DOWNSTREAM PROCESSING LABORATORY MANUAL

VII Semester (10BTL78)

Name of the Student :

Semester /Section :

USN :

Batch :

DAYANANDA SAGAR COLLEGE OF ENGINEERING
Accredited by National Assessment & Accreditation Council (NAAC) with 'A' Grade
(An Autonomous Institution affiliated to Visvesvaraya Technological University, Belagavi & ISO 9001:2008 Certified)
DEPARTMENT OF BIOTECHNOLOGY
SHAVIGE MALLESWARA HILLS , KUMARASWAMY LAYOUT
BENGALURU-560078

Vision of the Institute

To impart quality technical education with a focus on Research and Innovation emphasizing on Development of Sustainable and Inclusive Technology for the benefit of society.

Mission of the Institute

- To provide an environment that enhances creativity and Innovation in pursuit of Excellence.
- To nurture teamwork in order to transform individuals as responsible leaders and entrepreneurs.
- To train the students to the changing technical scenario and make them to understand the importance of Sustainable and Inclusive technologies.

DOWNSTREAM PROCESSING LABORATORY MANUAL

VII Semester (10BTL78)

Name of the Student :

Semester /Section :

USN :

Batch :

DAYANANDA SAGAR COLLEGE OF ENGINEERING
(An Autonomous Institution affiliated to Visvesvaraya Technological University, Belagavi)
DEPARTMENT OF BIOTECHNOLOGY
SHAVIGE MALLESWARA HILLS
KUMARASWAMY LAYOUT
BENGALURU-560078

DAYANANDA SAGAR COLLEGE OF ENGINEERING
(An Autonomous Institution affiliated to Visvesvaraya Technological University, Belagavi)
DEPARTMENT OF BIOTECHNOLOGY, BENGALURU-560078

VISION OF THE DEPARTMENT

To impart quality education, training and research in multidisciplinary domains of Biotechnology for the benefit of society and environment.

MISSION OF THE DEPARTMENT

- **To provide globally acceptable technical education in the field of biotechnology by encouraging innovative thinking with practical insights.**
- **To promote team work and nurture students to serve society with ethical and environmental responsibilities.**
- **To foster students for higher studies, R&D activities and professional career in emerging trends of biotechnology.**

PROGRAMME EDUCATIONAL OBJECTIVES [PEOs]

PEO-1: Skill -Enable our graduates to identify, analyse and solve industrial and environmental problems by implementing acquired skills.
PEO-2: Career - Encourage our graduates to apply their engineering knowledge as an individual or in a team to excel in higher studies, research, teaching and industry.
PEO-3: Lifelong learning - Instil in our graduates a desire to engage in lifelong learning that will foster their career with an impact on society.
PEO-4: Ethics - Inculcate in our graduates to develop high level of professionalism and ethical attitude with awareness of current issues in relation to safety, health and environment.

PROGRAMME SPECIFIC OUTCOMES [PSOs]

Graduates will be able to
PSO-1: Apply engineering principles to biological systems.
PSO-2: Analyze the genome and proteome by advanced molecular techniques and computational tools to address major challenges in Pharma and Health.
PSO-3: Develop eco-friendly solutions to address complex environmental problems.

DAYANANDA SAGAR COLLEGE OF ENGINEERING

(An Autonomous Institution affiliated to Visvesvaraya Technological University, Belagavi)

DEPARTMENT OF BIOTECHNOLOGY, BENGALURU-560078

DOWNSTREAM PROCESSING LABORATORY (SYLLABUS)

VII SEMESTER B. E (BT)

Sub. Code:10BTL78	**IA Marks : 25**
Hrs/Week : 3	**Exam Hrs : 3**
Total Hrs: 36	**Exam Marks : 50**

Course Objectives:

1. Students will be able to acquire the knowledge of experimental techniques related to the concepts of Bioseparations.
2. Students will be able to address centrifugation, cell disruption, chromatography, filtration, liquid – liquid extraction.
3. Students will be able to assess the impact of change in unit's operations and the impact on the process.
4. Students will be able to define the fundamentals of downstream processing for biochemical product recovery.

Syllabus:

1. Cell disruption techniques.
2. Solid-liquid separation methods: Filtration.
3. Solid-liquid separation methods: Sedimentation.
4. Solid-liquid separation methods: Centrifugation.
5. Product enrichment operations: Precipitation – (NH4)2 SO4 fractionation of a protein.
6. Product enrichment operations: Two – phase aqueous extraction.
7. Product drying techniques. 8. Staining Techniques (Coomassie Blue & Silver).
8. Separation of Amino acids / Carbohydrates by TLC.
9. Characterization of protein by Western blotting
10. Estimation of % of ethanol from fermented broth.
11. Estimation of Citric acid from fermented broth.
12. Separation of proteins by molecular sieving / Ion exchange chromatography.
13. Analysis of biomolecules by HPLC / GC (using standard spectra).
14. Native PAGE versus SDS PAGE (using BSA).

Course Outcomes:

1. Students are equipped with the knowledge of various methods of separation, isolation abd purification of byproducts.
2. Students will be able to understand the concept of bioprocess principles, methods, variables and parameters in product formation and recovery.
3. Students will be able to apply knowledge of cutting-edge pharmaceutical processing approaches and technologies.

DAYANANDA SAGAR COLLEGE OF ENGINEERING
(An Autonomous Institution affiliated to Visvesvaraya Technological University, Belagavi)
DEPARTMENT OF BIOTECHNOLOGY

DOWNSTREAM PROCESSING LABORATORY (10BTL78)

I - CYCLE

1. To determine the minimum thickener area required to concentrate a feed of --------% $CaCO_3$ slurry at a given rate.
2. To determine the percentage recovery of solute from a solution using a solvent.
3. To estimate the amount of ethanol present in an unknown (given) sample calorimetrically.
4. To study the filtration characteristics of a given leaf filter and to determine specific cake resistance and filter medium resistance.

II - CYCLE

5. To disrupt yeast cells by mechanical method using homogenize and estimation of total protein content by Biuret method.
6. To separate mixture of amino acids using thin layer chromatography.
7. To fractionate the proteins by precipitating it using the ammonium sulphate powder.
8. To estimate the amount of citric acid produced by Aspergillus niger using potato dextrose broth.
9. To separate the proteins by SDS page.

DAYANANDA SAGAR COLLEGE OF ENGINEERING
DEPARTMENT OF BIOTECHNOLOGY
BENGALURU – 560078

DO's

- Adhere and follow timings, proper dress code with appropriate foot wear.
- Bags and other personal items must be stored in designated place.
- Come prepared with the viva, procedure, and other details of the experiment.
- Secure long hair, loose clothing & know safety and emergency procedures.
- Do check for the correct ranges/rating and carry one meter/instrument at a time
- Inspect all equipment/meters for damage prior to use
- Conduct the experiments accurately as directed by the teacher.
- Immediately report any sparks/ accidents/ injuries/ any other untoward incident to the faculty /instructor.
- Handle the apparatus/meters/computers gently and with care
- In case of an emergency or accident, follow the safety procedure.
- Switch OFF the power supply after completion of experiment

DONT's

- The use of mobile/ any other personal electronic gadgets is prohibited in the laboratory.
- Do not make noise in the Laboratory & do not sit on experiment table.
- Do not make loose connections and avoid overlapping of wires
- Don't switch on power supply without prior permission from the concerned staff.
- Never point/touch the CRO/Monitor screen with the tip of the open pen/pencil/any other sharp object.
- Never leave the experiments while in progress.
- Do not insert/use pen drive/any other storage devices into the CPU.
- Do not leave the Laboratory without the signature of the concerned staff in observation book.

Experiment No: 1 Date:_____

BATCH SEDIMENTATION

Aim:

To determine the minimum thickener area required to concentrate a feed of --------% $CaCO_3$ slurry at a given rate.

Apparatus/Components required:

Measuring jar 1000 ml, stirrer and stopwatch.

Theory:

SEDMENTATION:
The separation of dilute slurry by gravity settling into a clear fluid and slurry of higher solid content is called sedimentation.

BATCH SEDIMENTATION:
There are several stages in the settling of a flocculated suspension and different zones are formed as sedimentation proceeds. Usually, the concentration of solids is high enough that sedimentation of individual particles or flocs is hindered by other solids to such an extent that all solids at a given level settle at a common velocity. At first the solids are uniformly distributed in the liquid. The total depth of the suspension is Zo.
After a short time, the solids have settled to give a zone of clear liquid (A) and a zone of settled solids (D). Above the zone of settled solids there is a transition layer (C) in which the solid content varies from that in original pulp to that in zone of settled solids. In zone 'B', the concentration is uniform and equal to the original concentration, since the settling is the same throughout the zone. The boundary between zones D and C and between zones C and B may not be distinguished but the boundary between zones A and B is usually sharp.
As settling continues the depth of zones D and A increases. The depth of zone 'C' remains nearly constant and that of zone 'B' decreases. Eventually, zone 'B' disappears and all the solids are in zones C and D. meanwhile, the gradual accumulation of solids put stress on the material at the bottom which compresses solids in layer 'D'. Compression breakesdown the structure of the flocs or aggregates and liquid is expelled into the upper zones. Sometimes liquid in the flocs spurts out of zone 'D' like small geysers, as layer 'D' compresses. Finally when the weight of the solid is balanced by the compressing strength of the flocs the settling process stops. The entire process is called sedimentation.
This laboratory batch settling test is the basis for the design for continuous thickness.

THEORIES OF SEDIMENTATION:

There are 2 theories

1. **Coe and Clevenger theory**:

This is the oldest theory proposed by Coe and Clevenger. For a given set of operating conditions, it is assumed that the settling rate is a function only of solids concentration. It was also assumed that

if batch sedimentation tests were run at different initial pulp concentration, the essential characteristics of the solids were unchanged. These assumptions may not be correct always.

2. **Kynch theory**:

First assumption of Coe and Clevenger theory has been taken here. This is based on the mathematical analysis of batch settling test presented by Kynch, which showed that the settling rate and the concentration of zone that limits the capacity can be determined for a single batch settling test.

RATE OF SEDIMENTATION:

The plot of interface height vs. time gives the rate of sedimentation. During the early stages of settling, the velocity is constant in the first portion of the curve. When zone 'B' disappears, the rate of settling starts decreasing and steadily drops until the ultimate height is reached.
Slurries vary greatly in their settling rates and in the relative heights of various zones during settling. The initial rate is a function of the feed concentration, but in the later stages, the settling rate is also depending on the initial height Zo, since compression effects are more important with the thickener sludge layer.

