DEPARTMENT OF BIOTECHNOLOGY

M.TECH

ADVANCED BIOPROCESS LABORATORY MANUAL

(481173L1)

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.

μ = 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

$$\ln X_t = \ln X_o + \mu t$$

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

EXP NO: **DATE:**

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

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

μ - Specific growth rate, hr^{-1}

μ_{max} - Maximum growth rate, h^{-1}

S - Limiting substrate concentration (g/L)

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

As S increases, μ also increases until it attains μ_{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 μ against S_o in the linear exponential growth region which is equal to specific growth μ (h) for that particular growth rate.

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

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

RESULT

Maximum specific growth rate, μ_{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

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

This value should be near zero for the dummy variables.

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

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

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

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

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								

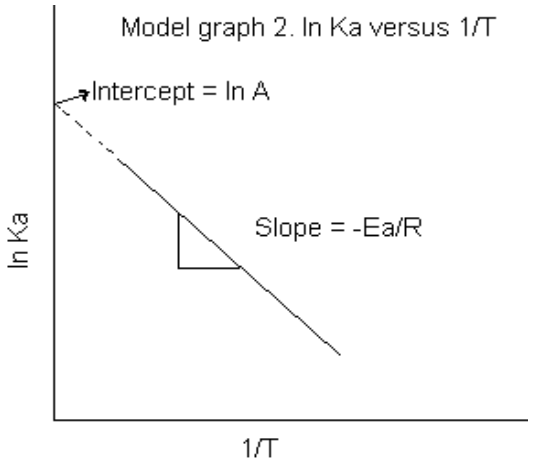
4.	Temperature (°C)	30	20	30	40	50	60	70
5.	Volume of DNS (ml)	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Test tubes were incubated in water bath at 90°C for 15 minutes								
6.	Volume of sodium potassium tartarate (ml)	1.0	1.0	1.0	1.0	1.0	1.0	1.0
7.	Optical density at 575 nm.							

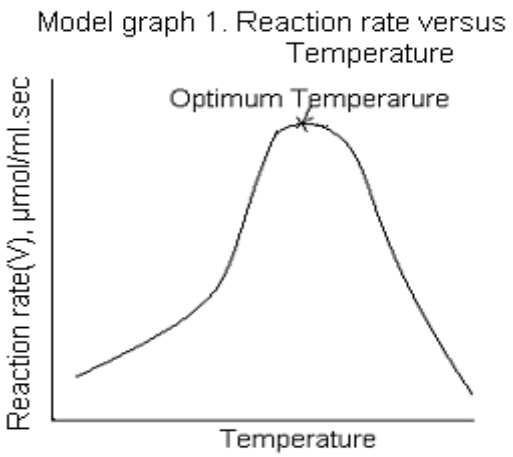
Tabulation-III

Temperature ˚c	Absorbance At 575 nm	Conc. Of Starch (g/l)	Rate of reaction (mol/ml. min)
30 20 30 40 50 60 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. DecreasingH_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.							

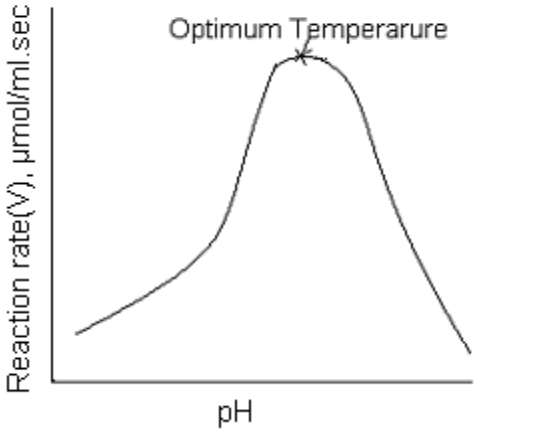
Tabulation-II For sample

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 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.							

Table 3

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:**

EXP NO: **DATE:**

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)

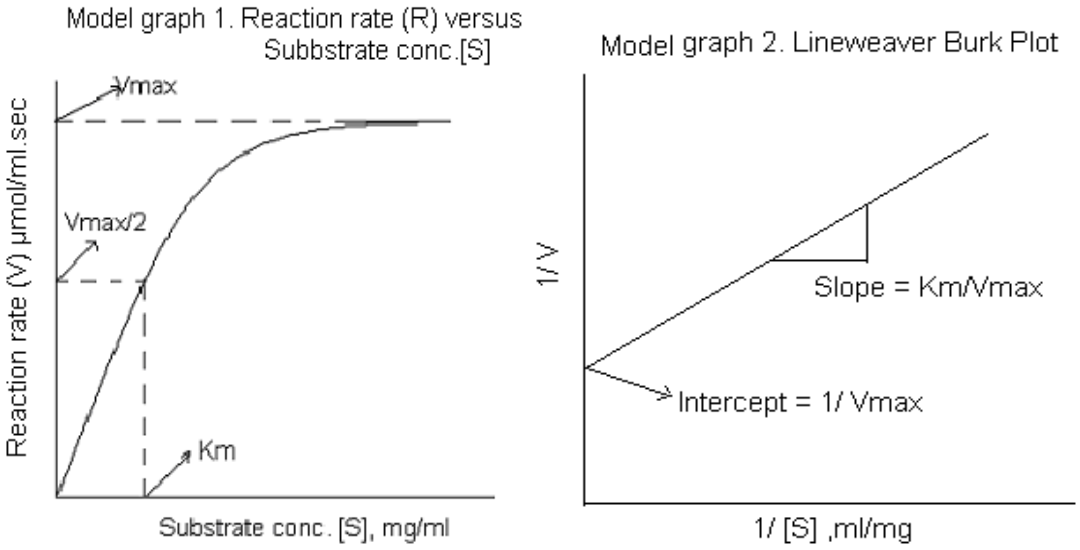
S.NO	PARTICULARS	B	S1	S2	S3	S4	S5	S6
1	Volume of glucose (ml)	-	0.5	1.0	1.5	2.0	2.5	3.0
2	Volume of distilled water(ml)	3	2.5	2.0	1.5	1.0	0.5	-
3	Volume of DNS	3	3	3	3	3	3	3
Incubate in water bath at 90º c for 15 minutes								
4	Volume of sodium potassium tartarate(ml)	1	1	1	1	1	1	1
5	Optical density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

6	tartarate(ml) 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:**

DETERMINATION OF K_La BY SULPHITE OXIDATION METHOD

AIM

To determine the oxygen mass transfer coefficient, K_La using sodium sulfite oxidation method.

