

8/1/23

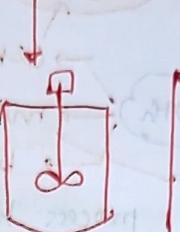
Bioprocess

buffer
C source
N source
trace elements

- ① Upstream → prep^n of medium, sterilization, grow microbes, strain selection, inoculum development, formulation of medium,
- ② Midstream
- ③ Downstream

isolate from soil, air, water
(pure culture)

or purchase



MTCC-
NCIM-

ISBD - freshwater microalgae

Upstream Processing

- ① Formulation of medium

- ② Sterilization of medium → autoclave (small reactors of 2-4 L)

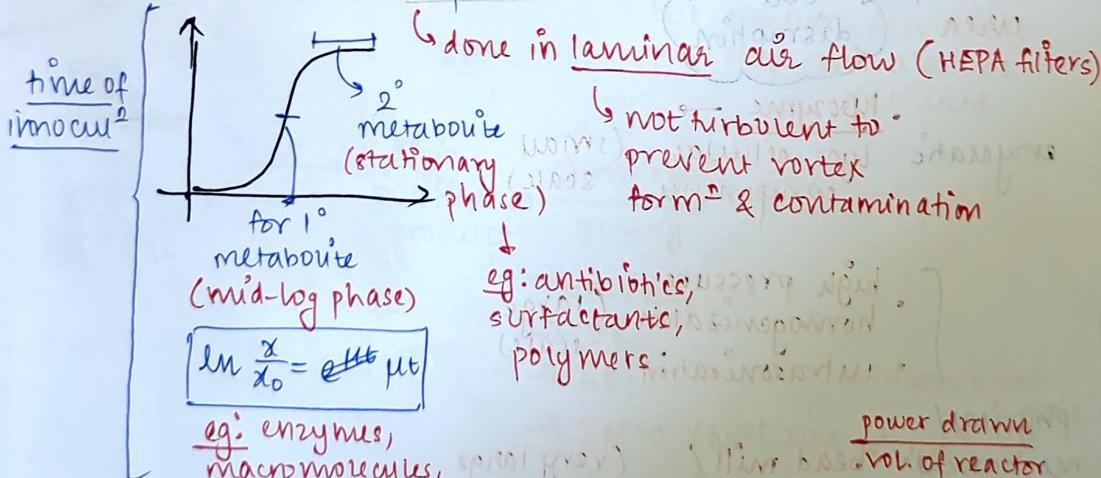
$$\ln \frac{N}{N_0} = kt$$

Design eqn. of sterilizer

microbes before steriliz. microbes after steriliz.

death rate const.

- ③ Inoculum development



Midstream

process scale-up → geometrical scaling

+ some const. factors

power drawn
vol. of reactor

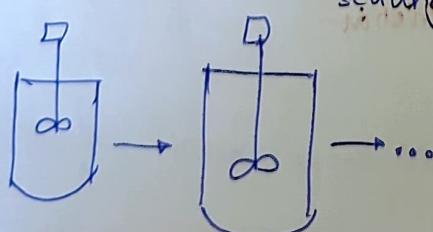
(most universal)