APPLICATIONS OF BATCH – SEDIMENTATION TEST FOR DESIGN OF CONTINUOUS THICKENERS:

The capacity of a continuous thickener is determined by the fact that the solids initially present in the feed must be able to settle through all zones of slurry concentration from that of initial feed to that of the underflow at the rate equal to that at which they are introduced into the thickener. If the area provided is not sufficient the solids will build up through the settling zone and into the clarification zone until finally some solids are discharged in the overflow. Further more, it is known at the start which zone will be the zone of minimum capacity.

KYNCH THEORY:

First assumption of Coe and Clevenger theory has been taken here. This method is based on the mathematical analysis of batch settling test presented by Kynch, which showed that the settling rate and the concentration of zone that limits the capacity can be determined for a single batch settling test. In a batch sedimentation test started with uniform initial concentration of solids, the concentration of solids in the zone 'C' must range between that of the initial slurry concentration in zone 'B' and that of the final slurry concentration in zone 'D'. If the solid handling capacity per unit area is lowest a some intermediate concentration, a zone of such concentration must start building up. It has been showed that the rate of upward propagation of such a zone is constant and is a function of the solid concentration.
By assumption of Kynch theory

$$V = C.\frac{dv}{dc} - V \qquad \text{_____ (1)}$$

Where V = upward velocity of propagation of the concentration gradient of minimum Settling rate with respect to vessel.
v = settling velocity of solids in concentration zone of minimum settling rate Wrt vessel.

C = concentration of solids, weight of solids per unit volume of pulp.

From the assumption that the settling rate is a function of solid concentration only i.e.

$$v = f(C)$$

$$v = Cf'(c) - f(c) \quad \text{_____ (2)}$$

suppose Co and Zo represents initial concentration and height respectively of a pulp in a batch settling test, the total weight of solids in this pulp is then CoAZo, where A is the test at the instant of the time when the layer corresponding to the limiting settling rate has reached the interface between the clean supernatant liquid and pulp. All the solids in the initial pulp must have passed through this layer, since the layer was propagated upward from the bottom of the column. If the concentration of this layer reaches the interface is C_L and the time of instant at which the layer reaches the interface is θ_L.

Then $C_L A (v_L + v_L) \theta_L = CoAZo$ _____ (3)

Where v_L and v_L refer to the respective velocities for a layer having a solid concentration of C_L. let Z_L corresponds to the height of interface at time θ_L .then

$$V_L = Z_L/ \theta_L \quad \text{_____ (4)}$$

Since from the equation (2) v is constant if 'C' is constant.

Substituting (4) in (3) and simplifying we get

$$C_L A (v_L + Z_L/ \theta_L) \theta_L = CoAZo$$
$$C_L (v_L \theta_L + Z_L) = CoZo$$
$$C_L = \frac{CoZo}{v_L\theta_L + Z_L} \quad \text{_____ (5)}$$

The value of settling velocity, VL is the slope of tangent to the curve (interface height vs. time). The tangent intercepts the vertical axis at Z = Z1 then slope of this line is

$$V_L = \frac{Zi - ZL}{\theta_L}$$
$$Z_i = Z_L + V_L\theta_L \quad \text{_____ (6)}$$

Comparing equations (5) and (6)

$$Z_i = \frac{CoZo}{C_L}$$

$$Z_i C_L = CoZo \quad \text{_____ (7)}$$

Equation (7) states that Z_i is the height of uniform slurry of concentration C_L which contains the same amount of solids as in the initial slurry.

The settling velocity as a function of concentration may be determined from a single settling test by the use of the above relationship. Using arbitrarily chosen values of the settling time θ, the corresponding tangents to the settling curve are located and the values of the intercepts are

used in equation (7) to determine the corresponding concentration, the respective settling rates are given by the corresponding slopes.

DETERMINATION OF THICKENER AREA:

Let 'F' be the volumetric feed rate of slurry per unit time.
'Co' be the initial concentration of solids in slurry.
'Cu' be the concentration of solids in underflow.
'L' be the volumetric floe rate of desired liquid.
'V' be the volumetric flow rate of clarified liquid.
'Cv' be the concentration of solids in overflow which is equal to zero.

The material balance for solids is

$$F\,Co = L\,Cu + V\,Cv \qquad \text{as } Cv = 0.$$

$$F\,Co = L\,Cu$$

$$L = \frac{F\,Co}{Cu} \qquad \text{_____ (8)}$$

Writing a liquid balance for the thickener

$$F(1 - Co) - L(1 - Cv) = V \qquad \text{_____ (9)}$$

Substituting (8) in (9)

$$F(1 - Co) - \frac{F\,Co}{Cu}(1 - Cu) = V$$

$$F - \frac{F\,Co}{Cu} = V$$

$$F\,Co\left(\frac{1}{Co} - \frac{1}{Cu}\right) = V \qquad \text{_____ (10)}$$

Dividing equation (10) by cross sectional area 'A' of the thickener

$$\frac{V}{A} = \frac{F\,Co}{A}\left(\frac{1}{Co} - \frac{1}{Cu}\right) \qquad \text{_____ (11)}$$

The term V/A represents the upward propagation velocity in the clarification zone of the thickener. When the thickener is operated at the capacity of the lowest value of the settling rate encountered must be equal to or greater than this value or otherwise solids will leave in overflow.

Consequently V/A must be replaced by V_L

$$V_L = \frac{F\,Co}{A}\left(\frac{1}{Co} - \frac{1}{Cu}\right) \qquad \text{_____ (12)}$$

The above equation may be written in terms of concentration of limiting layer which limits the capacity rather than in terms of feed concentration and the rate which is set by this capacity

i.e. $F\,Co = L_L\,C_L$

$$V_L = \frac{L_L\,C_L}{A}\left(\frac{1}{C_L} - \frac{1}{Cu}\right)$$

$$\frac{L_L C_L}{A} = \frac{V_L}{\left(\frac{1}{C_L} - \frac{1}{C_u}\right)} \qquad \text{_____ (13)}$$

By using settling velocity, concentration curve to obtain corresponding values of V_L and C_L and using the values in equation (13) the velocity values of $\frac{L_L C_L}{A}$, the solid Handling capacity per unit area of the thickener may be calculated. The lowest value calculated is to be used in determining the area of thickener.

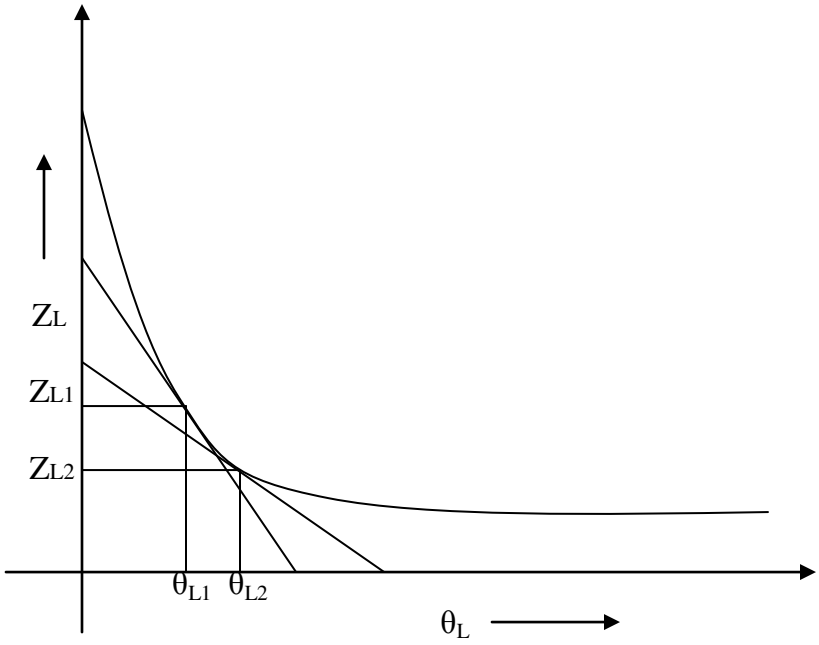
$$A = \frac{L_L C_L}{\left(\frac{V_L}{\left(\frac{1}{C_L} - \frac{1}{C_u}\right)}\right)_{min}}$$

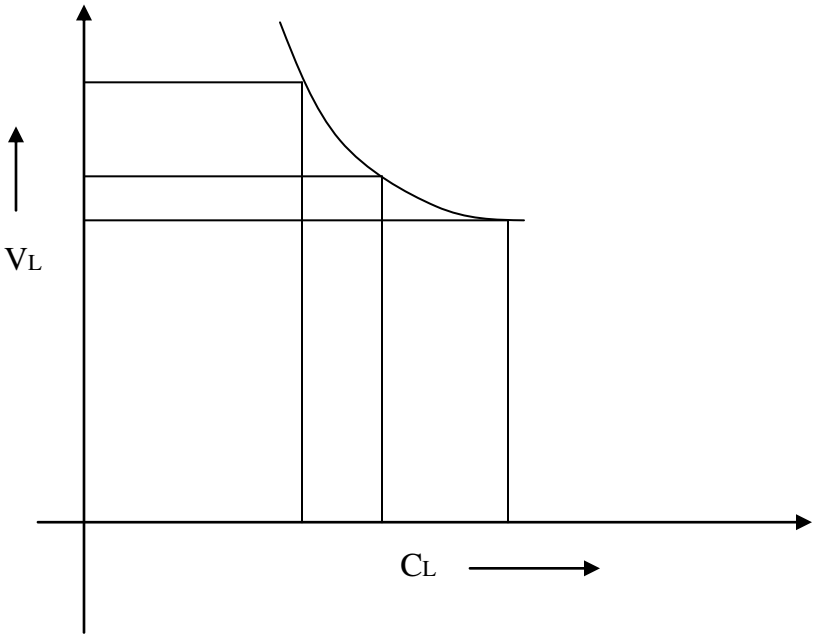
Procedure:

1. One litre of ----% (-----g/lit) $CaCO_3$ slurry was prepared and was poured into a measuring jar. The measuring jar was kept against a bright background so that the interface is clearly visible.
2. The suspension was stirred to achieve uniform distribution of particles. Note down the initial height of the slurry.
3. Initially for every 1 cm decrease in the height the time was noted. Later the time was noted for a decrease of 0.5 cm height and then for 0.2 cm was noted. This was noted till the settling was completed.

