graph between the concentration of ethanol and optical density

Calculate the concentration of ethanol in the wine sample at different time intervals from the standard graph (mg/mL)

**PREPARATION OF STANDARD CURVE FOR GLUOSE ESTIMATION:**

Prepare glucose standards ranging from 0.1 to 1 mL by using dry clean and labelled test tubes (Refer table)

Take 0.5 mL of wine sample collected at regular time intervals

Make all the samples to 3 mL with deionized water

3 mL of deionized water alone serves as a blank

Add 3 mL of Dinitro Salicylic Acid reagent to all the test tubes and vortex them for few seconds

Place all the test tubes in water bath at 90º C for 10-15 minutes to develop red-brown color

Cool the test tube in the running tap water and add 1 mL of 40% potassium sodium tartrate (Rochelle salt) solution to all the test tubes and vortex them for few seconds (Note: All the tubes must be cooled to room temperature before reading since the extinction is sensitive to temperature change)

Read the Optical density of the colored solutions at 540 nm using the solution in tube 1 as a blank

Plot the standard curve of the absorbance (Y- axis) against the glucose concentration (mg/mL) ( X-axis )

Calculate the concentration of glucose in the wine sample at different time intervals from the standard graph (mg/mL)

**Evaluation of ethanol yield and productivity**

The Stoichiometric equation from reducing sugars to ethanol can be written as

$$C_6H_{12}O_6 \longrightarrow 2\,C_2H_5OH + 2\,CO_2$$

The yield of ethanol can be calculated from the below equation

**Fermentation efficiency**: Ratio between the theoretical and observed yield of ethanol

$$Fermentation\ efficiency = \left(\frac{Observed\ ethanol\ yield}{Theoretical\ ethanol\ yield}\right) \times 100$$

**TABULATION**

**Table: 1 Biomass estimation**

| S. No | Hours | Weight of the cell biomass (g) |
|---|---|---|
| 1 | 0 | |
| 2 | 12 | |
| 3 | 24 | |
| 4 | 36 | |
| 5 | 48 | |
| 6 | 60 | |
| 7 | 72 | |
| 8 | 84 | |

| | | |
|---|---|---|
| 9 | 96 | |
| 10 | 108 | |

**Estimation of Ethanol**

**Table: 2 Standard table**

| S. No | Conc. of ethanol (mg/mL) | Volume of sample (mL) | Volume of water (mL) | Volume of chromic acid (mL) | | O.D at 590 nm |
|---|---|---|---|---|---|---|
| | | | | | | |
| | | | | | Incubate at 90°C for 15 mins | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |

**Table:3**

| S. No | Hours | Vol. of sample (mL) | Vol. of water (mL) | Vol. of chromic acid (mL) | | O.D at 590 nm | Conc. of ethanol (mg/mL) |
|---|---|---|---|---|---|---|---|
| | 0 | | | | | | |
| | 12 | | | | Incubate at 90°C | | |
| | 24 | | | | | | |
| | 48 | | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | 60 | | | | for 15 mins | | |
| | 72 | | | | | | |
| | 84 | | | | | | |
| | 96 | | | | | | |
| | 108 | | | | | | |

**Estimation of Glucose**

**Table: 4 Standard table**

| S. No | Vol. of standard solution (mL) | Conc. Of standard solution (mg/mL) | Vol. of Distilled water (mL) | Vol. of DNS reagent (mL) | | Vol. of 1%Na-K Tartrate (mL) | O.D at 540 nm |
|---|---|---|---|---|---|---|---|
| 1 | Blank | - | 3 | 3 | Incubate the tubes at 90°C for 10 - 15 minutes. | 1 | |
| 2 | 0.1 | | 2.9 | 3 | | 1 | |
| 3 | 0.2 | | 2.8 | 3 | | 1 | |
| 4 | 0.3 | | 2.7 | 3 | | 1 | |
| 5 | 0.4 | | 2.6 | 3 | | 1 | |
| 6 | 0.5 | | 2.5 | 3 | | 1 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | | | | |
| 7 | 0.6 | | 2.4 | 3 | | 1 | |
| 8 | 0.7 | | 2.3 | 3 | | 1 | |
| 9 | 0.8 | | 2.2 | 3 | | 1 | |
| 10 | 0.9 | | 2.1 | 3 | | 1 | |
| 11 | 1.0 | | 2.0 | 3 | | 1 | |