P/V

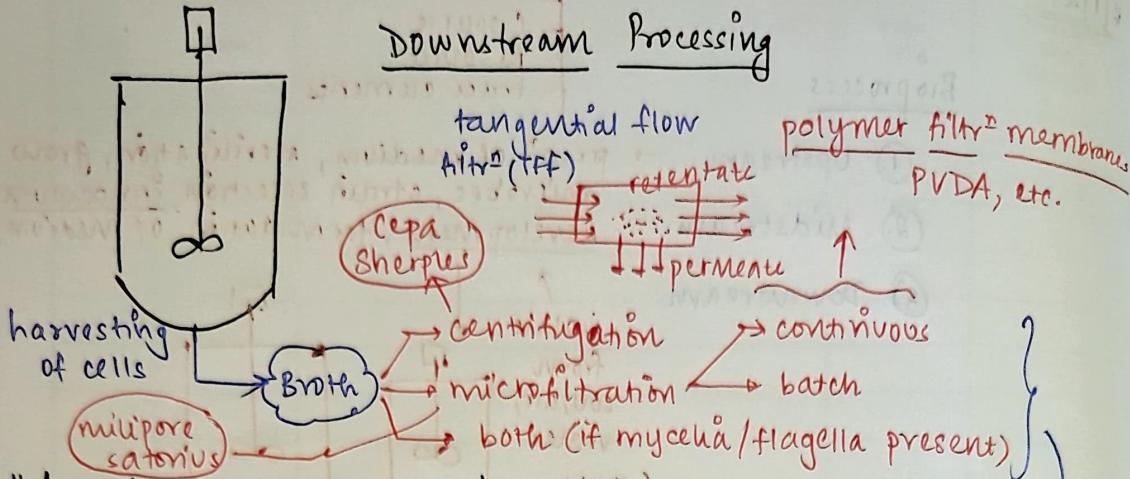
$k_{L,a}$
(vol. MT coeff.)

model fitting

- ✓ Monod
- ✓ Modified Monod



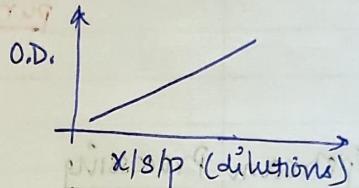
Downstream Processing



how do we know process has ended?

X, S, P ↓ product
 biomass ↓ substrate

} → standard curves



renaturing buffer

denaturing buffer if product is captured in inclusion bodies

if product is intracellular → Biomass & spent medium

if product is extracellular → (supernatant/filtrate)

dissolve TBS
Guanidinium, urea
cell disruption
lysosome → enzymatic lysis, cellular components (small scale)

- high pressure
- homogenization (large scale)
- ultrasonication

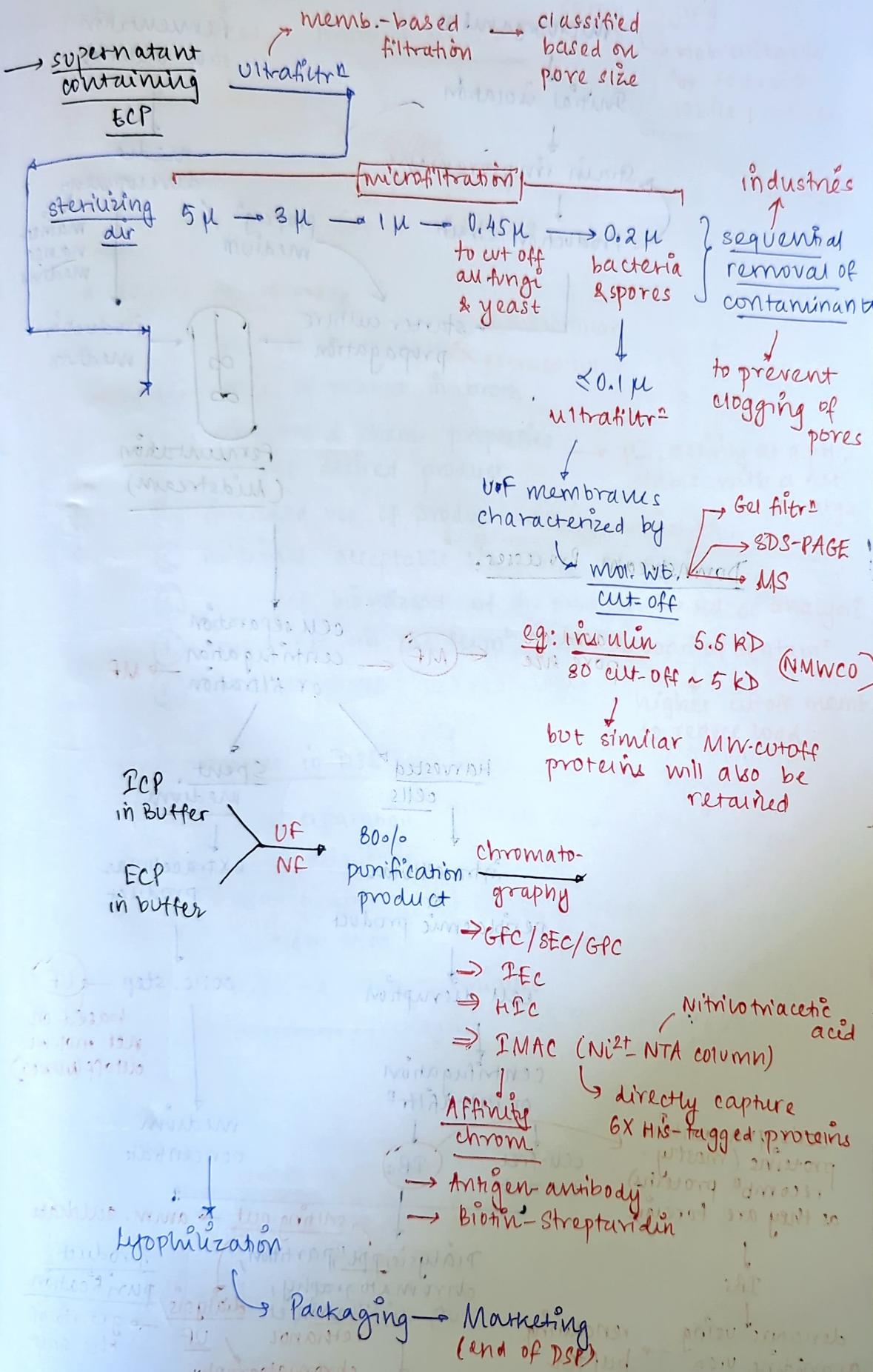
Physical/mechanical
bead mill / ball mill
Dynomil®