Nature of graph:

1. A graph of Z Vs θ was plotted. Then the tangents were drawn to the curve and corresponding Zi, Z_L and θ_L were noted down and the values of C_L and V_L were calculated using Kynch theory.
2. A graph of C_L Vs V_L was plotted and intermediate points were plotted and then $\frac{V_L}{\left(\frac{1}{C_L} - \frac{1}{C_u}\right)}$ was calculated.
3. A graph of $\frac{V_L}{\left(\frac{1}{C_L} - \frac{1}{C_u}\right)}$ Vs V_L was plotted and the minimum value of $\frac{V_L}{\left(\frac{1}{C_L} - \frac{1}{C_u}\right)}$ was found from the graph and the area of the thickener was then calculated.





From the graph,

$$\frac{V_L}{(1-1)} = \frac{}{C_L \text{ Cu min}}$$

$L_L C_L$ =

= -------kg/s.

Tabular Column:

FORMULAS:

1. $C_L = \frac{CoZo}{Z_i}$, kg/m^3.

2. $V_L = \frac{Zi - ZL}{\theta_L}$, m/s.

3. Area of thickener, $A = \frac{L_L C_L}{\frac{V_L}{(1 - 1)}}$ m^2.
   C_L C_U min

4. Given, $L_L C_L$ = ----- T/day.
   Cu = ------ kg/m^3.
   Co = -----% = ----- g/lit = ----- kg/m^3.

OBSERVATIONS:

1. $L_L C_L$ = _____ kg/sec.

2. Underflow concentration, C_U = -------
   C_U = _____ kg/m^3.

3. overflow concentration, C_O = -----
   C_O = _____ kg/m^3.

TABULAR COLUMN: 1

SL.NO:	Height Z_L (m)	Time θ_L (sec)
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.		
12.		
13.		
14.		
15.		

16.		
17.		
18.		
19.		
20.		
21.		
22.		
23.		
24.		
25.		
26.		
27.		
28.		
29.		
30.		
31.		
32.		
33.		
34.		
35.		
36.		
37.		
38.		
39.		
40.		
41.		
42.		
43.		
44.		
45.		
46.		
47.		
48.		
49.		
50.		
51.		
52.		
53.		
54.		
55.		
56.		

TABULAR COLUMN: 2

SL. NO:	Zi(m)	Z_L(m)	θ_L(s)	$C_L = \frac{CoZo}{Z_i}$, (kg/m^3)	$V_L = \frac{Zi - Z_L}{\theta_L}$, (m/s)	$\frac{V_L}{(\frac{1}{C_L} - \frac{1}{Cu})}$ (kg/m^{2}s)
1.						
2.						
3.						
4.						
5.						
6.						
7.						
8.						
9.						
10.						
11.						
12.						
13.						
14.						
15.						

TABULAR COLUMN: 3

SL.NO:	C_L(kg/m^3)	V_L (m/s)	$\frac{V_L}{(\frac{1}{C_L} - \frac{1}{Cu})}$ (kg/m^{2}s)
1.			
2.			
3.			
4.			
5.			
6.			
7.			
8.			
9.			
10.			
11.			

Results:

The area of thickener was found to be A = _____ m^2

Applications:

It is the basis for the design of continuous thickener.

Remarks:

Signature of Staff Incharge with date:

Probable viva questions:

1. What do you mean by minimum thickener area and how do you determine the area?
2. Write the theory behind batch sedimentation?
3. What is kynch theory and Cloe and Clevenger theory?
4. Write short notes on rate of sedimentation.
5. How to determine the thickener area?

Experiment No: _____ Date: _____

LIQUID – LIQUID EXTRACTION

Aim:

To determine the percentage recovery of solute from a solution using a solvent.

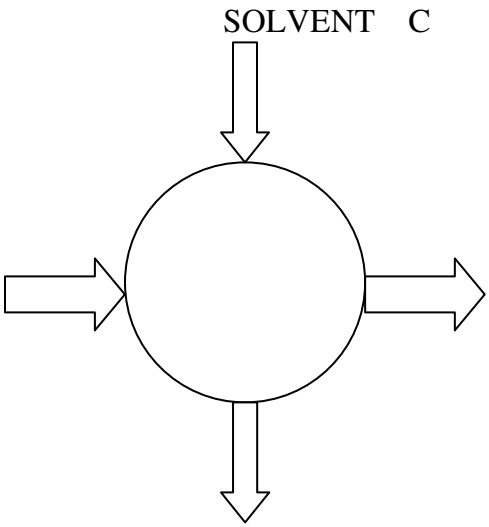
Apparatus/Components required:

Flask shaker, volumetric flask 250 ml, separating funnel, volumetric flask 500 ml, measuring jar 100 ml, burette, pipette 10 ml and beakers.

Theory:

Extraction is a terminology used in the mass transfer operations when a desired solute is selectively removed from rest of inerts or non desired materials by use of a liquid solvent. If the solute desired is present in another liquid mixture then the operation is termed as Liquid-liquid extraction and if the solute is present in a solid phase the operation is termed as leaching.

Liquid-liquid extraction is a mass transfer operation in which a solute dispersed or dissolved in a liquid media is separated in by use of a solvent which has differential solubiliies with media and solute and has a phase characteristics. The substance i.e. solute when dissolved in a medium can be taken into another liquid which is insoluble with the medium but has a higher solubility with solute. Due to the insolubility there will be two separate phases, one in which the solvent with a higher percentage of solute called as Extract and the other being the original medium in which the solute of lower percentage called as Raffinate.



Let F = kgs of feed.
E = kgs of Extract.
R = kgs of Raffinate.
B = solvent.
C = solute.
A = the media in which C is dissolved.

Then if Y is the mole fraction in the Extract

$$Y = \frac{\text{kg C}}{\text{Kg A+kg B+kg C}} \quad \text{__ (1)}$$

If X is the weight of C in the raffinate then,

$$X = \frac{\text{kg C}}{\text{Kg A+kg B+kg C}} \quad \text{__ (2)}$$

We can define X' and Y' which will be the ratios of mass of C to mass of A + B in raffinate and extract.

i.e. $X' = \frac{X}{1 - X}$, $Y' = \frac{Y}{1-Y}$

We can also define X = weight fraction of C on a B free basis.

i.e. $X = \frac{\text{Mass C in raffinate}}{\text{Mass A + B}}$ $Y = \frac{\text{Mass C in Extract}}{\text{Mass A + C}}$

And

$$N = \frac{\text{Mass C}}{\text{Mass A + C}}$$

Then NF, NR, NE will be the values o B/A+C in feed. Raffinate and Extract.

Liquid-liquid extraction is also known as solvent extraction and partitioning, is a method to separate compounds based on their relative solubilities in two different immiscible liquids, usually water and an organic solvent. It is an extraction of a substance from one liquid phase into another liquid phase. Liquid-liquid extraction is a basic technique in chemical

laboratories, where it is performed using a separatory funnel. This type of process is commonly performed after a chemical reaction as a part of the work-up.

In other words, this is the separation of a substance from a mixture by preferentially dissolving that substance in a suitable solvent. By this process a soluble compound is usually separated from an insoluble compound. Solvent extraction in an industrial application, the process is done continuously by pumping an organic and aqueous stream into a mixer. This mixes the organic component with the aqueous component and allows ion transfer between them. The mixing continues until equilibrium is reached. Once the ion transfer is complete (equilibrium is reached), the mixture flows into a vessel, where the organic and aqueous are allowed to separate, similar to the way oil and water would separate after mixing them. Fresh material is continuously fed into the mixer, and a two continuous streams is removed from the settler (one organic and one aqueous). The process is commonly used to process copper and uranium.

Liquid-liquid extraction is possible in aqueous systems: in a system consisting of a molten metal in contact with molten salt, metals can be extracted from one phase to the other. This is related to a mercury electrode where a metal can be reduced, the metal will often then dissolve in the mercury to form an amalgam which modifies its electrochemistry greatly. For example it is possible for sodium cations to be reduced at a mercury cathode to form sodium amalgam, while at an inert electrode (such as platinum) the sodium cations are not reduced. Instead water is reduced to hydrogen. If a detergent or fine solid can stabilize an emulsion which in the solvent extraction is known as a third phase.

The water-benzene and IPA constitutes a system of immiscible liquids i.e. water and benzene are insoluble in each other for all concentrations. For such a system if solvent (B) is added to a binary homogeneous liquid mixture (A-C) containing a diluent (A) and a solute (C), then C separates (distributes itself) between A and B with no mixing of A and B. such mixing and then separating two heterogeneous liquid constitutes a stage and if the exit streams are in equilibrium, then a combination of mixing and separating operations is known as an equilibrium stage. The two liquid phases are accordingly called as ‘Extract’ (solvent rich phase) and ‘Raffinate’ (diluent rich phase). If the operations are carried out in such a manner that if the fresh solvent is added to ‘raffinate phase’ then the operation is termed as cross-current operations.

ADVANTAGES:

1. Low cost.
2. Low energy consumption.
3. Variety of solvents is available.
4. Easy to scale-up.

DISADVANTAGES:

1. Emulsification may occur.
2. Effluent must be treated.

Procedure:

1. About 2 ml of Acetic acid is taken in 250 ml volumetric flask and is made to 250 ml by adding benzene, which forms a homogeneous solution acts as a feed solution to the experiment.
2. Two separating funnels are taken to which 50 ml of feed solution is added to each followed by 20 ml and 40 ml of water added to the two separating funnels respectively.
3. The separating funnels are then shaken for about 30 minutes and then allowed to settle.
4. On settling the mixture is going to form two separate phases. The bottom aqueous layer (Extract) is removed from the bottom and the volume is measured.
5. About 10 ml of the aqueous layer (Extract) is pipetted out into a conical flask and titrated against standardized NaOH solution using phenolphthalein as the indicator till the end point of pale pink is obtained, the volume of NaOH run down is recorded.
6. The volume of NaOH run down is used to determine the concentration of the acetic acid in the aqueous layer.
7. From the concentration of acetic acid in different extract layers the percentage recovery is calculated.

PREPARATION OF SOLUTIONS:

1. **0.1N oxalic acid solution**:

About 0.63gm of oxalic acid crystals is weighed and transferred into 100 ml volumetric flask and it is made up to the mark by adding water.

2. **0.5N NaOH solution**:

About 5 gm of NaOH pellets is weighed and transferred into 250 ml volumetric flask and it is made up to the mark by adding water.