**Table: 5**

| S. No | Hours | Vol. of standard solution (mL) | Vol. of Distilled water (mL) | Vol. of DNS reagent (mL) | | Vol. of 1% Na-K Tartrate (mL) | O.D at 540 nm | Conc. of glucose (mg/mL) |
|---|---|---|---|---|---|---|---|---|
| 1 | 0 | | | 3 | | 1 | | |
| 2 | 12 | | | 3 | | 1 | | |
| 3 | 24 | | | 3 | | 1 | | |
| 4 | 36 | | | 3 | | 1 | | |
| 5 | 48 | | | 3 | | 1 | | |
| 6 | 60 | | | 3 | | 1 | | |
| 7 | 72 | | | 3 | | 1 | | |
| 8 | 84 | | | 3 | | 1 | | |
| 9 | 96 | | | 3 | | 1 | | |
| 10 | 108 | | | 3 | | 1 | | |

**Incubate the tubes at 90°C for 10 - 15 minutes.**

**Graph:** Concentration of standard ethanol (mg/mL) vs Optical Density (@590 nm)

Concentration of ethanol (wine sample) (mg/mL) vs Optical Density (@590 nm)

Concentration of Standard Glucose (mg/mL) vs Optical Density (@540 nm)

Concentration of Glucose (wine sample) (mg/mL) vs Optical Density (@540 nm)

**EXP NO.** **DATE:**

# YOGURT FERMENTAION WITH *LACTOBACILLUS* CULTURES

## AIM

To demonstrate the use of microorganism in food processing by using yogurt as an example

## THEORY

The mushy substance formed during the prolonged precuring process in cheese manufacturing in which the natural action of lactose fermenting culture originally resident in butter milk was utilized to acidify milk. Of course, this custard - textured substance was none. Actually this experiment has already been performed. One may have noticed in Experiment other than yogurt, sometimes spelled yoghurt or yoghourt.

Other than cheese, buttermilk, and yogurt, lactic starter cultures are also used to help prepare or manufacture a wide variety of food products such as sour dough bread, pickles, and sausages. As implied by the name "lactic cultures," they belong to a category of microorganisms that can digest the milk sugar lactose and convert it into lactic acid. For the cells to utilize lactose, deriving carbon and energy from it, they must also possess the enzymes needed to break lactose into two components sugars: glucose and galactose. Some representative strains are *Streptococcus lactis, S. cremoris, thermophilus, Lactobacillus bulgaricus, L. acidophilus,* and *L. plantarum.* These cultures can be purchased directly from local health food and drug stores in tablet form. These tablets, taken orally during the intake of dairy products, help those people who have digestive tract disorder and cannot tolerate lactose. The major steps involved in a large scale production of lactic starter cultures are the following: media preparation (constitution, mixing, straining, and sterilization), inoculum preparation, fermentation, cell concentration by centrifugation, liquid nitrogen freezing, and packaging.

In summary, commercial yogurt production is composed of the following steps: pretreatment of milk (standardization, fortification, and lactose hydrolysis),

homogenization, heat treatment, cooling to incubation temperature, and inoculation with starter, fermentation, cooling, post-fermentation treatment (flavoring, fruit addition, and pasteurization), refrigeration/freezing, and packaging. For set yogurt, the packaging into individual containers is carried out before fermentation. In addition to the above steps, the starter culture is propagated in parallel. Although a batch process is followed in this illustrative experiment, the commercial production of yogurt is carried out in an automated continuous fermentation process. A good strain of starter culture not only affects the flavor and aroma, it can also speed up the process and thus reduces the effective equipment cost.

**MATERIALS REQUIRED**

(i) Beakers

(ii) Heat source

(iii) Incubator, 43°C

(iv) Thermometer

(v) Milk

(vi) Starter culture or plain yogurt from local stores

**PROCEDURES**

(i) 1L (approximately 1 quart) of milk in a beaker was heated slowly to 85°C and maintained at that temperature for 2min. This step kills undesirable contaminant microorganisms. It also denaturizes inhibitory enzymes that retard the subsequent yogurt fermentation. If you are attempting this procedure at home with a saucepan, use caution so as not to allow the milk to boil over and make a mess on your kitchen stove.