(very large scale)

cells are ruptured by attrition (or friction)

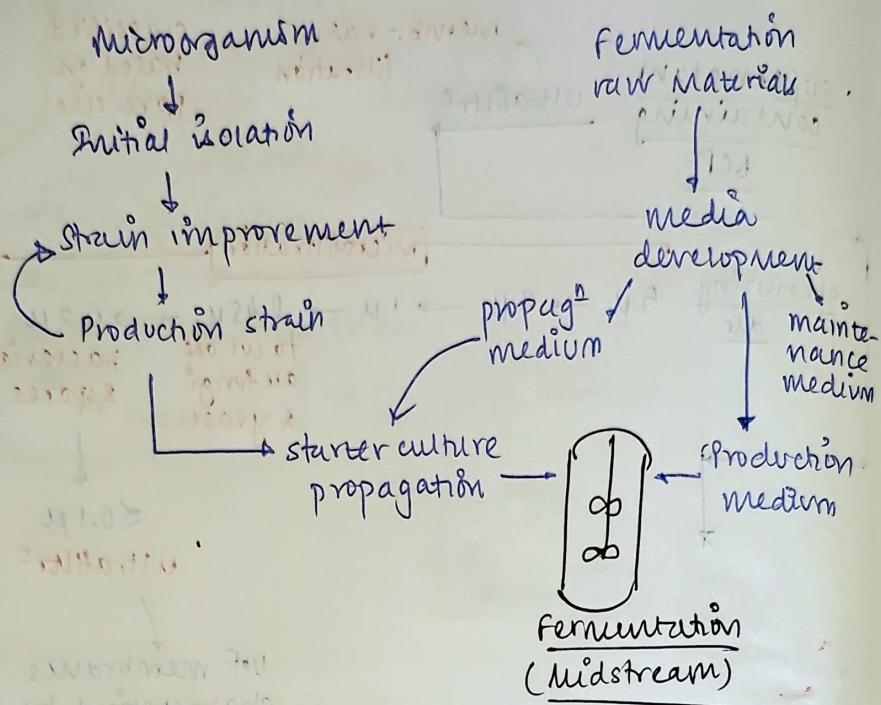
ICP in

→ Buffer → important to stabilize the product (protein) to a particular conformation