3. **Feed solution**:

4 ml of Acetic acid is measured using a measuring jar and it is added to a 500 ml volumetric flask and it is made up to the mark by adding benzene.

4. **Standardization of NaOH solution**:

About 10 ml of oxalic acid is pipetted out into a conical flask and it is titrated against NaOH solution using phenolphthalein indicator till the end point of pale pink is reached.

Tabular Column:

OBSERVATIONS AND CALCULATIONS:

1. Room temperature = _____°C.
2. Molecular weight of Acetic acid = 60 g
3. Density of Benzene = 0.879 gm/cc.

TABULAR COLUMN:

Sl.no	Volume of feed (ml)	Volume of water (ml)	Volume of aqueo layer (ml)	Volume of NaOH run down (ml)
1.	50	20		
2.	50	40		

STANDARDISATION OF NaOH:

TABULAR COLUMN:

Burette reading	**I**	**II**
Initial burette reading		
Final burette reading		
Volume of NaOH run down		

$$(N_1V_1)_{NaOH} = (N_2V_2)\ \text{Oxalic acid}$$

$$(N_1)_{NaOH} = \frac{(N_2V_2)\ \text{Oxalic acid}}{(V_1)\ NaOH}$$

Where N_2 = Normality of oxalic acid **= 0.1N.**

V_2= Volume of oxalic acid **= 10 ml.**

V_1= Volume of NaOH run down**.**

Burette: **NaOH** solution.
Conical flask: 10 ml oxalic acid + phenolphthalein indicator (2-3) drops.
Indicator: phenolphthalein indicator.
End point: colorless to pale pink.

CALCULATION: FOR (50 ml feed solution + 20 ml water)

Normality of Acetic acid (**CH_3COOH**) in extract phase:

TABULAR COLUMN:

Burette reading	**I**	**II**
Initial burette reading		
Final burette reading		
Volume of NaOH run down		

$$(N_1V_1)\text{Extract} = (N_2V_2)\ \text{NaOH}$$

$$(N_1)\ \text{Extract} = \frac{(N_2V_2)\ \text{NaOH}}{(V_1)\ \text{Extract}}$$

Where N_2 = Normality of **NaOH.**
V_1= Volume of **aqueous layer** pipetted out = 10 ml**.**
V_2= Volume of **NaOH** run down**.**

Burette: **NaOH** solution.
Conical flask: 10 ml of Extract + phenolphthalein indicator (2-3) drops.
Indicator: phenolphthalein indicator.
End point: colorless to pale pink.
Amount of Acetic acid present in the Extract phase =

$$\frac{N_1 * \text{Molecular weight of Acetic acid} * \text{volume of Extract collected}}{1000}$$

Concentration in Extract phase =

$$Y' = \frac{C}{B+C}$$

Where C = Amount of Acetic acid in Extract phase.
B + C = Amount of Extract (aqueous layer).

Feed solution = 50 ml.

Weight of feed solution = Volume * Density
= 50 * 0.879
= 43.95 gm.
A $(1+X_F)$ = 43.95 gm.

$$A = \frac{43.95}{1 + X_F}$$

$$X_F = \frac{\text{Amount of Acetic acid taken} * \text{Density of Acetic acid}}{\text{Volume of benzene taken} * \text{Density of benzene}}$$

$$= \frac{4 * 1.049}{496 * 0.879}$$

$$A = \frac{43.95}{1+}$$

Weight of Acetic acid in feed = 43.95-A.

%Extraction = $\frac{\text{Amount of Acetic acid present in Extract phase}}{} * 100$

Amount of Acetic acid in feed

Results:

Sl. No.	SAMPLE	PERCENTAGE RECOVERY
1.	50 ml Feed + 20 ml Distilled water	
2.	50 ml Feed + 40 ml Distilled water	

Applications:

Solvent extraction is used -

1. In nuclear reprocessing and ore processing.
2. In the production of fine organic compounds.
3. In the processing of perfumes.

Remarks:

Signature of Staff Incharge with date:

Probable viva questions:

1. What is solvent, solute and solution in the given experiment?
2. What is raffinate and extract?
3. Write a formula to find the percentage of extraction.
4. Write a Material balance for liquid – liquid extraction.
5. Write the application of liquid – liquid extraction in Biotechnology.

Experiment No: _____ Date: _____

ESTIMATION OF ETHANOL FROM FERMENTED BROTH

Aim:

To estimate the amount of ethanol present in an unknown (given) sample calorimetrically.

Apparatus/Components required:

Standard Alcohol solution (0.2 to 1.0 %), Potassium dichromate, Cone H_2S04, Distilled water, Test tubes, volumetric flask, Beakers.

Theory:

Potassium dichromate is a powerful oxidizing agent. $K_2Cr_2O_7$ is reduced by organic substance forming green solution containing chromium salt with alcohol in pressure of cone. H_2SO_4 it gives green colored chromic sulphate and acetaldehyde. The green color is calorimetrically read at 600nm.

$K_2Cr_207+4\ H_2SO_4$—*$K_2\ SO_4$+ $Cr_2\ (S0_4)_3$ + 4 H_20+3[0] Chromic salt

C_2H_5OH+ [O]--------►$CH3CHO + H_20$ Acetaldehyde

Procedure:

1. Blank Preparation: 10ml of distilled water and 10ml of $K_2Cr_2O_7$ served as the reagent blank (taken in test tube).
2. 10ml of std. alcohol solution of cone. 0.2, 0.4 ..... 1.0% were taken in the test tubes, numbered as 2 to 6. To each of the test tube 10ml of potassium dichromate reagent was added.
3. To the given sample (10 ml), 10ml potassium dichromate reagent was added. The test tubes were air sealed with foil. All the tubes were incubated at 60°C for 20min.The absorbance of the content in each of the tube was read calorimetrically at 600nm against reagent blank.

Reagents Preparation:

1. **Potassium dichromate solution:**
   Solution A. 34 gm of potassium dichromate was dissolved in 400 ml of distilled water.
   Solution B. 325 ml of cone sulphuric acid was added slowly with constant stirring to 200 ml of distilled water and cooled. Solution A and B were mixed and the volume was made up to 1000 ml with distilled water.
2. **Std. alcohol solutions:** Std. alcohol solutions of concentration 0.2 to 1.0 % were prepared using absolute alcohol.

Results:

The yeast cells were disrupted using a homogenizer; proteins were precipitated out and estimated. It was found that amount of total protein increased with increase in the homogenization time.

Applications:

Remarks:

Signature of Staff Incharge with date:

Probable viva questions:

1. What are the methods for production of ethanol? Name any microorganism responsible for it.
2. What are the methods of estimation of ethanol? Explain any one.
3. Potassium dichromate is a powerful oxidizing agent. How?
4. What is the unit of specific gravity?
5. Briefly explain the principle of specific gravity method for the estimation of ethanol.

Experiment No: _____ Date: _____

VACUUM LEAF FILTER

Aim:

To study the filtration characteristics of a given leaf filter and to determine specific cake resistance and filter medium resistance.

Apparatus/Components required:

Plate and frame filter press, watch glass, balance, weight box, and stopwatch.

Theory:

FILTRATION:

Filtration is the removal of solid particles from a fluid by passing the fluid through a filtering medium or septum on which the solids are deposited.

Filtration is the separation of solids from liquids when the amount of solids is relatively small as compared to the liquid.

Filters are of 3 types:

i. Those which operate with pressure above atmosphere on upstream side of filter medium.
ii. Those which operate with atmospheric pressure on the upstream and
iii. Vacuum on the down stream side.

Pressure above the atmosphere may be developed by the force of gravity acting on a column of liquid. By a pump, or blower or by centrifugal force. Most individual filters are pressure filters, vacuum filters or centrifugal separators. They are also either continuous or discontinuous depending on the discharge of filtered solids is steady or intermitted.

During filtration operation a bed of solids is formed and the filtrate passes under laminar flow conditions.

The Hagen-Poisellue equation for laminar flow of fluid is given by

$$\Delta P = \frac{32\mu VL}{De^2} \qquad \text{_____ (1)}$$

Where V = velocity of the fluid
L = Thickness of the bed
μ = viscosity of the fluid
ΔP = Pressure drop for the flow.
De = equivalent dia of the flow path.

The equation (1) can be rearranged and written as

$$\overline{V} = \frac{\Delta P\, De^2}{32\mu L}$$

Also we have

$$V = \frac{1}{A'}\frac{du}{d\theta}$$

Where A' = area of the voids.
V = volume of filtrate collected.
θ = time taken to collect V.

$$\frac{du}{d\theta} = V A' = \frac{\Delta P\, De^2}{32\mu L} . A' \qquad \text{_____ (2)}$$

The equivalent dia De is defined as

De = Δ * hydraulic radius

$$= \frac{\Delta * \text{cross sectional area of flow}}{\text{Wetted perimeter}}$$

Multiply by the thickness of the bed

$$De = \frac{\Delta * \text{cross sectional area of flow} * L}{\text{Wetted perimeter} \quad L}$$

$$= \frac{\Delta * \text{void volume}}{\text{Total surface area of the particles for flow}}$$

We select a unit volume of packed bed then the volume of passage will be the porosity 'Є' and the area of the passage will be So(1- Є)
Where So = the surface area per unit volume of bed.

Then $P = \frac{4\,Є}{So\,(1- Є)}$ _____ (3)

Where Є = void fraction i.e. $\frac{\text{volume of void}}{\text{Total volume}}$

So = specific surface area i.e. surface area per unit volume.

Substituting (3) in (2) we get

$$\frac{du}{d\theta} = \frac{\Delta P}{\mu L} * \frac{A\ 16\ Є^3}{So^2 (1- Є)^2 * 32} = \frac{\Delta P\ A\ Є^3}{2\ \mu L\ So^2 (1- Є)^2} \qquad \text{_____ (5)}$$

This is kozney equation. The above equation does not take into account the fact that the depth of granular bed is less than the actual length of the path traversed by the fluid, since actual flow path is not straight through the bed. Hence the constant '2' is represented by 'K' by 'Carman'.

$$\frac{du}{d\theta} = \frac{\Delta P\ A\ Є^3}{K\mu L\ So^2 (1- Є)^2} \qquad \text{_____ (6)}$$

the above equation cannot be used for constant pressure filtration directly because the thickness of the cake goes on increasing as the time passes and thus resistance offered by the cake goes on increasing. The above equation is applicable only at a particular instant of time. To use the equation for the entire operation it is modified by replacing the values of 'L' by volume of filtrate and other parameter.

$$\frac{du}{d\theta} = \frac{\Delta P\ A\ Є^3}{K\mu L\ So^2 (1- Є)^2}$$

CONSTANT PRESSURE FILTRATION:

Expressing the thickness of the cake in terms of volume of filtrate, writing a material balance at any time when the cake thickness is 'L'

Weight of solids in the cake = $LA(1-\epsilon)\rho s$.