THEORY

The volumetric liquid mass transfer coefficient (K_La) is a useful parameter to characterize the bioreactor's capacity for aeration. This helps the reactor design, optimization of technologies and scaling up or scaling down processes. The sodium sulfite combines with oxygen to give sodium sulfate, with CuSO4 as a catalyst in the reactor. The concentration of sodium sulfite at various time points is inversely proportional to the oxygen transfer rate.

$$Na_2SO_3 + \tfrac{1}{2}\,O_2 \xrightarrow{Cu^{2+}} Na_2SO_4$$

The above reaction is

A. Independent of sodium sulfite concentration within the range of 0.04N to 1.0N

B. The rate of the reaction is much faster than the O_2 transfer rate.

Thus, the rate of oxidation is controlled by the rate of mass transfer alone.

The reaction consumes oxygen at a rate that is sufficiently fast so that transport of O_2 from gas to a liquid through the liquid film is the rate-limiting step. The rate of the reaction is zeroth order in Na_2SO_3. If the reaction is not fast enough, the reaction occurs in the liquid film around the gas bubbles. This would decrease apparent film thickness and give incorrectly high values of K_La. Concentrations of unreacted sulfite are determined by reacting to the sulfite with excess iodine and then back titration of the iodine with thiosulphate. It is important to note that the dissolved oxygen is zero through the reaction.

By titrating sodium sulfite present in the reactor (by taking a sample at fixed intervals of time) against sodium thiosulphate, the quantity of sodium sulfite that would have reacted according to the equation can be measured as the difference between successive rate instants of time. Then based on stoichiometry, the corresponding number of moles of O_2 that would have been consumed can be determined.

The Oxygen transfer rate

$$\mathbf{OTR = K_La\ (C^*-C_L)}$$

Where $C^* = 8.43X10^{-3}$g/l at 25^oC

C_L = Dissolved Oxygen content.

In this case, $C_L = 0$, since the DO is maintained such that it is not saturated.

$$\mathbf{OTR = K_La\ (C^*)\ ----\ (1)}$$

$$\mathbf{K_La = OTR/\ C^*}$$

Thus K_La can be determined.

PROCEDURE

- Take 100 ml of 0.5M Sodium sulphite & 0.002M copper sulphate solutions in a 250ml conical flask.
- Keep the conical flask with sodium sulfite solution in a magnetic stirrer for mixing and take the '0^{th} h' sample for titration.
- Add 15 mL of Iodine solution and few drops of starch indicator to the 1 ml of sample withdrawn from the flask.
- Titrate the sample against 0.5N Sodium thiosulphate. (It becomes straw yellow color and then turn into dark blue color)
- Repeat the analysis for 5,10,15,20…….minutes samples (till constant titer value reached to continue the sampling)
- Plot the graph between titer volume vs. time
- Calculate the OTR by using the slope of the graph
- Calculate the Kla by using the given formula

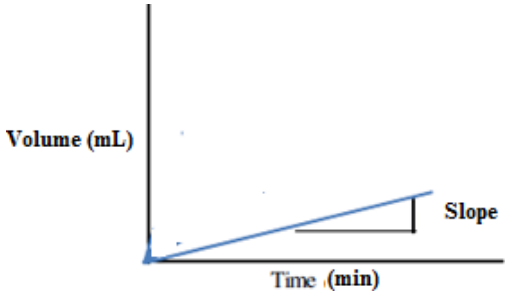
REACTION:

$O_2 + 2\ Na_2SO_3 \longrightarrow 2\ Na_2SO_4$

$2\ Na_2SO_3 + 2I + 2\ H_2O \longrightarrow 2\ Na_2SO_4 + 4HI$

$4\ Na_2SO_3 + 2I_2 \longrightarrow 2Na_2S_4O_6 + 4NaI$

MODEL GRAPH:



CALCULATION:

$$OTR = \frac{\text{Slope x Molarity of } Na_2S_2O_3 \text{ x Molecular weight of } O_2}{\text{Sample volume x Molecular weight of } Na_2S_2O_3}$$

$$K_la = OTR / C^*$$

$$kLa = \frac{\text{Slope x Molarity of Sodium thio sulphate x Mol.Weight of } O_2 \text{ x 1000 x 60}}{\text{Volume of sample (ml) x Mol. Weight of Sodium thio sulphate x } C^*} \; h^{-1}$$

TABULATION:

Sample time (min)	Titer volume (ml)

RESULT: The oxygen mass transfer coefficient, K_La is determined using sodium sulfite oxidation method and found to be ……

EXP NO: **DATE:**

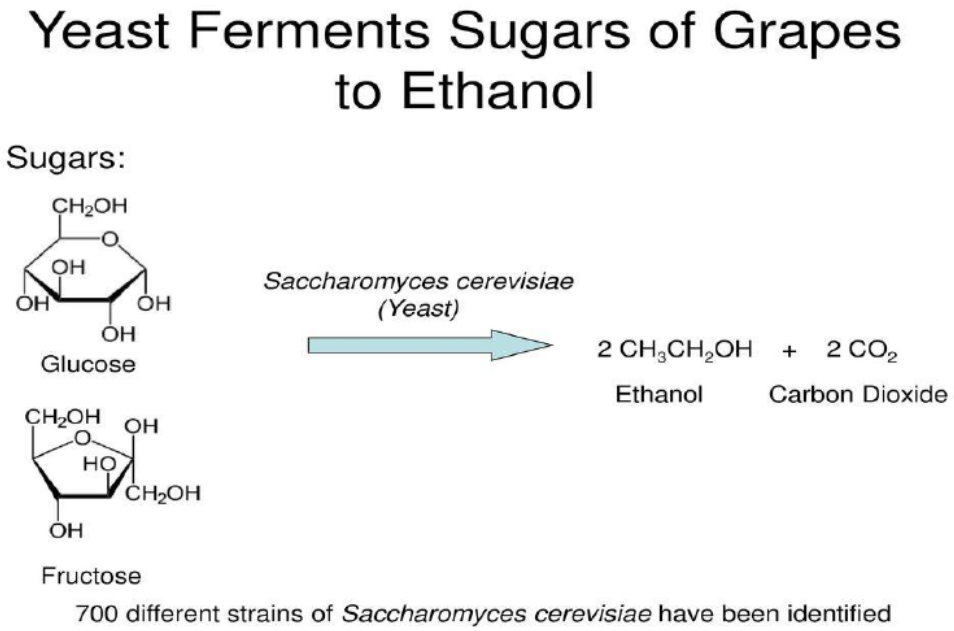
PRODUCTION OF ETHANOL BY YEAST

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 carbon dioxide. 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
- KH2PO4 – 1 gm
- MgSO4- 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.

BATCH BIOREACTOR OPERATION

To study the design, construction and control systems of a bioreactor.