(ii) The milk was cooled in a cold-water bath to 42 – 44 °C. The cooling process should take about 15min.

(iii) 5g of starter culture was added to the cooled milk and mix with a glass rod.

(iv) The container was covered to minimize the possibility of contamination. It was incubated at 42°C for 3 to 6h undisturbed until the desired custard consistency is reached. Yogurt is set when the mixture stops flowing as the container is tipped slowly. Fluid yogurt results if the mixture is stirred as the coagulum is being formed.

(v) The fresh made yogurt is ready for consumption when it is set. However, it may be required to refrigerate it first. Refrigeration also stops the growth of the lactic acid culture, which is thermophilic. (Thermophilic cultures grow best at high temperatures.)

vi) Use of *Lactobacillus acidophilus* : Grind 4 yogurt tablets (about 1g) into fine powder. Repeat Steps 3 –5.

vii) For entrepreneurs or simply hungry / thrifty students : You can recycle a small part of the finished product as the starter culture for the next batch. Theoretically, you can multiply or maintain your supply of yogurt indefinitely. However, in actuality, extended recycling is not recommended because the composition of the mixed culture will gradually deviate from the ideal one, and hence the flavor.

## RESULT

Thus the use of microorganisms in food processing is demonstrated by using yogurt as an example.

EXP NO. DATE:

# WINE PRODUCTION

## AIM

To become acquainted with wine production by fermentative activites of yeast cells

## THEORY

Wine is a product of the natural fermentation of juices of grapes and other fruits such as peaches, plums, and apples by the action of yeast cells. This biochemical conversion of juice to wine occurs when the yeast cells enzymatically degrades the fruit sugars fructose and glucose, first to acetaldehyde and then to alcohol as indicated in the equation.

Grapes containing 20 – 30% sugar concentration will yield wine with an alcoholic content of approximately 10 – 15%. Also present in grapes are acids and minerals whose concentration are increased in the finished product and that are responsible for the characteristics taste and bouquets of different wines. For real wine the crushed grapes must be fermented with their skins to allow extraction of their colour into the juice.

Commercial production of wine is long extracting process. First, the grapes are crushed or pressed to express the juice which is called must. Potassium metabisulphite is added to must to retard growth of acetic acid bacteria, moulds and wild yeast that are endogenous to grapes in the wine yard. A wine producing strain of yeast, *Saccharomyces cervisiae varellipsoides* is inoculated under aerobic conditions at 21– 32°C for a period of 2 weeks. Then wine is aged for a period of 1 – 5 years in aging tanks or wooden barrels. During this time, the wine is clarified of any turbidity, thereby producing volatile esters, which are responsible for characteristics flavors. The clarified products are then filtered, pasteurized at 60°C for half an hour and bottled.

This experience is a modified method by which red wine is produced from red grape juice. The fermenting wine was examined at one week interval during the incubation period.

(i) Total Acidity (Expressed as % Tartaric acid)

To a 10mL aliquot of fermenting wine add 10mL of distilled water and 5 drops of 1% Phenolphthalein solution. Mix and titrate the first persistent pink colour with 0.1N NaOH. The total acidity was calculated using the formula

$$\% \text{ Tartaric Acid} = \frac{\text{mL Alkali x Normality of Alkali x 7.5 x 100}}{\text{Weight of Sample in g}}$$

(ii) Volatile Acidity (Expressed as % Tartaric acid)

Following titration, calculate volatile acidity using the formulae

$$\%\text{Acetic acid} = \frac{\text{mL Alkali x Normality of Alkali x 6 x 100}}{\text{Weight of Sample in g}}$$

(iii) Aroma

Fruity, Yeast like, Sweet, none

(iv) Clarity

Clear, Turbid

**MATERIALS REQURIED**

i) **CULTURE**

50mL of red grape juice broth culture of *Saccharomyces cervisiae varellipsoides* is incubated for 48hrs at 25°C.

ii) **MEDIA**

500mL of pasteurized red grape juice.

iii) **REAGENTS**

1% Phenolphthalein solution, 1N NaOH, Potassium metabisulphite and sucrose.

(i) 1L Erlen – Meyer flask
(ii) One holed rubber stopper containing a two inch glass tube plugged with cotton
(iii) Balance
(iv) 10mL graduated cylinder
(v) Burette or pipette for titration.