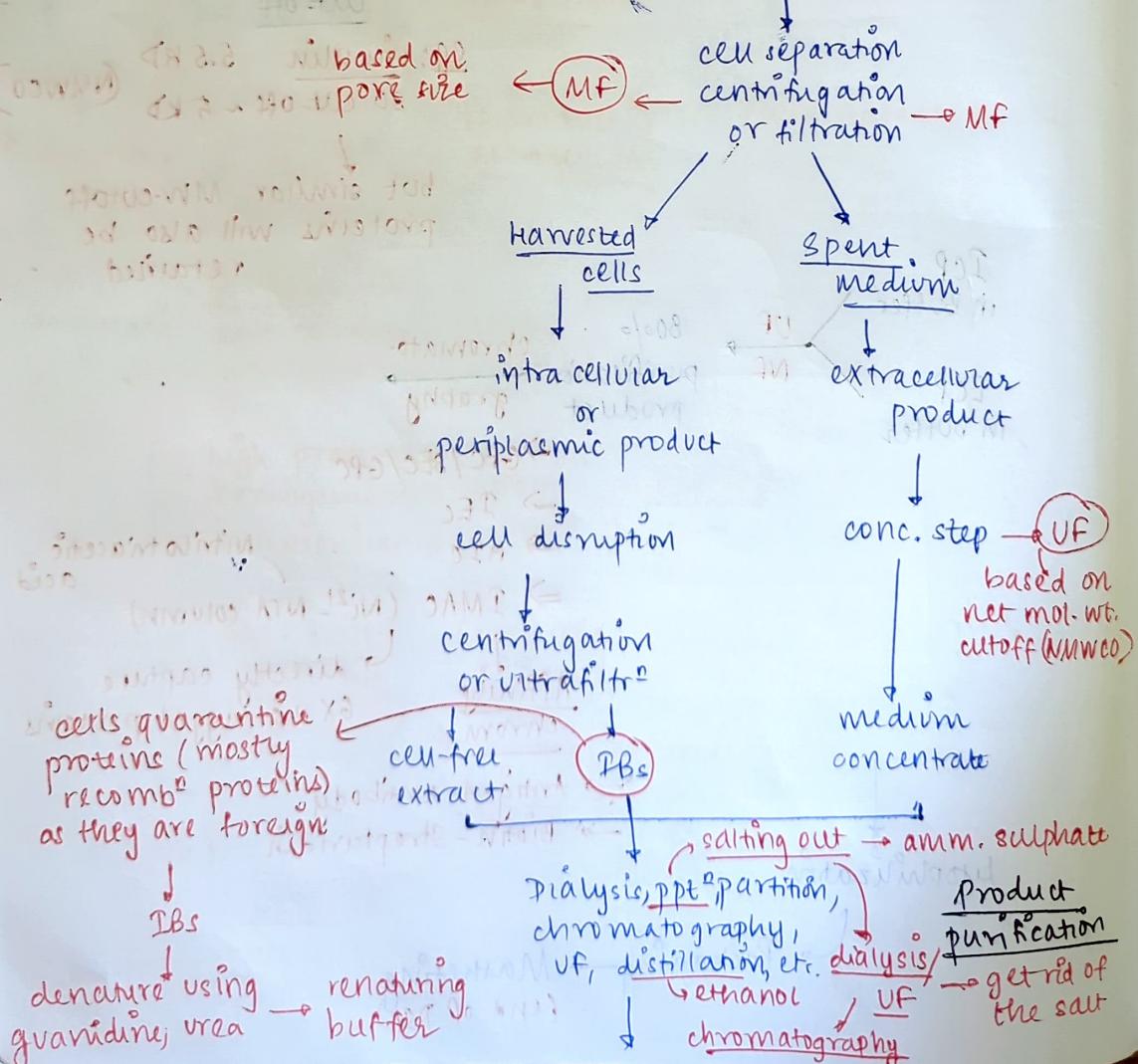


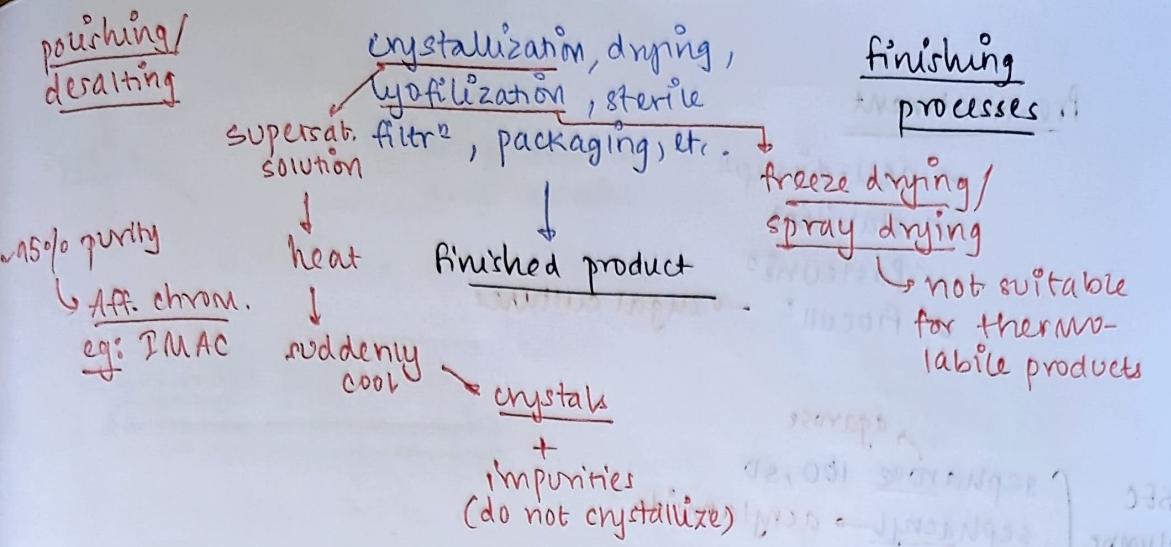
17/1/24

Upstream processes:



Downstream Processes:





Choice of recovery:

- ① location of product $\begin{cases} \text{intracellular} \\ \text{extracellular} \end{cases}$
 - ② conc. of product in broth
 - ③ physical & chem. properties of the desired product \rightarrow PI, activity at a pH, stable with a net charge
 - ④ intended use of product \rightarrow therapeutic, etc.
 - ⑤ minimal acceptable standard of purity
 - ⑥ magn. of biohazard of the product \rightarrow Hazoe analysis
 - ⑦ impurities in the fermentⁿ broth \rightarrow load of contamⁿ
 - ⑧ market prices
- ↓
Higher cutoff memb. to reduce load

Unit processes in DSP:

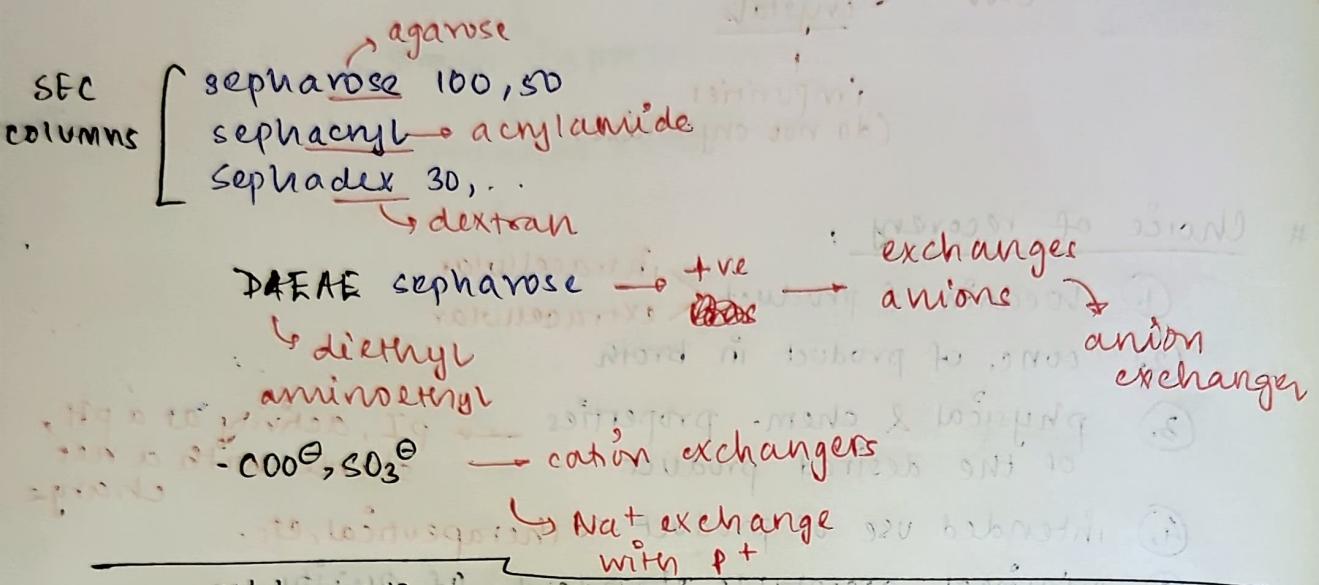
- ① cell separation
- ② cell disruption
- ③ clarification
- ④ concentration
- ⑤ high-resolution techniques
- ⑥ finishing / packaging

partition coefficient

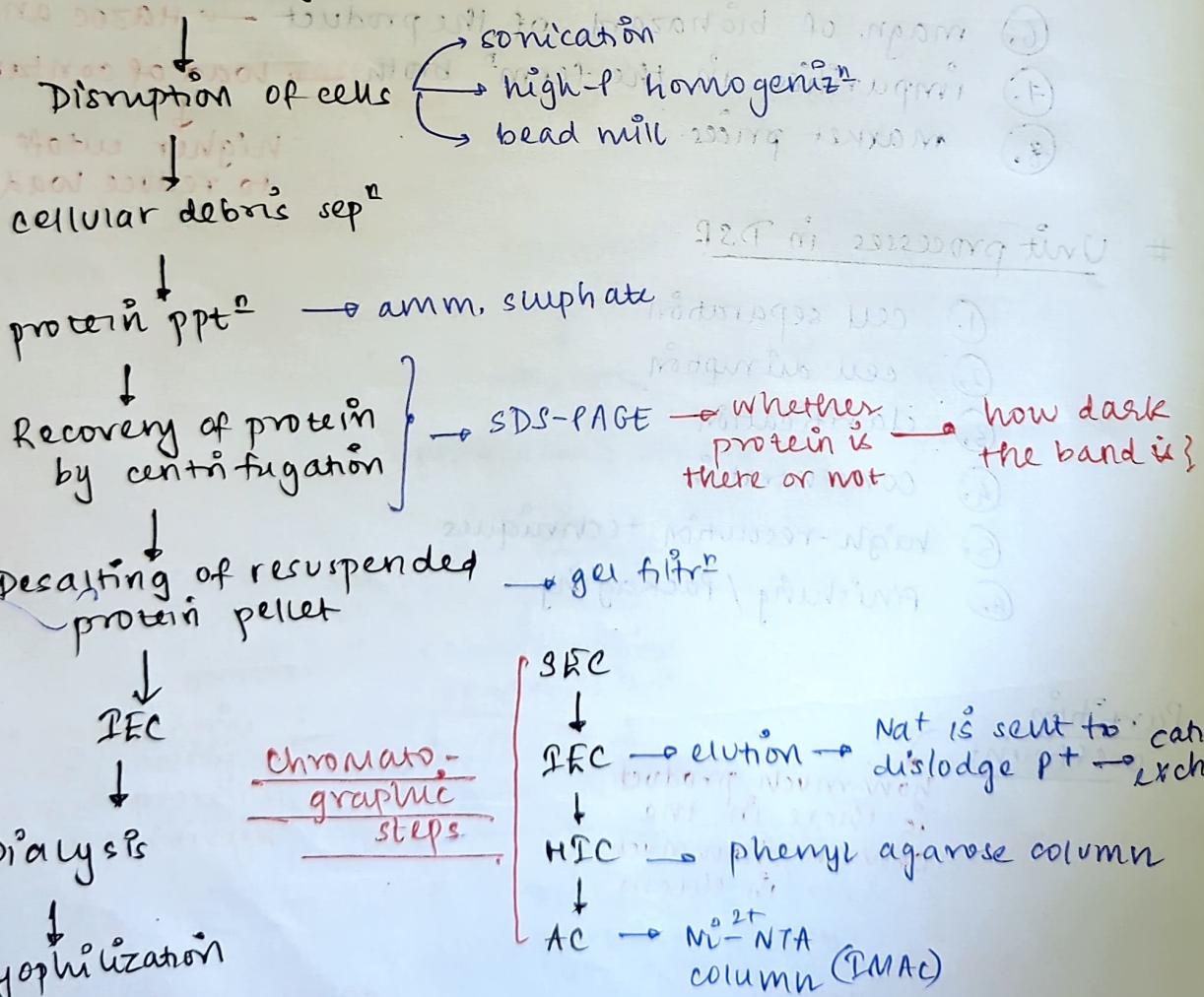
how much product is there in the column; how much in eluent