PRINCIPLE:

A bioreactor is a device in which a substrate of low value is utilized by living cells or enzymes to generate a product of higher value. Bioreactors are extensively used for food processing, fermentation, waste treatment, etc. On the basis of the agent used, bioreactors are grouped into the following two broad classes: (i) those based on living cells and, (ii) those employing enzymes. But in terms of process requirements, they are of the following types: (i) aerobic, (ii) anaerobic, (iii) solid state, and (iv) immobilized cell bioreactors.

A bioreactor should provide for the following: (i) agitation (for mixing of cells and medium), (ii) aeration (aerobic fermenters; for O_2 supply), (iii) regulation of factors like temperature, pH, pressure, aeration, nutrient feeding, liquid level, etc., (iv) sterilization and maintenance of sterility, and (v) withdrawal of cells/medium (for continuous fermenters). Modern fermenters are usually integrated with computers for efficient process monitoring, data acquisition, etc.

BASIC FUNCTIONS OF A FERMENTER:

1. It should provide a controlled environment for optimum biomass/product yields.

2. It should permit aseptic fermentation for a number of days reliably and dependably, and meet the requirements of containment regulations. Containment involves prevention of escape of viable cells from a fermenter or downstream processing equipment into the environment.

3. It should provide adequate mixing and aeration for optimum growth and production, without damaging the microorganisms/cells. The above two points (items 2 and 3) are perhaps the most important of all.

4. The power consumption should be minimum.

5. It should provide easy and dependable temperature control.

6. Facility for sampling should be provided.

7. It should have a system for monitoring and regulating pH of the fermentation broth.

8. Evaporation losses should be as low as possible.

9. It should require a minimum of labour in maintenance, cleaning, operating and harvesting operations.

10. It should be suitable for a range of fermentation processes. But this range may often be restricted by the containment regulations.

11. It should have smooth internal surfaces, and joints should be welded wherever possible.

12. The pilot scale and production stage fermenters should have similar geometry to facilitate scale-up.

13. It should be contrasted using the cheapest materials that afford satisfactory results.

Key parts of the bioreactor:

Agitator – This facilitates the mixing of the contents of the reactor which eventually keeps the "cells" in the perfect homogenous condition for better transport of nutrients and oxygen for adequate metabolism of cell to the desired product(s).

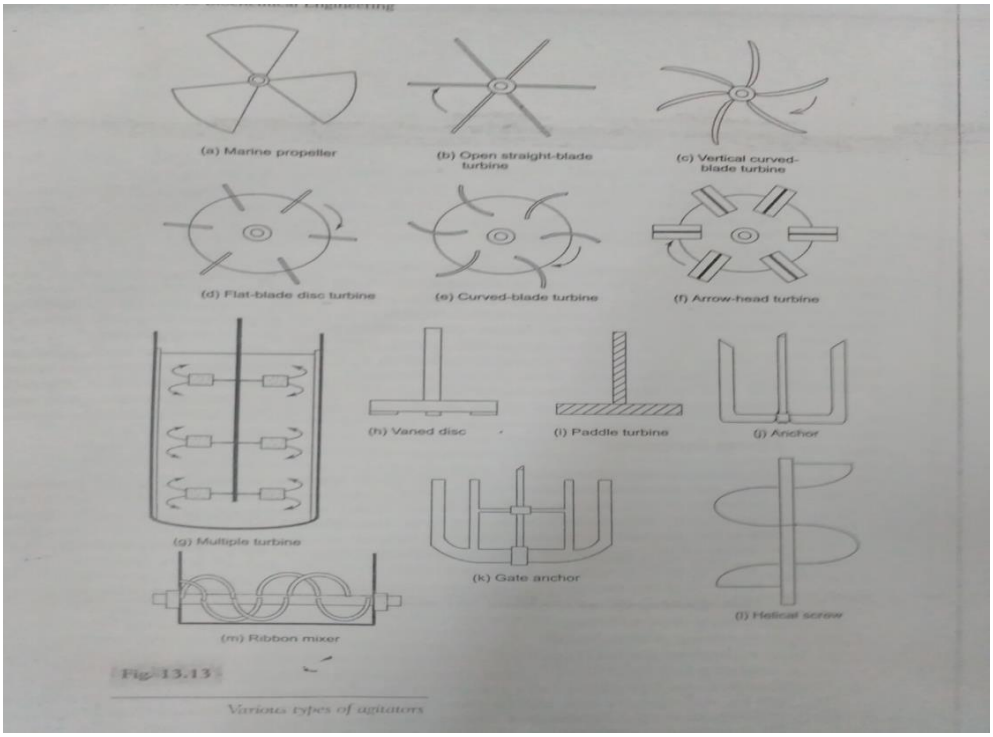
The agitator can be top driven or bottom which could be basically magnetic / mechanically driven. The bottom driven magnetic /mechanical agitators are preferred as opposed to top driven agitators as it saves adequate space on the top of the vessel for insertion of essential probes (Temperature, pH, dissolved oxygen foam, CO_2 etc) or inlet ports for acid, alkali, foam, fresh media inlet /exit gases etc. However mechanical driven bottom impellers need high quality mechanical seals to prevent leakage of the broth.

* Types of agitators:

- Disc turbine

- Open turbines of variable patch.

- Propellers



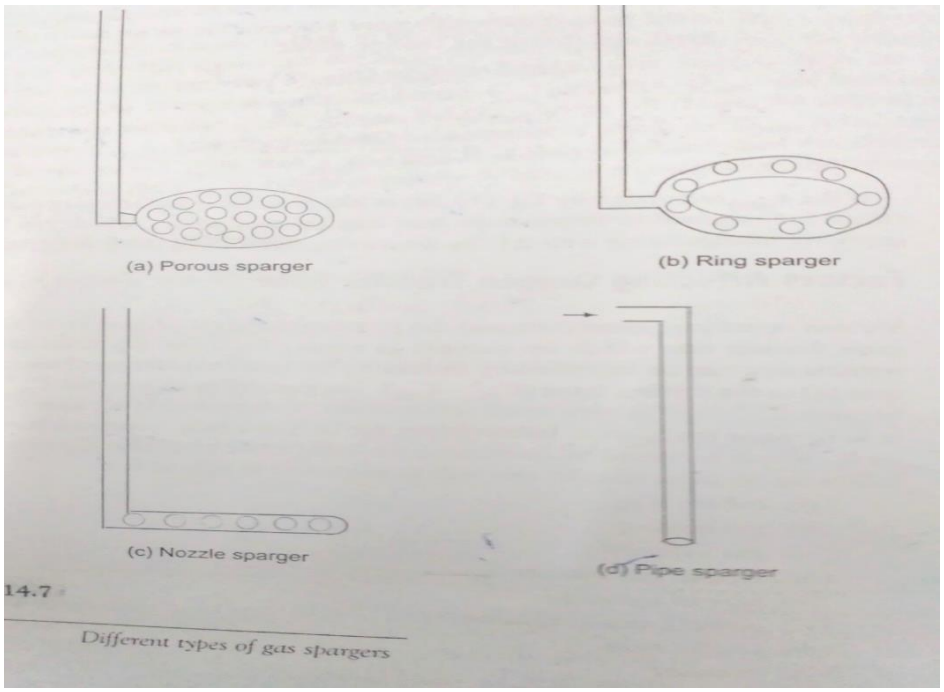
Baffle – The purpose of the baffle in the reactor is to break the vortex formation in the vessel, which is usually highly undesirable as it changes the centre of gravity of the system and consumes additional power.