Where ρs = the density of the solid particles. A = area of the cake.

Weight of solids in slurry feed = $(V+LA\epsilon)\ \rho\ \frac{w}{1-w}$

Where V = volume of filtrate discharged.

ρ = weight fraction of solids in slurry.

Equating the above 2 equations

$$LA(1-\epsilon)\rho s = (V+LA\epsilon)\ \rho\ \frac{w}{1-w}$$

ϵLA = volume of filtration in the cake.

$$LA(1-\epsilon)\rho s = (V\rho + \rho LA\epsilon)\ \frac{w}{1-w}$$

$$LA(1-\epsilon)\rho s(1-w) = V\rho w + w\rho LA\epsilon$$

$$LA(1-\epsilon)\rho s(1-w) - w\rho LA\epsilon = V\rho w$$

$$L[A(1-\epsilon)\rho s(1-w) - w\rho A\epsilon] = V\rho w$$

$$L = \frac{V\rho w}{A[(1-\epsilon)\rho s(1-w) - w\rho\epsilon]}$$

Since the value of 'L' in (6)

$$\frac{du}{d\theta} = \frac{\epsilon^3}{K\,So^2(1-\epsilon)^2} * \frac{A^2[(1-\epsilon)\rho s(1-w) - w\rho\epsilon]\Delta P}{V\rho\mu w} \quad \text{(7)}$$

Where ΔP = pressure difference across the cake.

An alternate form of the equation which is used is

$$\frac{du}{d\theta} = \frac{A^2(1-mw)\Delta P}{V\rho\mu w\alpha} \quad \text{(8)}$$

Where m = ratio of wet cake to dry cake

α = specific resistance of cake.

CORRECTION FOR FILTER CLOTH RESISTANCE:

Equation (7) and (8) are usually modified since the pressure difference across the cake cannot be measured directly. Only the difference between the pressure at the inlet to the filter and the pressure at the filtrate discharge can be measured. In such a case the resistance of filtrate medium must be added to that of the filter cake to obtain the total resistance which may be treated as being equivalent to that offered by a fiatitious weight of cake of thickness 'L_f'. Denoting the volume of filtrate corresponding to this fiatious weight of cake by V_f then

$$L + L_f = \frac{(V + V_f)\rho_w}{A\,[(1-\epsilon)\,\rho_s(1-w) - w\rho\epsilon]}$$

In the cake filtration the liquid passes through the resistances in series. The cake resistance is zero at the start and increases with time as filtration proceeds, the overall pressure drop at anytime is the sum of the pressure drop over the medium and cake. If Pa is the inlet pressure, Pb is the outlet pressure and P' is the pressure at the boundary between the cake and medium then

$$\Delta P = P_a - P_b = (P_a - P') + (P' - P_b)$$
$$= \Delta P_c + \Delta P_m \qquad \text{_____ (1)}$$

Where ΔP = total pressure drop.
ΔP_c = pressure drop over the cake
ΔP_m = pressure drop across the medium.

The figure shows diagrammatically a section through a filter cake and filter medium at a definite time't' from the start of the flow of filtrate. At this time the thickness of the cake measured from the filter medium is Lc, the filter area measured perpendicular to the direction of flow is 'A'. consider a thin layer of cake of thickness dL lying at a distance 'L' from the medium. Let pressure at this point be 'P'. This layer consists of a thin bed of solid particles through which the filtrate is flowing. In this filter bed the velocity is sufficiently low to ensure 'laminar flow'. As a starting point for treating the pressure drop through the cake we can write Kozney Carman equation noting that $\frac{\Delta P}{}$ = $\frac{dP}{}$ and the velocity of filter is 'u'.

$$\frac{dP}{dL} = \frac{150\mu u(1-\epsilon)^2}{\Phi s^2 Dp^2 \epsilon^3} \qquad \text{_____ (2)}$$

the pressure drop is expressed as a function of specific volume ratio instead of the particle size.

Substituting WKT $\Phi s = \frac{6Vp}{SpDp}$

Or $\Phi s Dp = \frac{6Vp}{Sp}$

$$\frac{dP}{dL} = \frac{150\mu u(1-\epsilon)^2}{(6Vp/Sp)^2\epsilon^3}$$

$$= \frac{4.17\mu u(1-\epsilon)^2(Sp/Vp)^2}{\epsilon^3} \qquad \text{_____ (3)}$$

Where $\frac{dP}{dL}$ = pressure gradient at thickness 'L'.
μ = viscosity of filtrate
Vp = volume of single particle.
ϵ = porosity of the cake.

The linear velocity is given by $Vp = \frac{dV/dt}{A}$ _____ (4)

Where V = volume of filtrate collected in time 't'. Since the filtrate must pass through the entire cake, V/A is the same for all layers and V is independent of L. the volume of solids on the layer under consideration is $A(1 - \epsilon)\, dL$ and if Sp is the density of the particles, then the mass of solids in this layer is $dm = Adc(1 - \epsilon)Sp$

$dm = Ade(1 - \epsilon)Sp$ or $dc = dm/ A(1 - \epsilon)Sp$ _____ (5)

Eliminating de from (3) and (5) gives

$$dp = \frac{K1\, \mu u(1 - \epsilon)^2 (Sp/Vp)^2}{\epsilon^3} \cdot \frac{dm}{\mathbf{A(1 - \epsilon)Sp}}$$

$$dp = \frac{4.17 \mu u(1 - \epsilon)(Sp/Vp)^2\, dm}{\epsilon^3}$$ _____ (6)

Where K is replaced by 4.17.

COMPRESSIBLE AND INCOMPRESSIBLE FILTER CAKE:

In the filtration under low pressure drop of slurries continuing rigid uniform particles are the factors on the right hand side of the equation (6) except 'm' are independent of 'L' and the equation is integrable directly over the thickness of the cake. If ne is the mass of total solids in the cake

$$\int_{P'}^{Pa} dp = \frac{K_1 \mu u(1 - \epsilon)(Sp/Vp)^2}{\epsilon^3} \int_{0}^{mc} dm$$

$$Pa - P' = \Delta Pc = \frac{K_1 \mu u(1 - \epsilon)(Sp/Vp)^2 mc}{\epsilon^3}$$ _____ (7)

Filter cake of this type are called incompressible. For the use of equation (7) a specific cake resistance 'α' is defined as

$$\alpha = \frac{\Delta PcA}{\mu u mc}$$ _____ (8)

By comparing (7) and (8)

$$\alpha = \frac{K_1(1 - \epsilon)(Sp/Vp)^2}{\epsilon^3}$$ _____ (8a)

The cake resistance 'α' may also be expressed in terms of particle dia Dp with a new co-efficient K2.

$$\alpha = \frac{K_2(1 - \epsilon)}{(\Phi s Dp)^2 \epsilon^3 Sp}$$ _____ (8b)

For incompressible cake 'α' is independent of pressure drop and position of the cake.

From definition 'α' from equation (8)

" α is the resistance of the cake that gives a unit pressure drop when μu and mc/A are equal to one". A filter medium resistance 'Rm' can be defined by analogy with cake resistance αmc. The equation is
A

$$Rm = \frac{\Delta Pm}{\mu u} \qquad \text{_____ (9)}$$

The dimension of Rm is L^{-1}

COMPRESSIBLE FILTER CAKE:

In compressible filter cake, the factors Є, K2 and Sp/Vp vary from layer to layer, because of usual slurry in a mixture of agglomerates or flocs, consisting of loose assemblies of very small particles and the resistance of the cake depends on the properties of flow rather than on the geometry of the individual particles. The flocs are deposited from the slurry on the upstream face of the cake and form the complicated network of channels to which the equation (6) does not apply. Therefore 'α' varies with the distance from the septum, since the cake near the septum is subjected to the greatest compressive force and has the lowest void fraction. This makes the pressure gradient non linear. The local value of 'α' may also vary with time. In consequence equation (7) does not strictly apply. In practice this variation in 'α' with the time and location are ignored. An average value of 'α' is obtained experimentally for the materials to be filtered using equation (8) for the calculation.

FILTER MEDIUM RESISTANCE:

By definition of filter medium resistance the equation is $Rm = \frac{P' - Pb}{\mu u} = \frac{\Delta Pm}{\mu u}$

The Rm may vary with the pressure drop. Since the higher liquid caused by a large pressure drop may force additional particles of solid into the filter medium but since it is important only during the early stages of fitration, it is nearly always satisfactorly to assume that it is constant, During any given filtration. When 'Rm' is treated as an empirical constant, it also includes any resistance to flow that may exist in the pipes leading to and from the filter.
From equation (8) and (9)

$$\Delta P = \Delta Pc + \Delta Pm = \frac{\alpha \mu u mc}{A} + Rm\mu u$$

$$= \mu u\left(\frac{mc\alpha}{A} + Rm\right) \qquad \text{_____ (10)}$$

Strictly, the cake resistance 'α' is a function of ΔPc rather than of ΔP. During the important stage of filtration, when the cake is of appreciable thickness, ΔPm is small in comparison with ΔPc and the effect on the magnitude of carrying the intrgration of equation (7) over a range of ΔP instead of ΔPc can be safely ignored. In equation (10) then 'α' is taken as a function of ΔP.

In using equation (10) it is convenient to replace 'u', the linear velocity of the filtrate and mc, the total mass of the solid in the cake, by a function of 'V' the total volume of filtrate collected at time 't'.