## PROCEDURE

(i) Pour 500mL of white grape juice into the 1Liter Erlen – Meyer flask.
(ii) 20g of sugar and 50mL of *Saccharomyces cervisiae* grape juice broth culture was added to the fresh grape juice.
(iii) Close the flask with stopper containing cotton plug.
(iv) After 2days and 4days of incubation, add 20g of sucrose to the fermenting wine.
(v) Incubate the fermenting wine for 25 days at 20°C.
(vi) Using a uninoculated red grape juice, titration is done to determine the total acidity and volatile acidity.
(vii) The aroma and clarity were also observed.
(viii) At 7days interval, using sample of fermenting wine. The above two steps were repeated.

## RESULTS

Wine production by the fermentation activities of yeast cells was acquainted

**EXP NO.** **DATE:**

# PRODUCTION OF AMINO ACID

## AIM

To produce and estimate the Glutamic acid using Bacillus subtilis

## THEORY

Amino acids react with ninhydrin to give a colored complex called hydrindantin ammonia and carbondioxide. The amount of hydrindantin produced is equal to the amount of amino acid present In the solution

## MATERIALS REQUIRED

(i) Conical flask

(ii) Test tube

(iii) Pipette

(iv) Ninhydrin

(v) Phenol

(vi) Ethanol

## MEDIA COMPOSITON

- Glucose – 20 g
- Ammonium chloride – 20 g
- $KH_2PO_4$ – 1 gm
- $MgSO_4$- 0.5 gm
- Distillated water – 1 litre
- pH, 0.5% $CaCO_3$ was sterilized separately

(i) The chemical is added orderly in known volume of distilled water, pH was adjusted using 1N NaOH or 1N HCl to pH 7.

(ii) The medium was dispersed in two 250mL conical flask containing 100mL each. It is autoclaved at 15 pounds for 15 – 20min.

(iii) The sterilized media was cooled, to that 0.1mL *Bacillus* culture medium is added and incubated overnight.

(iv) Incubate the culture at 37 °C in rotary shaker for 24 – 48h.

(v) Culture was centrifuged at 5000 rpm for 20min.

vi) The supernatant was collected and used as aminoacid source. The pellet was collected and fresh weight of *Bacillus* cell was determined

**ESTIMATION OF AMINO ACID FROM THE CULTURE FILTRATE**

To 0.5mL culture filtrate containing aminoacid, add 0.2mL of 80% phenol and heat the contents for 10min at 80°C in a water bath. Cool the contents to room temperature and add 0.5mL of ninhydrin. Again, heat the contents for colour development for 10min. The blank solution is prepared as above with the help of distilled water instead of culture filtrate. Using the blank, measure the optical density of the solution at 575nm in Spectrophotometer. The optical density of the culture filtrate was measured using the standard curve, using aminoacid estimation. Thus, the concentration of amino acid can be measured.