- Baffles are metal stripes roughly $1/10^{th}$ of the vessel diameter and are attached radially to the wall.
- Normally 4 baffles are used, but in vessel over 3 dm^3 diameter 6-8 baffles may be used.

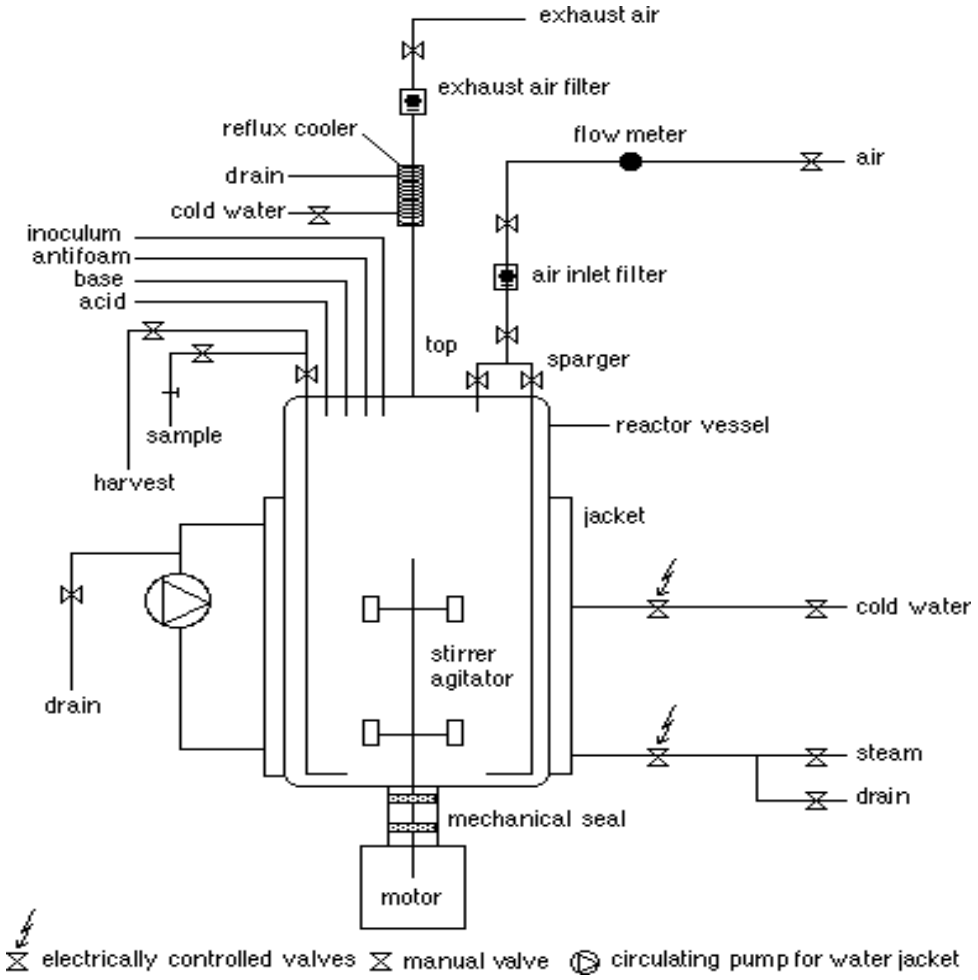
Sparger – In aerobic cultivation process the purpose of the sparger is to supply oxygen to the growing cells. Bubbling of air through the sparger not only provide the adequate oxygen to the growing cells but also helps in the mixing of the reactor contents thereby reducing the power consumed to achieve a particular level of (mixing) homogeneity in the culture.

Three basic types of sparger are used:

- Porous sparger.
- Orifice sparger.
- Nozzle sparger



14.7 Different types of gas spargers



Bioreactor (Bottom Driven)

Jacket – The jacket provides the annular area for circulation of constant temperature water which keeps the temperature of the bioreactor at a constant value. The desired temperature of the circulating water is maintained in a separate Chilled Water Circulator which has the provision for the maintenance of low/high temperature in a reservoir. The contact area of jacket provides adequate heat transfer area wherein desired temperature water is constantly circulated to maintain a particular temperature in the bioreactor.

Body construction:

For a small scale (1 to 30 dm^3) glass and/ or stainless steel is used because it can withstand repeated steam sterilization cycles.

Two basic types are used:

- A glass vessel with a round or flat bottom and top flanged carrying plates.
- A glass cylinder with stainless steel top and bottom plates. This bioreactor may be sterilized in situ. (AISI graded steel are now commonly used in bioreactor construction).

Peripheral parts:

*Reagent pumps

- Pumps are normally part of the instrumentation system for pH and antifoam control.
- Peristaltic pumps are used and flow rate is usually fixed with a timed shot and delay feed system of control.

*Medium feed pumps and reservoir bottles

Medium feed pumps are often variable speed to give the maximum possible range of feed rates. The reservoir bottles are usually larger, but are prepared in the same as normal reagent bottles.

*Rotameter

A variable area flow meter indicates the rate of gas flow into a bioreactor. A pressure regulator valve before the rotameter ensures safe operation.

*Stirrer glands and bearings:

These are used for the sealing of the stirrer shaft assembly and can be operated aseptically for a long duration. Four basic types of seal assembly have been used,

- The stuffing box (packed gland seal)
- The simple bush seal
- The mechanical seal
- Magnetic drive.

Basic control systems for the operation of the bioreactor are described below:

- **Temperature Measurement and control –** The measurement of the temperature of the bioreactor is done by a thermocouple or Pt -100 sensor which essentially sends the signal to the Temperature controller. The set point is entered in the controller which then compares the set point with the measured value and depending on the error, either the heating or cooling finger of the bioreactor is activated to slowly decrease the error and essentially bring the measured temperature value close to the set point.