Equation (4) relates 'u'⟶'V' and a material balance relates mc and V. if 'C' is the mass of the particles deposited on the filter per unit volume of filtrate, the mass of solids in the filter at any time 't' is 'V*L' and $m_c = Vc$ _____ (11)

Substituting 'u' from equation (4) and mc from equation (11) in equation (10)

$$\Delta P = \mu u\left(\frac{Vc\,\alpha}{A} + Rm\right) = \mu \frac{du}{dt}\left(\frac{Vc\,\alpha}{A} + Rm\right)$$

$$\frac{dt}{du} = \frac{\mu}{A\,\Delta P}\left(\frac{Vc\,\alpha}{A} + Rm\right) \qquad \text{_____ (12)}$$

CONSTANT PRESSURE FILTRATION:

When ΔP is constant the only variable in equation (12) are 'V' and't'. when t=0, v=0 and ΔP = ΔPm hence

$$\left(\frac{dt}{du}\right) = \frac{1}{qo} = \frac{\mu Rm}{A\Delta P} \qquad \text{_____ (13)}$$

Equation (12) may be therefore written as

$$\left(\frac{dt}{du}\right) = \frac{1}{q} = KcV + \frac{1}{q} \qquad \text{_____ (14)}$$

$$\text{Where } Kc = \frac{\alpha\mu C}{A^2\Delta P} + \frac{1}{qo} = \frac{\mu Rm}{A\Delta P} \qquad \text{_____ (15)}$$

Integrating equation (15) between the limits (0,0) and (t,u) we get

$$\frac{t}{V} = \frac{KcV}{2} + \frac{1}{qo} \qquad \text{_____ (16)}$$

A plot of t/V vs. V will be linear, with a slope equation to Kc/2 and an intercept of 1/qo. From such plot using the value of intercept and slope in equation (12) and (14) the value of **α** and **Rm** may be calculated.

CHARACTERISTICS OF FILTER MEDIUM:

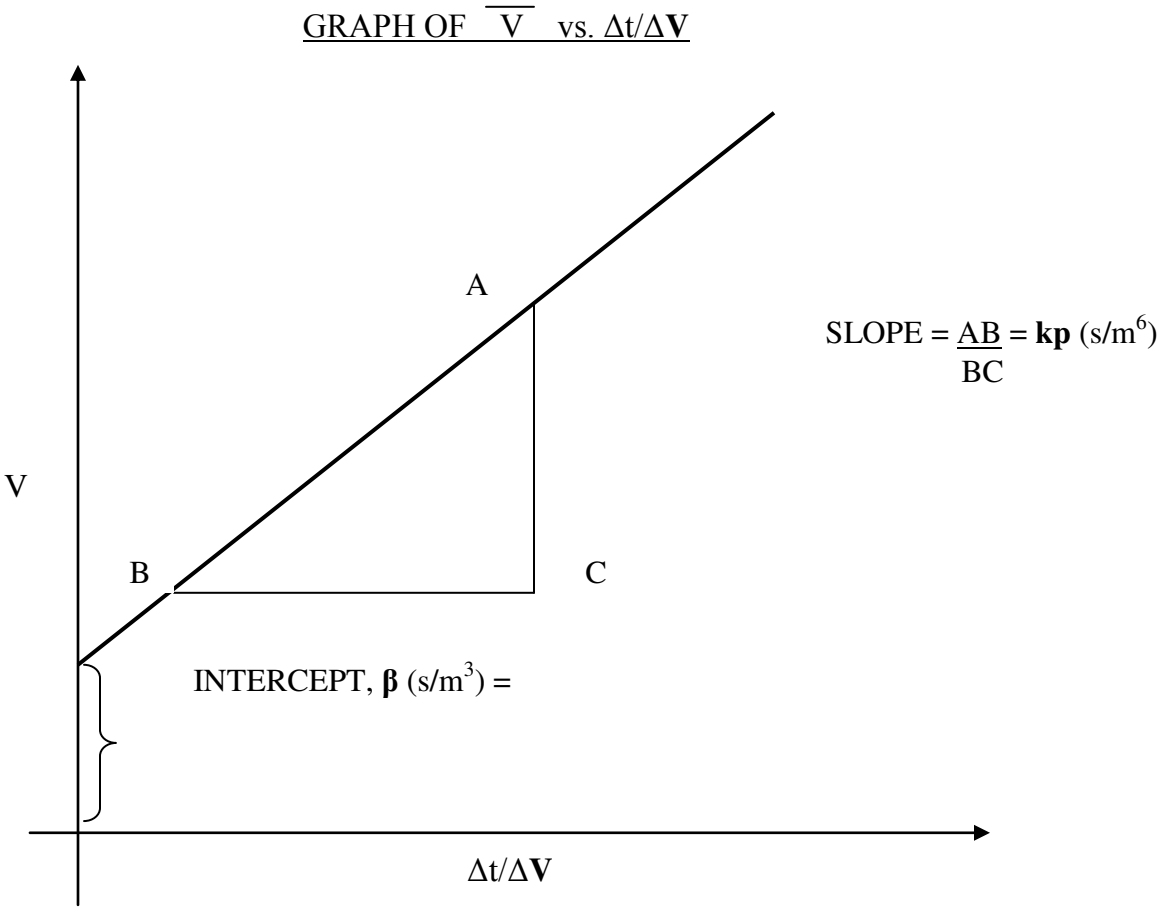
In case of cake filtration, the choice of a filter medium is often the most important consideration in assuring satisfactory operation of a filter. The filter medium in any filter must meet the following requirements:

1. It should retain the solids to be filtered, giving a reasonably clear filterate.
2. It should not plug or blind (low rate of entrapment of solids within its interstices).
3. It should be mechanically strong to withstand the process conditions.
4. It should be resistant to the corrosive action of fluid.
5. It should offer as little resistance as possible to the flow of the filtrate.

6. It should possess ability to discharge cake easily and cleanly.
7. It should have acceptable resistance to mechanical wear.
8. It should be cheap.
9. It should have long life.

In cake filtration, the filter medium is frequently a textile fabric. Canvas cloth, woolen cloth, metal cloth of monel or stainless steel, glass cloth and synthetic fibre cloth, nylon, polypropylene etc. are commonly used as filter media in industrial filtration practice depending upon the process conditions.
For alkaline slurry, nylon cloths are used while for an acidic slurry, polypropylene cloths are used as a filter medium.

Nature of graph:



Tabular Column:

SPECIFICATIONS:

1. Filter leaf = 12.3 cm.
2. Slurry $CaCO_3$ = 2.5% = 25g/lt.

3. Area of filter leaf, A = 118.82 cm^2.
4. Filtration tank dia = 5 cm.
5. Area of filtration tank, A' = 19.63 cm^2.
6. Manometric fluid = Mercury.

OBSERVATIONS:

1. Mercury level = _____ cm.
2. Weight of watch glass = _____g.
3. Weight of watch glass + wet cake = _____g.
4. Weight of watch glass + dry cake = _____g.
5. Mass of wet cake, m_f = _____g.
6. Mass of dry cake, m_c = _____g.
7. Diameter of the leaf = _____ m.
8. Initial concentration of $CaCO_3$ = 2.5% = 25g/lt.
9. Area of filter leaf, A = _____ m^2.
10. Filtration tank diameter = _____ m.
11. Area of filtration tank, A' = _____ m^2.
12. Density of filtrate (water) = _____ kg/ m^3.
13. Manometric fluid density (mercury) = _____ kg/ m^3.
14. Viscosity of filtrate = _____ kg/m.s.

FORMULAS:

1. Filtration volume, V = A'h cm^3

2. $\Delta P = \rho_m g h_m$ (N/m^2).

3. Average volume, $\overline{V} = \frac{V_1+V_2}{2}$ cm^3

4. $\Delta t = t_2 - t_1$ sec.

5. Specific cake resistance, $\alpha = \frac{kp\Delta PA^2}{C\mu}$ (m/kg)

6. Medium cake resistance, $Rm = \frac{\beta\Delta PA}{\mu}$, ($m^{-1}$).

7. $C = \frac{Cs}{1-(\frac{mf}{mc}-1)\frac{Cs}{\rho_w}}$, ($kg/m^3$).

TABULAR COLUMN 1:

SL. NO:	Height h (m)	Time (sec)	Δt (sec)	V = A'h (m^3)	Δ**V** (m^3)	$\overline{V} = \frac{V_1+V_2}{2}$ (m^3)	Δt/Δ**V**
1.							
2.							
3.							
4.							
5.							
6.							
7.							
8.							
9.							
10.							
11.							
12.							
13.							
14.							
15.							
16.							
17.							
18.							
19.							
20.							
21.							
22.							
23.							
24.							
25.							
26.							
27.							
28.							
29.							
30.							
31.							
32.							
33.							

TABULAR COLUMN 2:

Mercury level = _____ cm.

SL. NO:	h (m)	Time (sec)	Δt (sec)	V = A'h (m^3)	Δ**V** (m^3)	$\overline{V} = \frac{V_1+V_2}{2}$ (m^3)	Δt/Δ**V** (s/ m^3)
1.							
2.							
3.							
4.							
5.							
6.							
7.							
8.							
9.							
10.							
11.							
12.							
13.							
14.							
15.							
16.							
17.							
18.							
19.							
20.							
21.							
22.							
23.							

Results:

Sl. No.	**Mercury level**	**Specicfic cake resistance, α**	**Medium resistance, Rm**

Applications:

1. Optimization of individual process by
   i. Maximizing surface area of mass transfer.
   ii. Adjusting flow feeds

Remarks:

Signature of Staff Incharge with date:

Probable viva questions:

1. What is specific cake resistance?
2. What are the various filtration methods?
3. Give a brief introduction to filtration theory.
4. What are the characteristics of medium resistance?
5. What is filter medium resistance? Write a filter equation for filter medium resistance.

Experiment No: _____ Date: _____

CELL DISRUPTION TECHNIQUE: YEAST CELL DISRUPTION USING HOMOGENIZER

Aim:

To disrupt yeast cells by mechanical method using homogenize and estimation of total protein content by Biuret method.

Apparatus/Components required:

Centrifuge tubes, Commercial Baker's yeast, 0.1 M phosphate buffer, pH 6.9, Homogenizer, Cooling centrifuge, 20%TCA, Acetone , Biuret reagent, Standard protein solution (5mg/ml), 0.1 N NaOH, Test tubes, Measuring cylinder

Theory:

Various mechanical and non-mechanical methods are available for '.the disruption of microbial cells for product recovery of intracellular contents like enzymes, metabolic products and recombinant **DNA** products. Homogenizers have been found to be particularly effective to provide effective release of cellular constituents while maintaining a high degree of biological activity in the product.

A homogenizer is a piece of laboratory equipment used for the homogenization of various types of material, such as tissue, plant, food, soil, and many others. Many different models have been developed using various physical technologies for disruption. The mortar and pestle, already used for thousands of years, is a standard tool even in modem laboratories. More modem solutions are based on blender type of instruments (also known in the kitchen), bead mills, ultra sonic treatment, high pressure, and many other physical forces. Whereas older technologies just focused on the disruption of the material, newer technologies also address quality or environmental aspects, such as cross-contamination, aerosols, risk of infection, or noise.