**RESULT**: Thus amino acid is produced by the above method using Bacillus subtilis.

# FERMENTOR

## OBJECTIVES OF FERMENTOR

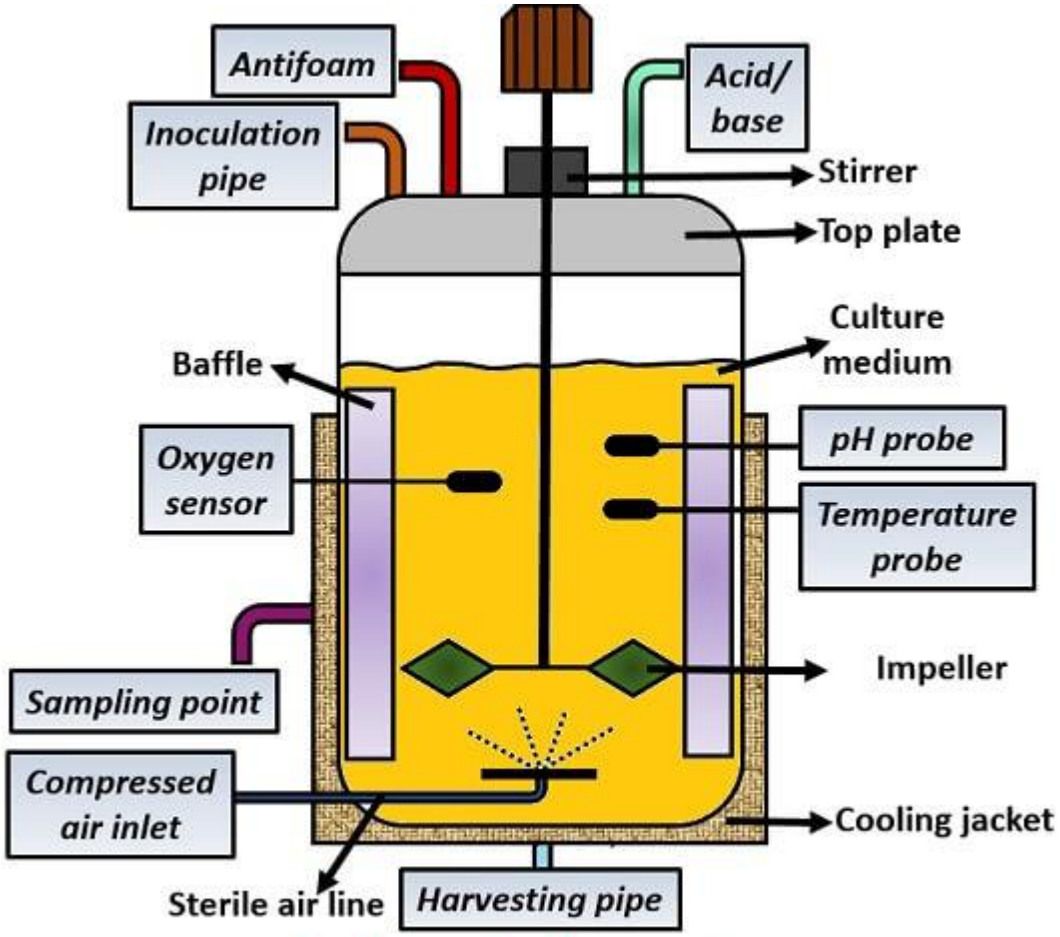
The objectives include the production of metabolites, enzymes, microbial biomass and recombinant product.

## IDEAL PROPERTIES OF A FERMENTOR

- A fermentor should be made of a good quality material that can withstand all the conditions inside the vessel.
- It should give high productivity.
- It should be able to handle the stream sterilization pressure.
- There should be all the control parameters to monitor the fermentation process like pH electrode, temperature probe etc.
- A material used in the fermentor should be cheap that could give satisfactory results.

*Basic Elements*

Basic components are necessary for the construction of fermentor, which involves:




Design of a fermentor

**Top-plate**: It is the cover that is generally made of stainless steel.
**Inoculation pipe**: It helps to port the inoculum inside the fermentor.
**Drive motor**: It drives the impeller shaft.
**Impeller shaft**: Holds the agitator centrally.
**Impeller**: Acts as an agitating device for mixing up the nutrients and microorganisms uniformly.
**Stirrer**: Mixes the gas bubbles throughout the liquid culture medium.
**Baffle**: Prevents the counterflow or vortex formation by breaking down the gas bubbles to improve aeration efficiency.
**Sparger**: It supplies oxygen into the culture medium through the perforated tubes.
**Drain point**: Withdraws cells or medium for the continuous fermentation.
**Cooling jacket**: It is fitted externally to the fermentation vessel which allows the passage of steam or cold water to balance the heat generated during the process.

*Controlling Elements*

Controlling elements monitor the parameters like (temperature, pH, acid, bases, oxygen supply, pressure etc.) that are necessary for the product formation and it includes:

**Pt-100**: Monitors the temperature in the culture vessel.
**Foam probe**: It senses foam formation.
**pH electrode**: Monitors the pH in the culture vessel.
**Oxygen sensor**: Maintains the dissolved oxygen content level.
**Heating pad**: Provides heat to the medium.
**Cold finger**: It is a pipe that passes cold water inside a vessel to cool the contents.
**Rotameter**: Provides variable airflow into the culture vessel.
**Pressure valve**: Maintains the pressure.
**Air pump**: Supplies air throughout the medium.
**Peristaltic pump**: It pumps acid, base and antifoam into the medium.