- **pH measurement and control –** The measurement of pH in the bioreactor is done by the autoclavable pH probe. The measured signal is compared with the set point in the controller unit which then activates the acid or alkali to bring the measured value close to the set point. However before the pH probe is used, it needs to be calibrated with two buffers usually in the pH range which is to be used in the bioreactor cultivation experiment. The probe is first inserted in (let us say) pH 4 buffer and the measured value is corrected by the zero knob of the controller. Thereafter the probe is put in pH 7 buffer and if needed the measured value is corrected by the asymmetry knob of the controller. The pH probe is now ready for use in the range 0-7 pH range.

- **Dissolved oxygen controller –** The dissolved oxygen in the bioreactor broth is measured by a dissolved oxygen probe which basically generates some potential corresponding to the dissolved oxygen diffused in the probe. Before the measurement can be done by the probe it is to be calibrated for its zero and hundred percent values. The zero of the probe is set by (zero knob) the measured value of the dissolved oxygen when the broth is saturated with nitrogen purging. Similarly the hundred percent of the instrument is calibrated by the measured value of dissolved oxygen when broth is saturated with purging air in it. After calibration the instrument is ready for the measurement of the dissolved oxygen in the broth. In the event of low oxygen in the fermentation broth, more oxygen can be purged in the bioreactor &/or stirrer speed can be increased to enhance the beating of the bubbles which essentially enhances the oxygen transfer area and net availability of oxygen in the fermentation broth.

- **Foam control –** The fermentation broth contains a number of organic compounds and the broth is vigorously agitated to keep the cells in suspension and ensure efficient nutrient transfer from the dissolved nutrients and oxygen. This invariably gives rise to lot of foam. It is essential that control of the foam is done as soon as possible.
- **Speed control-** Speed control relies on the feedback from tachometer located with drive motor determining the power delivered by the speed controller to maintain the speed set point valve set by the user. A digital display shows the actual speed in rpm, as determined by the tachometer signals.

Table 1: Measurements of various parameters in a bioreactor

Measurements	Methods	Remarks
Agitator speed	Frequency counter tacho generator	More precise less reliable.
Agitator power	Torque sensor. Electrical power.	Difficult. Recommended.
Temperature	Resistance Thermometer	Probably best Fragile

	Thermistor Thermocouple	Satisfactory Not recommended
Flow rate	Rota meter Orifice meter Thermal mass flow meter	Satisfactory Less accurate Set-point control
Dissolved oxygen	Galvanic probe Polarographic probe	Widely used Widely used
pH	pH electrode	Widely used
Foam	Conductivity probe	Widely used
Redox	Redox electrode	Empirical valve
Turbidity	Turbidity sensor	Complex
Liquid feed rate	Peristaltic pump Syringe pump Magnet flow meter	Widely used Limited capacity Large
Pressure	Pressure transducer	Satisfactory

EXP NO **DATE**

FERMENTOR

OBJECTIVES OF FERMENTOR

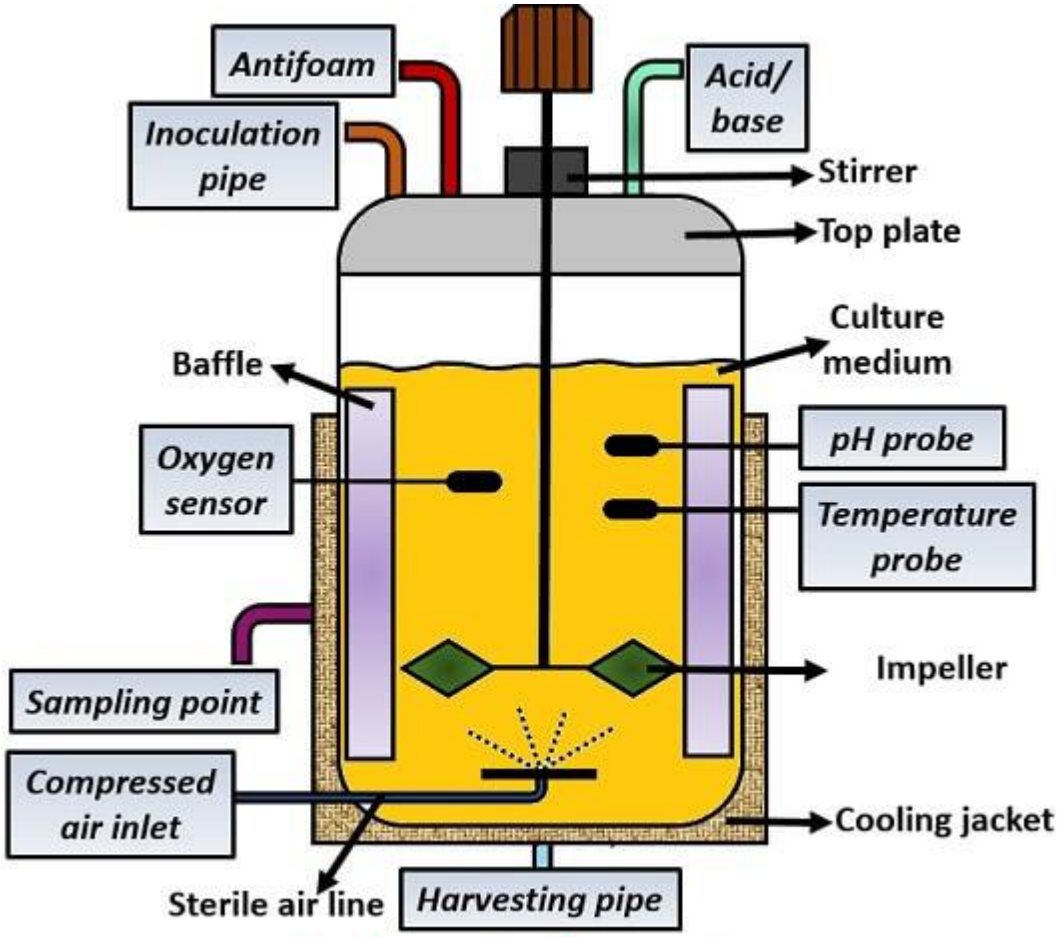
The objectives include the production of metabolites, enzymes, microbial biomass and recombinant product.