Pre-treatment

- ✓ cell-disrupt
- ✓ stabilize
- ✓ Pasteurize
- ✓ flocculate → algal cultures



cell harvesting



ppt → solvent → ethanol
 acetone
 ethyl acetate
 amyl acetate
 salt → ammonium sulphate

Expanded Bed Adsorption Chromatography :

24/11/24

PRECIPITATION

cell harvesting → centrifugation
 → multifiltration → pore size, sequential serial filtr²
 intracellular products → cell disruption
 → isolation from IBs

Intracellular Products:

significance of cell lys.

$$(\alpha - \beta)TA = T$$

- ① Intracellular product → most enzymes & proteins
→ biopolymers (PHAs)
→ metabolites
→ IBs (recombⁿ proteins)
- ② membrane proteins
- ③ periplasmic products

Methods of cell lysis

preferred by industries.

Chemical

- Osmotic shock → Lysozyme
- Enzyme digestion (expensive)
- Solubilisation
- Lipid dissolution
- Alkaline treatment

Mechanical

- Homogenization
- Grinding
- Ultrasonication
- Pressure cell
- Ball mill
- Heat shock

Alkali treatment

✓ Marsh method → does not disrupt only cells → also disrupt intracellular proteins → not preferred much

Disadvantages of enzymes

✓ works well with cultured cells

✓ buffer has to be removed → may not be effective with tissues

before DSP (filtration)

✓ physical disruptⁿ → more efficient in lysis

however, protein denaturation & aggregation may occur

Osmotic Shock:

$$\Pi = RT(C_i - C_o)$$

total solute molarity outside

inside cell

osmotic transmemb. pressure

total solute molarity inside cell

Enzymes

Protoplast

removing the cell wall

Spheroplast

Lysozyme

cell is still intact

Detergents

use in disruption (Ampipathic/ amiphilic molecules)

SDS, Tween-80

(anionic)

(non-ionic)

LABS: Linear Alkyl Benzene Sulphonates

CTAB (cationic)

Chaotropic agent:

- ✓ Guanidine hydrochloride
- ✓ Urea

Alkalii treatment :

Physical methods

small-scale

- mech^o homogenizer
- ↳ polytron

large-scale

- high P homogenizer

- ball mill

↳ Manton-Gaulin
homogenizer

periplasmic prot.

released faster

intracellular

slow release

memb - bound

several passes

high Pressure Homogeniz^z

$$R = \frac{Cr}{Cr_{\max}}$$

principles

conc. of released prod.

max. or conc. of prod. that can be released

→ 1st order process

unreleased product

released product

$$dR = (R - 1) dN$$

→ rate of release \propto conc. of unreleased product rate const.