Homogenization is a very common sample preparation step prior to the analysis of nucleic acids, proteins, cells, metabolism, pathogens, and many other targets. The degree of cell disruption and the release of the products depends on microorganism used, location of the product and the type of homogenizes.

Under constant conditions the rates of product release for bacteria and yeast can be described by first order kinetics. The concentration of the released product (Cr) will be proportional to the concentration of disrupted cells. The rate of release dCr /dt will be proportional to the concentration of unreleased product, Cr max - Cr, so

$$\frac{dCr}{dt} = K_h (Cr\ max - Cr) \qquad - (1)$$

Where K_h is the first order release constant for homogenizer, which depends on operating pressure and t being the homogenization time.

Integration of (l) gives,

$$\ln \frac{(Crmax)}{(Cr\ max - Cr)} = -K_h t \quad \text{- (2)}$$

$$\frac{Cr}{Crmax} = (1 - \exp(-K_h t) \quad \text{- (3)}$$

$$Cr = Cr\ max\ (1 - \exp(-K_h t) \quad \text{- (4)}$$

The release of intracellular enzymes is more complex compared to the release of proteins because the enzymes may be located at different regions within the cell. The rate of release of an enzyme to the total protein release is independent of disruption pressure, temperature and cell concentration.

Enzymes located outside the cell membrane are released at a rate faster than the total protein, and those soluble in the cytoplasm like glucose -6- phosphate dehydrogenate are released approximately at the same rate as total protein. Membrane bound enzymes such as fumarase are released more slowly than total protein and enzymes such as inverts and a- glycosidase, located in the periplasmic space are released faster than cytoplasm proteins. Thus the rate of release of the enzyme can be an indication of its location within the cell.

Procedure:

PREPARATION OF REAGENTS:

1. Biuret reagent::
Dissolve 3g. of copper sulphate and 9g of sodium potassium tartarate in 500ml of 0.2M sodium hydroxide; add 5g of potassium iodide and make up to 1 litre with 02M sodium hydroxide.

2. 0.1N NaOH:
Dissolve 0.4 grams of NaOH in 100ml of distilled water.

3. Standard protein: solution (5mg/ml):
Dissolve 0.5 grams of **BSA** in 20ml of distilled water. Transfer this solution to 100ml volumetric flask and! make up to the mark with distilled water.

4. 0.1 M phosphate buffer:
Solution 1: Prepare 200ml of 0.1M **NaH_2P0_4**.
Solution 2: Prepare 200ml of 0.1M **Na_2HP0_4**.
Mix 68.5ml of solution 1 with 31.5ml of solution 2 and check the pH of the solution using pH meter.

5. 20% TCA:
Dissolve 20grams of TCA in 50mi of distilled water. Transfer this solution to 100ml volumetric flask and make up to the mark with distilled water.

Procedure:

1. Preparation of homogenate:

1. Weighed two grams of commercial baker's yeast into 5 .different centrifuge tubes labeled 1min, 2min, 3min, 4min and 5 min.
2. Added 5ml of phosphate buffer to each of the centrifuge tube prior to homogeni2ation.
3. The yeast solution in the centrifuge rube was homogenized for different times as labeled using a homogenizer keeping the rpm constant.
4. The homogenized suspension was centrifuged at 12,000 rpm for 10 min at 4°C using a cooling centrifuge.
5. Supernatant was measured and was made-up to 5 ml using phosphate buffer.

2. Extraction of protein from the homogenate.

1. Added 5ml of 20% TCA to 2mi of each homogenate and ^incubated for 30 minutes on ice to precipitate out proteins.
2. Centrifuged at 12,000 rpm for 10 min at 4°C.
3. Discarded the supernatant and the protein pellet was washed .with 5ml of acetone.
4. Centrifuged at 12,000 rpm for 10 min at 4°C.
5. Discard the acetone and dissolved the pellet in lml.
6. The protein solutions obtained at different time interval were stored in refrigerator for estimating the total protein.

3. Preparation of Standard BSA carve.

1. Pipette out standard BSA solution in the range of 0.0 to 1.0ml to dT ssnt test tubes.
2. Made up the contents to 4.0ml using distilled water.
3. Added 6.0ml of Biuret reagent to all the test tubes followed by keeping al est tubes at room temperature for ten minutes.
4. O.D was read at 520 nm against the blank solution.
5. Standard graph was plotted using O.D v\s amount of protein in mg.

4. Estimation of total protein by Biuret method.

1. Pipette out 1ml of protein solutions obtained at different time interval to different test tubes and for the blank test tube 1ml of 0. 1N NaOH.
2. Added 3ml of distilled water to all test tubes followed by the addition of 6ml of Biuret reagent.
3. Incubated all the test tubes at room temperature for ten minutes.
4. O.D was read at 520nm against the blank.
5. Estimated the total protein present in 1ml each of extract (=2ml of each homogenate) in mg using standard graph.
6. Calculated the total protein present in 5ml each of homogenate.
7. Graph was obtained by plotting amount of total protein in mg Vs. homogenization time.

Results:

The yeast cells were disrupted using a homogenizer; proteins were precipitated out and estimated. It was found that amount of total protein increased with increase in the homogenization time.

Applications:

Remarks:

Signature of Staff Incharge with date:

Probable viva questions:

1. What is the principle behind the homogenizer?
2. What are the methods of cell disruption?
3. Briefly explain the enzymatic cell disruption.
4. What is the importance of cell disruption?
5. Briefly explain the extraction process of protein.

Experiment No: _____ Date: _____

THIN LAYER CHROMATOGRAPHY

Aim:

To separate mixture of amino acids using thin layer chromatography.

Apparatus/Components required:

Glass plate, Capillary tubes, Spraying unit, beakers, Chromatography chamber.

Theory:

PRINCIPLE: Thin Layer Chromatography is similar to paper chromatography in experimental methodology except that the support for the stationary phase is a glass plate or aluminum foil. The plate is coated with fine particles of an absorbent such as alumina or silica gel as a thin layer of about 0-2 mm thickness. The thin layer of absorbent is formed by coating slurry of the absorbent in water and allowing the water to evaporate by drying at room temperature or a higher temperature.
Drying at room temperature allows the absorbent to hold water. The experimental set up and the procedures are exactly the same for the paper chromatography. The solute components- get separated due to their different migration rates. The Separation mechanism is partition and /or adsorption depending on the amount of water immobilized[1] during drying. The R_f values of the individual solute components are determined as in paper chromatography. The RF spots may be detected by exposing TLC plate to iodine vapors, particularly when organic samples arc spotted.

THEORY:- In the TLC technique ,a thin layer of a finely divided substance *is* deposited on to a flat glass.he plate is dipped into the solvent in a glass jar and the development carried .out by the ascending technique. After the development, the lawyer can fee dried and the components detected by various methods.

TLC may be either carried out by the adsorption principle JOT by partition principle. Steps involved in **TLC** techniques are:-

1) **Preparation of the Layer** : The glass plate on which the thin layer prepared should be even and is thoroughly washed and dried before layer application . Modern thin layer chromatography kits provide plastic or foil plates in lieu of a glass plate. The materials of which the thin layer is made usually mixed with water in such a proportion that a thick suspension known .as stony results. The slurry is applied to the plate surface uniformly as a thin layer by the means of a spreader starting at one end of the plate and moving to an another end in an unbroken manner , The nature of desired chromatographic separation dictates the thickness of the slurry layer used. Thus for the analytical separations the thickness of the layer might be about 5mm. Although the thin layer technique can be used for many different types of chromatographic separations such as adsorption gel filtration, ion exchange etc. The list of the commonly used adsorbents are;- Kieselghar G, Silica Gel G etc.

2) **Sample Application:** The sample is applied to the paper as a small spot using a capillary tube. This is done before dipping the glass plate into the eluting solvent, the care should be taken not to scrape the thin layer while applying the sample.

3) **Plate development** :- The choice of solvents and the methods of elution are much the same as for paper chromatography. The procedure must of course be in a closed chamber to prevent the evaporation of the solvent. Two dimensional chromatography may also be carried out much in the same way as the of the paper chromatographv.

4) **Detection:** Generally used detection methods are ultraviolet adsorption, fluorescence autoradiography, if the components are radio labeled or production of colors by chemical treatment. Those specific for TLC are:-1) Spraying the plate with 25-50% sulphuric acid in ethanol and Seating. This results in charring of most of the compounds, which show up as "brown spots. ii) Iodine vapors, is used extensively as a universal reagent for organic compounds. The iodine spots disappear rapidly but can be made permanent by spraying with 0.5%, benzidine solution in absolute ethanol. Iodine vapor is been concentrated in the form of cloud over the region where the components have separated. These spots can thus be scraped out, eluted and analysed quantitatively.

Procedure:

1. Preparation of stationary phase: 5 gram of silica gel was dissolved in 20 ml of distilled water. ii covered a 20 X 20 cm: plate with 250µm Thick layer . Then before using the glass plate, it was coated with silica gel and kept in oven at 105 ℃ for activation for 30 minutes.
2. The line of origin was drawn at a distance of 1.5 cm from one of the edges of the plate.
3. Then the mixture of amino acids was spotted on TLC using a capillary tube with a gap of at least 2 cm between them.
4. Then the Spotted plate was placed in chromatography chamber saturated with solvent- for about 2-3 hours.
5. The solvent front was noted down after the development of the chromatogram
6. The plates were dried in hot air oven for 4-5 minutes at 100 °C.
7. Then the ninhydrin solution was developed to develop purple colour
8. The distance travelled by the samples was noted.
9. The R_f values for each component was calculated.

Preparation of reagents:

1. Solvent system: Butanol: Acetic Acid: Water (60:20:20)
2. Ninhydrin solution; 1% in acetone
3. Amino Acid solution 1% Glycine solution, 1% Arginine solution, 1% Glycine + 1% Arginine mixture (1:1)

Observation:

1. Distance travelled by the solvent front = _____ cm.
2. Distance travelled by Glycine = _____ cm.
3. Distance travelled by Arginine = _____ cm.
4. Distance travelled by Glycine in mixture = _____ cm.
5. Distance travelled by Arginine in mixture = _____ cm.

Retardation factor, R_f = Distance travelled by sample / Distance travelled by solvent front

1. R_f (Glycine) =
2. R_f (Arginine) =
3. R_f (Glycine in mixture) =
4. R_f (Arginine in mixture) =

Results:

From the spots observed in the paper, it is found corresponding to the sample ___ and the spot 2 is corresponding to ____.