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

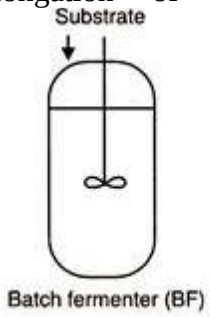
A fermentor is mainly of five types:

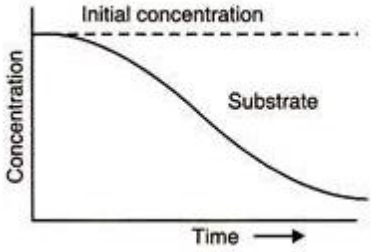
1. Stirred tank fermentor
2. Airlift fermentor
3. Fluidised bed fermentor
4. Packed bed fermentor
5. Photo fermentor

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

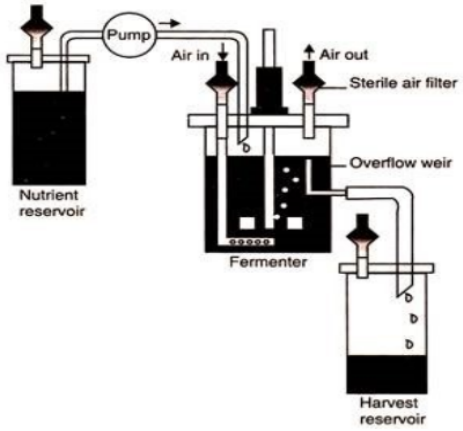
(biomass and metabolites) as the microbes undergo different phases of growth and metabolism. The culture broth is harvested, separated and purified when the desired product is formed. Batch fermentation are widely used in primary and secondary metabolites production under definite culture conditions 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

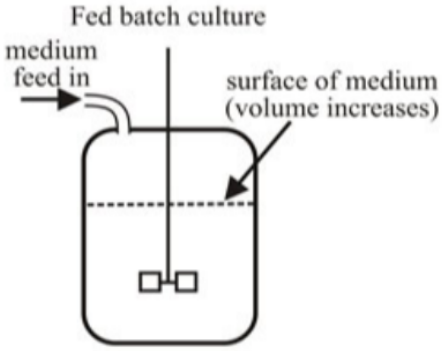
In this process, the nutrient medium and culture are kept in continuous mode of operation by balancing the input and the output of the harvested culture and the nutrient medium in a single fermenter.

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.



EXP NO: **DATE:**

CENTRIFUGATION STUDIES DURING SETTLING OF YEASTCELLS

AIM

To study the effect of:

i) Increasing speeds of centrifugation on the settling of the yeast cell particles.

ii) Increasing centrifugal times on the settling of yeast cell particles.

PRINCIPLE

Centrifugation is a basic separation technique used to separate material of different densities when gravitational force is insufficient for their separation. Normally a suspension of slowly under the influence of gravity. This process is called sedimentation. In centrifugation, the process of settling is added by centrifugal forces.

The centrifugal force, F is given by

$F = m\omega^2 r$

Where m = mass of the particle on which it is acting, w = angular velocity, r = radius.

It has been observed that by fixing the type (density and size) of particles, to be settled, under the given study and also the type of rotor, the settling of a particle subjected to centrifugation depends on the speed of centrifugation as well as the time spent by the particles at that speed. Hence, varying these two parameters (keeping either constant) we can study centrifugation profiles of particles.

MATERIAL REQUIRED

Yeast, Centrifuge, UV-visible Spectrophotometer, Centrifuge tubes,

PROCEDURE

1. Prepare 100 ml of stock yeast solution by dissolving 2 g of yeast in 10 ml of warm water (distilled) and make it up to 100 ml using normal water (distilled) (Since yeast is not easily soluble in normal water use warm distilled water).

2. Pipette out 10 ml of the stock solution into 10 centrifuge tubes
3. The first 5 tubes are centrifuged for 5 minutes each, with varying centrifugal speeds of 1000, 1500, 2000, 2500, 3000 rpm. The O.D's of supernatant are recorded at 540 nm.
4. The next 5 tubes are centrifuged at 1500 rpm with varying times of centrifugation of 1, 2, 3, 4, 5 min respectively. Here also, supernatants are collected and optical densities are noted.
5. Graphs of OD_{540} vs rpm and OD_{540} vs Centrifugation time are plotted.
6. Based on these graphs, the perfect speed for centrifugation and duration for the same for efficient separation of all particles can be measured.

RESULT

The profiles of OD vs rpm and OD vs centrifugation time are plotted. the perfect speed for centrifugation and duration for the same for efficient separation of all particles was found to be…….

OBSERVATION AND TABULATION

Concentration	Speed (rpm)	Time(min)	Optical Density (540 nm)
2 g / 100 ml	1000	5	
	1500	5	
	2000	5	
	2500	5	
	3000	5	
	3500	5	

Concentration	Speed (rpm)	Time(min)	Optical Density (540 nm)
2 g / 100 ml	1500	1	
	1500	2	
	1500	3	
	1500	4	
	1500	5	

EXP NO: **DATE:**

PRECIPITATION OF PROTEIN BY SALTING OUT (AMMONIUMSULPHATE PRECIPITATION)

AIM

To find % protein recovery from precipitation of proteins from the given solution by adding Ammonium sulfate

PRINCIPLE

Ammonium sulphate precipitation is a method of protein purification by altering solubility of protein. It is a specific case of a more general technique known as salting out. Ammonium sulfate is commonly used as its solubility is so high that salt solutions with high ionic strength are allowed.

The solubility of protein varies according to the salt concentration. Two distinct effects are observed: at low salt concentrations, the solubility of the protein increases with increasing salt concentration (i.e. increasing ionic strength), an effect termed salting in. As the salt concentration (ionic strength) is increased further, the solubility of the protein begins to decrease. At sufficiently high ionic strength, the protein will be almost completely precipitated from the solution (salting out).

Since proteins differ markedly in their solubilities at ionic strength, salting out is a very useful procedure to assist in the purification of a given protein. The commonly used salt is ammonium sulfate, as it is very water soluble and has no adverse effects upon enzyme activity. It is generally used as a saturated aqueous solution which is diluted to the requiredconcentration, expressed as a percentage concentration of the saturated solution (a 100 % solution)

MATERIALS REQUIRED

Test tubes, Graduated cylinder, Pipettes, UV spectrophotometer, Centrifuge

REAGENTS

- Prepare a known concentration protein solution(BSA – 2 mg / 10 ml) and raw hen white (collected)

- Ammonium sulphate

PHOSPHATE BUFFER SALINE (PBS) – 0.01M pH 7.2

- 28 mL of KH_2PO_4 (2.78 gm in 100 mL normal saline)
- 72 mL of Na_2HPO_4 (34.5 gm in 100 mL normal saline)

PROCEDURE

1. 10 ml of microbial cell culture/protein solution was pipette out in a test tube.
2. Weigh 6.97 gm of Ammonium sulphate salt (required saturation level) and add to the solution
3. Add the ammonium sulphate salt to the protein solution until the protein start precipitate.
4. Weigh the remaining amount of Ammonium sulphate salt and calculate the amount ofammonium sulphate added to the protein solution.
5. The solution was centrifuged for 10,000 rpm for 10 mins
6. The precipitate was collected carefully discarding as much supernatant as possible
7. To the precipitate 1 ml of PBS buffer (desired amount) was added to dissolve the pellet
8. The protein concentration measured by Lowry's method
9. Protein Recovery is calculated by using the formula

$$\% \text{ Protein Recovery} = \frac{\text{Final protein content}}{\text{Initial protein content}} 100$$

RESULT

Maximum amount of protein was recovered by using ---------- % of ammonium sulphate

Percentage Protein recovery at 37 ^{0} C was found to be ------

EXPT. NO. :

DATE :

LIQUID-LIQUID EQUILIBRIUM

AIM

To determine the liquid-liquid equilibrium for the given tertiary system (Benzene-Acetic acid- water) and to draw the bimodal diagram for the above system

MATERIALS REQUIRED

(i) Conical flasks
(ii) Burette
(iii) Pipette
(iv) Measuring cylinders
(v) Benzene
(vi) Acetic acid
(vii) Distilled water.