**PROPERTIES OF A FERMENTOR**

- It should be reliable for long-term operation.
- A fermentor should be capable of being operated aseptically or should provide sterile conditions.
- The bioreactor provides adequate aeration and agitation for uniform mixing of the contents in the vessel.
- It should consume less power.
- A fermentor must be equipped with controlling probes that can maintain the temperature, pH, oxygen level etc.
- It facilitates the passage of inoculum and media into the vessel.
- A bioreactor does not allow excessive evaporation loss.
- It minimizes the labour input for the operation, harvesting, cleaning and maintenance.

**Types of Fermentor**

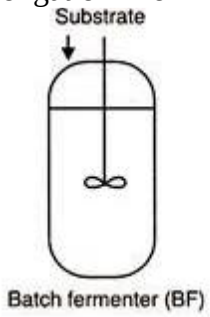
A fermentor is mainly of five types:

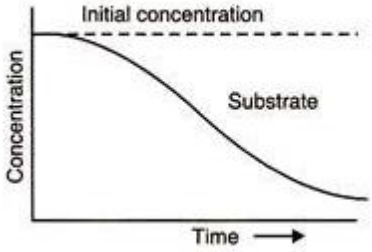
1. Stirred tank fermentor
2. Airlift fermentor
3. Fluidised bed fermentor
4. Packed bed fermentor
5. Photo fermentor

**Batch fermentation**

In batch fermentation, a closed system where an initial and limited amount of sterilized mediuis introduced into a fermenter. The nutrient medium is inoculated with the microorganism and kept for incubation for a definite period under optimum conditions. Oxygen is provided in the form of air and pH is controlled by acid, base or any antifoaming agent, are being added during fermentation process. During fermentation a change in the composition of the culture medium

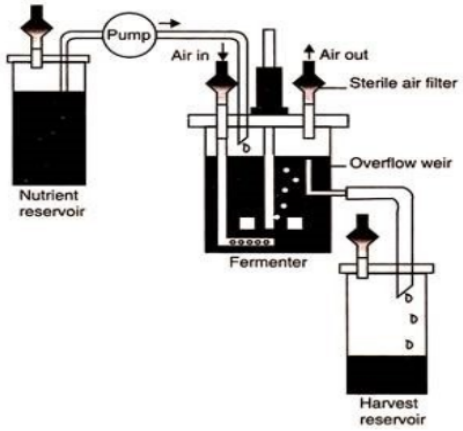
(biomass and metabolites) as the microbes undergo different phases of growth and metabolism. The culture broth is harvested, separated and purified when the desired product is formed. Batch fermentation are widely used in primary and secondary metabolites production under definite culture conditions that supports the fastest growth rate and the maximum growth could be utilized for the biomass production. In order to obtain optimum yield of primary metabolites, prolongation of exponential growth phase is required whereas it should be




**Continuous fermentation**

A closed system of fermentation that operated for an indefinite period of time. Here fresh sterilized nutrient medium is added intermittently or continuously and at the same time in order to recover cells and fermented products, an equivalent amount of spend medium along with the microbes are withdrawn either intermittently or continuously. As a result, concentration and volume of the nutrient medium is optimally maintained in an automatic manner. However, continuous fermentation process lowers the operating cost as it has high productivity with less down time. In this mode of operation, medium and inoculum are initially added to the reactor and constant volume of the broth is maintained. This process has been generally used for organic solvents, starter culture, antibiotics and single cell protein production.




Continuous fermentation can be carried out in three ways as follows,

**Single stage fermentation**

In this process, the nutrient medium and culture are kept in continuous mode of operation by balancing the input and the output of the harvested culture and the nutrient medium in a single fermenter.

**Multiple stage fermentation**

Two or more fermenters are operated simultaneously where growth phase and synthetic phase of the fermentation process are being carried out in different fermenters. This process is more suitable for those fermentation in which the different phases (growth and synthetic) of the microbes are not simultaneous.

**Fed batch fermentation**

A modified form of batch fermentation process where substrate is periodically added in regular intervals as the fermentation process progress. Hence, an optimal concentration of the substrate is maintained. This process is essential for those fermentation processes where the secondary metabolites are being subjected to catabolic repression by the presence of high nitrogen, carbohydrates or glucose concentration in the nutrient medium. The critical elements of the medium are provided in lesser amount during the start of the fermentation process while during the production phase the substrate are being added in small amount. This process is mostly widely employed for the penicillin production. Fixed, variable and cyclic were the three types of fed batch fermentation process.