SAMPLE	GLYCINE	ARGININE	IN MIXTURE GLYCINE + ARGININE
R_f			

Applications:

1. It is used as pilot technique to quickly determine the complexity of a mixture.
2. It may be used as an aid in order to find out the least conditions for large scale chromatography.
3. It is used to follow the course of reactions due to its speed and simplicity.
4. It is used to identify drugs, contaminants and adulterants.
5. It is widely used to resolve plant extracts and many other biochemical preparations.

Remarks:

Signature of Staff Incharge with date:

Probable viva questions:

1. What is partition co – efficient?
2. Briefly explain the distribution co – efficient?
3. What are the causes for capillary action in TLC?
4. Briefly explain the process of preparation of thin layer.
5. What are the detecting agents for TLC?

Experiment No: _____ Date: _____

PROTEIN PRECIPITATION USING AMMONIUM SULPHATE

Aim:

To fractionate the proteins by precipitating it using the ammonium sulphate powder.

Apparatus/Components required:

Centrifuge tubes, Commercial Baker's yeast, 0.1 M phosphate buffer, pH 6.9, Homogenizer, Cooling centrifuge, 20%TCA, Acetone , Biuret reagent, Standard protein solution (5mg/ml), 0.1 N NaOH, Test tubes, Measuring cylinder

Theory:

Ammonium sulfate is widely used &r precipitation and fractionation of proteins as well as for the crystallization of proteins and protein-nucleic acid complexes. Ammonium sulfate is also utilized in hydrophobic interaction chromatography and antibody purification. Ammonium sulfate acts by pulling water molecules away from the non-polar units of proteins. The decrease in available water molecules increases the surface tension and enhances hydrophobic interactions, thus allowing the protein to precipitate from a solution or bind to a hydrophobic column. The use of ammonium sulfate confers the following advantages: 1) High concentrations of ammonium sulfate inhibit microbial growth and maintain the protein in a folded state. 2) The low density of saturated solutions (1.25 g/cm") allows plating of proteins by centrifugation. 3.) A low heat of solubilization avoids the risk of protein denaturation that can occur when the sample temperature, increases. The saturation concentration of an ammonium sulfate solution is temperature dependent. Lower temperatures will decrease the concentration at which the solution is saturated. A saturated solution of ammonium sulfate is 4.1 M at.25°C, but is 3.8 M at 0 °C, the difference in molarity being 7%.

Accordingly, a saturation table based on the addition of crystalline ammonium sulfate varies with temperature. For this reason, an aliquot from a saturated solution at 25 °C may be stored at the temperature desired for the application. For the original concentration to be maintained with accuracy, the 'saturated solution at 25 °C should contain no insoluble ammonium sulfate crystals when aliquot.

Procedure:

For protein purification, often two precipitation steps are earned out on a given protein sample. The first step is performed at an ammonium sulfate concentration below that required to precipitate the protein(s) of interest. Accordingly the protein(s) of interest remain in the supernatant, while other proteins precipitate and are collected .in the pellet upon centrifugation. The second step is performed at an ammonium sulfate concentration high enough to precipitate or pellet the protein(s) of interest. Additional proteins may remain in the supernatant. Two ammonium sulfate precipitation- steps are performed, involving the addition of the saturated

ammonium sulfate solution to raise the percent saturation of the sample solution initially from 0% to,35% and then in the second step, from 35% to 70%.

1. Prepare a protein sample at a concentration of at least 1 mg/ml in a buffered solution (>50. mM buffer). Cool the protein sample and the saturated ammonium sulfate solution to the desired working temperature. The ammonium sulfate solution typically has a pH in the range of 5 to 6, so a slight pH shift may occur if the protein sample is not adequately buffered.
2. Determine the-volume of the saturated ammonium sulfate solution required to give the desired ammonium sulfate concentration (percent saturation) in the protein sample using Table of saturation. For this example the protein sample has a. starting volume of 100 ml and an ammonium sulfate concentration of 0%. The first precipitation step will be performed at 35% saturation with, ammonium sulfate. Using the left side of Table of saturation, locate the row corresponding to initial ammonium sulfate concentration (0%). Follow this row across the table to the column corresponding to the final ammonium sulfate concentration (35% saturation). Factor corresponding to raising the ammonium sulfate concentration from 0 to 35% saturation is 0.538. Multiply the initial sample volume (100 ml) by the multiplication factor (0.538) to calculate the volume of the saturated ammonium sulfate solution required (53.8 ml).
3. Slowly add the calculated volume (53.8 ml) of the saturated ammonium sulfate solution to 100 ml of the protein sample with gentle stirring.
4. Allow the solution to incubate at the desired temperature for a minimum of 20 minutes. Some proteins may require a longer period of time to precipitate, so the optimal length of time for precipitation should be determined empirically for each sample.
5. At the desired temperature, centrifuge the sample at a minimum of 1,000 x ***g*** for at least 5 minutes. Repeat the centrifugation step as needed to ensure the complete precipitation of protein. Carefully decant or pipette the supernatant from the pellet.
6. For this example the protein of interest remains in the supernatant from step 5 (35% saturation) and a second precipitation step at 70% saturation is required to pellet the protein of interest. Repeat the calculations detailed in step 2 to determine the volume of saturated ammonium sulfate solution required to bring the ammonium sulfate concentration to 70% saturation. Locate the row corresponding to ammonium sulfate concentration, now 35% saturation, from Table of saturation and move across the table to the column corresponding to the desired final ammonium sulfate concentration (70% saturation). The multiplication factor corresponding to raising the ammonium sulfate concentration from 35 to 70% saturation is 1.167. *Multiply* the current sample volume (153.8 ml) by the multiplication factor (1.167) to calculate the volume of the saturated ammonium sulfate solution required (179.5 ml).
7. Slowly add the calculated volume (179.5 ml) of the saturated ammonium sulfate solution to the 153.8 ml of protein sample (supernatant from step 5) with gentle stirring.
8. Allow the solution to incubate at the desired temperature for a minimum of 20 minutes. Some proteins may require a longer period of time to precipitate, so the optimal time for precipitation should be determined empirically for each sample.
9. At the desired temperature, centrifuge the sample at a minimum of 1,000 x ***g*** for at least 5 minutes. Repeat the centrifugation step as needed to ensure the complete precipitation of protein. Carefully decant or pipette me supernatant from the pellet.
10. Dissolve the protein pellet in the desired buffer and store at the appropriate temperature

Results:

The protein was analyzed in UV-VIS spectrophotometer at 280 nm precipitated at different saturation levels of ammonium sulphate powder .

Applications:

Remarks:

Signature of Staff Incharge with date:

Probable viva questions:

1. What are the methods of protein precipitation?
2. Briefly explain organic solvent and salting – out and salting – in process in protein precipitation.
3. What is electrophoric precipitation?
4. Briefly explain the action of Ammonium sulphate in protein precipitation.
5. What are the applications of protein precipitation?

Reference:

1. Protein Purification by Scopes R.K., IRL Press.
2. Rate controlled separations by Wankat P.C., Elsevier.
3. Bioseparations by Belter P.A. and Cussier E., Wiley.
4. Product Recovery in Bioprocess Technology - BIOTOL Series, VCH.
5. Separation processes in Biotechnology by Asenjo J. and Dekker M.
6. BIOSEPARATION S: SCIENCE & ENGINEERING by Roger G Harrison, Paul Todd, Scott R Rudge, Demetri P Petrides, Oxford University Press.

Dayananda Sagar College of Engineering
Department of Biotechnology
Bengaluru-560078

DOWNSTREAM PROCESSING LABORATORY (10BTL78)

PROBABLE/SUGGESTED QUESTION BANK

1. Write a equation for concentration of released product to the concentration of disrupted cells.
2. Write the rate of cell disruption.
3. What is principle behind vacuum leaf filter?
4. Write a equation for specific cake resistance & Medium cake resistance.
5. What are the downstream process steps in ethanol production?
6. What are actions of invertase and zymase enzyme in ethanol production?
7. Explain the principle behind liquid – liquid extraction.
8. Write the phase diagram for liquid – liquid extraction.
9. What is chromatography?
10. What is the importance of Thin layer chromatography in Biotechnology?
11. How proteins will be separated in Thin layer chromatography?
12. What is the principle behind Thin layer chromatography?
13. How do you determine the protein concentration after protein precipitation?
14. Write a short note on Ammonium sulphate precipitation table.
15. Write a material balance on batch sedimentation of the particle.
16. Write the applications of batch sedimentation in Biotechnology.
17. Briefly explain the action of Ammonium sulphate in protein precipitation.
18. Briefly explain the process of preparation of thin layer.
19. What are the detecting agents for TLC?
20. What are the methods of cell disruption?
21. Briefly explain the enzymatic cell disruption.
22. Write a formula to find the percentage of extraction.
23. Write a Material balance for liquid – liquid extraction.
24. Write short notes on rate of sedimentation.
25. How to determine the thickener area?

DAYANANDA SAGAR COLLEGE OF ENGINEERING
DEPARTMENT OF BIOTECHNOLOGY, BENGALURU-560078

CONTINUAL EVALUATION FORMAT

DOWNSTREAM PROCESSING LABORATORY (10BTL78)

(Academic Year - 2016)

Semester /Section : **Batch :**

S No.	USN	student Name	Expt. No: 1			Expt. No:2			Expt. No:3			Expt. No:4		
			Date:			Date:			Date:			Date:		
			Viva (05)	Record (10)	Total (15)	viva (05)	Record (10)	Total (15)	Viva (05)	Record (10)	Total (15)	Viva (05)	Record (10)	Total (15)
Faculty Signature With Date														
Name of the Faculty Incharge			(1) (2) (3)											

Note:
(1) Viva questions to be asked w.r.t the current experiment of the particular week.
(2) The above same page format is used for next set of experiments i.e. 5, 6,….expts.
(3) Separate sheets must be used for different batches.

DAYANANDA SAGAR COLLEGE OF ENGINEERING
DEPARTMENT OF BIOTECHNOLOGY, BENGALURU-560078

FINAL IA MARKS FORMAT

DOWNSTREAM PROCESSING LABORATORY (10BTL78)

Year: 2016

Semester /Section : VII **Batch :**

SN	USN	Name Of The Student	Continual Evaluation Marks (15)	IA Test Marks (10)	Final Marks (25)	Signature of Student