THEORY

Liquid-Liquid extraction also called solvent extraction is the separation of constituents of liquid solution by contact with another insoluble liquid. If the substances' consisting of the original solution distributes themselves differentially between the two liquid phases, a certain degree of separation will result.

Extraction involves the use of system composed of at least three substance and two phases are chemically quiet different which leads to a separation of the components according to physical or chemical properties. Generally all three components appear to some extent in both phases. In all such operation, the solution which is to be extracted is called feed and the liquid with which feed is contacted is the solvent. The solvent rich product of the operation is extract and the residual liquid from which the solute has been removed is called raffinate. Solvent extraction can

sometimes used as an alternative to separation by distillation. For example, Acetic acid can be removed from water by liquid-liquid extraction using organic solvent. The resulting organic solvent and acetic acid solution is the distilled.

Equilibria and Phase Composition

One of the mostly common type of system in extraction whose one pair is partially soluble, we observe that liquid C dissolves in A and B. A and B pair is partially soluble.

PROCEDURE

(i) Clean all conical flasks and dry. In first five conical flasks add 25 mL of water.

(ii) Add 4,8,12, 16 and 20 mL of acetic acid in each flask containing water.

(iii) Agitate the flask containing the solution for half an hour.

(iv) Titrate each solution against benzene taken in a burette.

(v) In the remaining five conical flasks add 25 mL of benzene.

(vi) Add the acetic acid in the same manner in each flasks containing benzene solution.

(vii) Agitate the sample and titrate against water taken in a burette.

(viii) The end point is turbidity.

RESULT

The liquid - liquid equilibrium for tertiary system is determined and the binodal diagram is drawn.

OBSERVATION

Density of acetic acid = g / mL

Density of water = g / mL

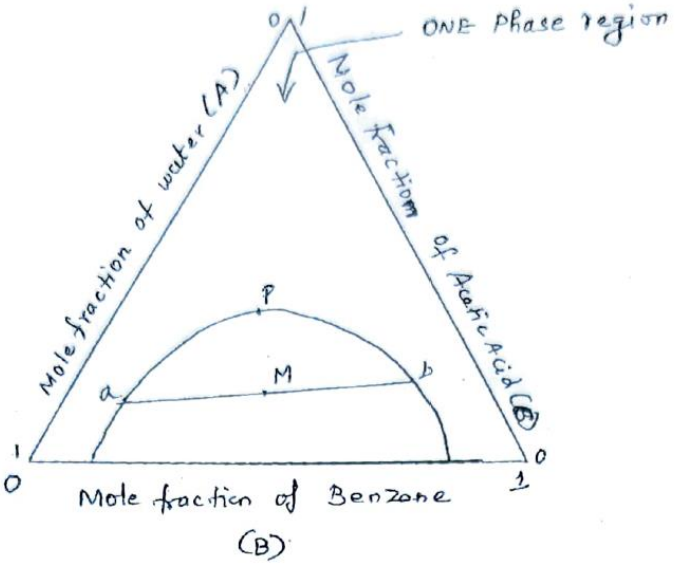
Density of benzene = g / mL

Tabular Column

S. No.	Volume of Water, mL	Volume of Acetic Acid, mL	Volume of Benzene, mL	Weight Fraction of Water	Weight Fraction of Acetic Acid	Weight Fraction of Benzene

S. No.	Volume of Benzene, mL	Volume of Acetic Acid, mL	Volume of Water, mL	Weight Fraction of Water	Weight Fraction of Acetic Acid	Weight Fraction of Benzene

LIQUID-LIQUID EQUILIBRIUM



A and B – Partially miscible system

Component C: Soluble in both A & B

P: plait point

EXPT. NO. :

DATE :

BATCH ADSORPTION

AIM

(i) To study the adsorption of oxalic acid from aqueous solution on activated carbon and to draw the adsorption isotherms

(ii) To determine the constants of Freundlich and Langmuir isotherm equations.

MATERIALS REQUIRED

(i) Burette
(ii) Conical flasks
(iii) Pipette
(iv) Measuring cylinders
(v) Standard flasks
(vi) Oxalic acid
(vii) Activated carbon
(viii) Sodium hydroxide.

THEORY

In adsorption processes one or more components of a gas or liquid stream are adsorbed on the surface of a solid adsorbent and a separation is accomplished. Adsorption is a surface phenomenon; the adsorption processes may be physical or chemical dependent on the binding force involved. Another classification is reversible and irreversible depending on the use of desorption on the bound molecules. In commercial processes, the adsorbent is usually in the form of small particles in a fixed bed. The fluid is passed through the bed and the solid particles adsorb components from the fluid. When the bed is almost saturated, the flow in this bed is stopped and the bed is regenerated thermally or by other methods, so desorption occurs. The

adsorbed material (adsorbate) is thus recovered and the solid adsorbent is ready for another cycle of adsorption. Adsorption from a solution is usually monomolecular (i.e.) adsorption ceases when the surface is completely covered. The amount of adsorption varies with the concentration of the solution. An expression representing the variation of the amount adsorbed with equilibrium concentration is known as adsorption isotherm. Numerous expressions have been proposed to reproduce experimental isotherms. The linear law can be expressed by an equation

$q = Kc$, q = kg adsorbate (solute) / kg adsorbent (solid)

c = kg adsorbate/m^3 of fluid.

The Freundlich isotherm equation often approximates data for physical adsorption and is given by

$$q = Kc^n$$

Where K and n are constants

The plot of q vs. c is practically a straight line at low concentration there by indicating a direct proportionality of the amount of adsorption, with the concentration of low values. At high values of concentration the curve becomes convex towards the axis of q, which shows that at the higher concentration the amount adsorbs increases less than proportionality occurring to the gradual saturation of the surface. On plotting log q vs. logc, a straight line is obtained, slope of the line gives n and intercept will be logK from which constant K is obtained.

A more significant isotherm for physical adsorption derived on theoretical basis, is Langmuir isotherm and is given by

$$q = abc / (1 + ac)$$

a and b are constants.

The constant a is proportional to the heat of adsorption. Constant b is amount of adsorption. The equation was derived assuming there are only a fixed number of active sites for adsorption that only a monolayer is formed and that the adsorption is

reversible and reaches an equilibrium condition. By plotting c /q vs. c, a straight line is obtained with intercept 1 / (ab) and slope 1/ b from which a and b are calculated.

Uses

Applications of liquid phase adsorption include removal of organic compounds from water or organic solution, coloured impurities from organics and fermentation products from fermentor effluents.

Gas-phase adsorption includes removal of water from hydrocarbon gases, sulfur compounds from natural gas, etc.

PROCEDURE

(i) Prepare 1 N solution of sodium hydroxide and 1 N solution of oxalic acid.

(ii) Weigh 2 gm of activated carbon.

(iii) Take five cleaned conical flasks (region bottles), and in the first flask add

i. 10mL of oxalic acid and 90mL of water

(iv) Fill the rest of the flasks with varying proportions of the oxalic acid and water.

(v) In each flasks, add 2 gm of accurately weighed activated carbon

(vi) Agitate the flasks in the bottle shaker for 10 min.

(vii) After 10 min. of shaking, stop the shaker and leave for half an hour. Filter the contents of the flasks.

(viii) Titrate 20mL of filtrate against standard NaOH solution using phenolphthalein indicator.

(ix) Calculate the normality of oxalic acid c (filtrate) and x /m or q, weight of oxalic acid adsorbed by 2 gm of activated carbon.

GRAPHS

Freundlich isotherm

Plot q Vs. c to find slope and intercept which is given by
Slope = n
Intercept = log K
K , n – Freundlich constants

Langmuir isotherm

Plot c/q vs. c
Slope = 1 / b: intercepts = 1 / (ab)

RESULT

Freundlich isotherm constants n =
K =
Langmuir isotherm constants a =
b =

OBSERVATIONS

Tabular Column 1

S. No.	Volume of Titrate Solution, mL	Volume of Sodium Hydroxide, mL	Normality of Oxalic acid c_f, N	Normality of Oxalic acid (Filtrate), c, g Oxalic acid / m^3 Solution

Tabular Column 2

Bottle No.	m g	x g	c, N	q = x / m	c / q	log c	log q

CALCULTION

Volume of NaOH V_1 = mL

Normality of NaOH N_1 = N

Volume of filtrate V_2 = mL

Normality of filtrate or oxalic acid N_2 = $\{V_1 \times N_1\} / V_2$, N

g of oxalic acid adsorbed, x = $\frac{(c_f - c) \times V \times \text{Equivalent weight}}{1000}$

Freundlich constants

x / m or q = Kc^n

q = g oxalic acid /g of activated carbon.

x = g of oxalic cid adsorbed, g

m = g of activated carbon used = 2 g

Take log on both sides

$\log q$ = $\log x + n \log c$

Slope = n: intercept = log K, Calculate K

Langmuir constants

q = $abc / (1 + ac)$

c / q = $1/(ab) + (c/b)$

Slope = 1/b: intercepts = $1 / ab$

MODEL GRAPHS

log q Vs. log c

c/q Vs. c

BATCH ADSORPTION
1
10 mL : OXALIC ACID ; 90 mL DISTILLED WATER
2 g : ACTIVATED CARBON
2
20mL: OXALIC ACID
80mL : DISTILLED WATER
2g : ACTIVATED CARBON
3
30mL: OXALIC ACID
70 mL : DISTILLED WATER
2g : ACTIVATED CARBON
4
40mL : OXALIC ACID
60mL : DISTILLED WATER
2g : ACTIVATED CARBON
5
50mL : OXALIC ACID
50mL : DISTILLED WATER
2g : ACTIVATED CARBON

EXPT. NO. :

DATE :

BATCH SEDIMENTATION

AIM

To determine the minimum area of a continuous thickener required to concentrate the feed of 5% $CaCO_3$ slurry at the rate of 175 tons / day and solids to give an underflow concentration 40% by carrying out batch sedimentation.

MATERIALS REQUIRED

(i) Graduated measuring jar
(ii) $CaCO_3$ slurry
(iii) Stop clock.

THEORY

Sedimentation is a process of separation of dilute slurry by gravity settling into a clear fluid and the slurry of higher solid content. When the particle is at sufficient distance from the boundaries of the container and from other particles so that it falls without being effective then, the process is called free settling. If the motion is impeded by other particles, it is called hindered settling.

For lower $N_{Re,}$ drag force on the particle obey's stroke's law. The law is valid at low velocities when the particle moves through the fluid by deforming it. The wall shear is the result of viscous force only. In gravitational settling 'g' is a constant and drag force increases with velocity, acceleration decreases with time and approaches zero. The particle then reaches a constant velocity called terminal settling velocity.

Newly prepared slurry having uniform concentration is taken in a cylinder as shown in fig. As the process begins the particles start settling. Different Zones of varying concentration are obtained. Zone B consists of heavier particles that settle

faster. Zone C is called transition layer consisting of variable size distribution and non uniform concentration. The layers are present as channels through which the fluid raises upward and the particles settles down. Zone B is of uniform concentration. Zone A the topmost layer is of clear liquid. As the sedimentation progresses, Zone A and Zone D grow larger at the expense of Zone B and the Zone C disappears which is known as critical point.

At this stage, solids present in these layers stops the settling process when the force of compression is equal to the weight of solid particle.

PROCEDURE

i. Prepare 5% $CaCO_3$ slurry by taking 50gm of solid in a beaker. Minimum amount of H_2O was added and the contents were transferred to the graduated cylinder.
ii. Stir the content in a vertical manner until the concentration is uniform throughout the cylinder. Start the stop clock after stirring. Note down the time for each centimeter traveled by the solids.
iii. Measure the height using a scale fixed to the sides of the beaker. Note the time for every 1 cm, for about half the length after which the time is recorded for 0.5 cm until it reaches the ultimate bed height.

RESULT

The minimum thickener area of continuous thickener is ……m^2

OBSERVATIONS

Z_o = Initial height of interface = cm
C_f = Initial solid concentration =
C_u = Concentration of underflow =
L_{cL} = Rate of dry solids =

Tabular Column 1

S. No.	Z, cm	T, sec

Tabular Column 2

S. No.	Z_i, cm	T, sec	Rate of Sedimentation $V = (dz / dt) \times 10^{-3}$, m / s	$C_L = C_F \times (Z_o / Z_i)$

Tabular Column 3

S. No.	$V \times 10^{-3}$, m / s	C_L g / cc	$L_{cL} / A = (V \times 10^{-3}) / (1 / C_L - 1 / Cu)$, g / $cm^2 s$

BATCH SEDIMENTATION