$$\frac{dCr}{dN} = K_h (Cr_{\max} - Cr)$$

no. of passes thru homogenizer

empirical const.

$$K_h = k P^\alpha$$

integrate

$$Cr = Cr_{\max} (1 - e^{-K_h N})$$

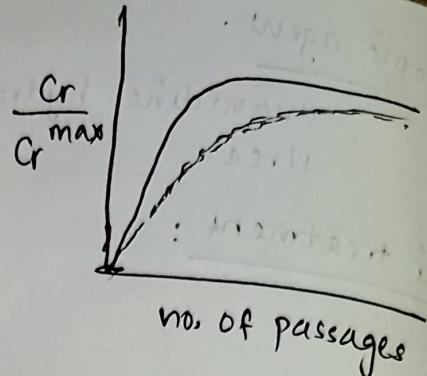
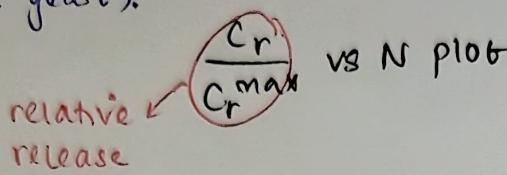
$$\ln \left(\frac{Cr_{\max} - Cr}{Cr_{\max}} \right) = -K_h N$$

$$\ln (1 - R) = -K_h N$$

$$\ln (1 - R) = -k P^\alpha N$$

S. cerevisiae
(Baker's yeast).

$$\alpha = 2.9$$



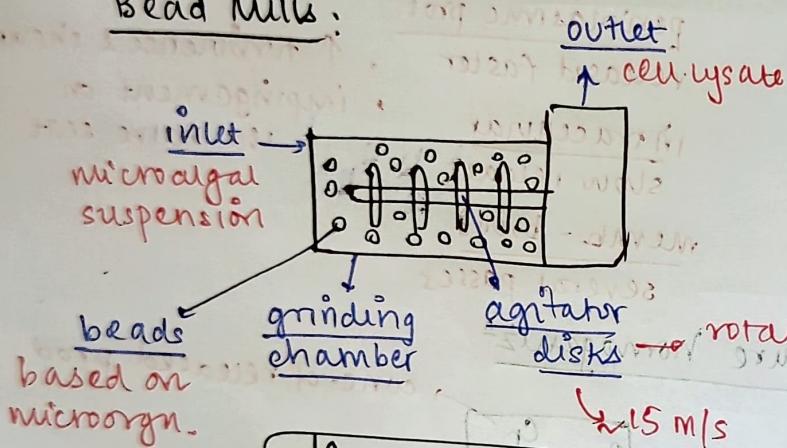
Ultrasonication:

✓ ↑ freq. vibrⁿ (\approx 20 kHz)

✓ cavitation

↑ formⁿ of vapor cavities

Bead Mill:



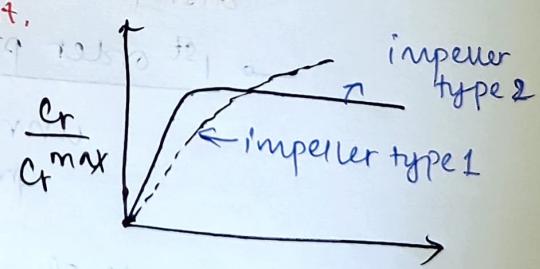
beads based on microorgn.

$$\frac{dCr}{dt} = K_b(C_r^{\max} - C_r)$$

processing time

similar to PFR but here

$\frac{Cr}{Cr^{\max}}$ vs t plot



$$\text{batch: } \ln(1-R) = -K_b t$$

$$\text{cont.: } \frac{1}{1-R} = 1 + \left(\frac{K_b t}{j}\right)^j$$

equiv. to no. of CSTRs in series

$$(n-1)^{j-1} \cdot j = 10$$

$$V_{out} = (n-1) V_i$$

$$n_{out} = (n-1) n_i$$

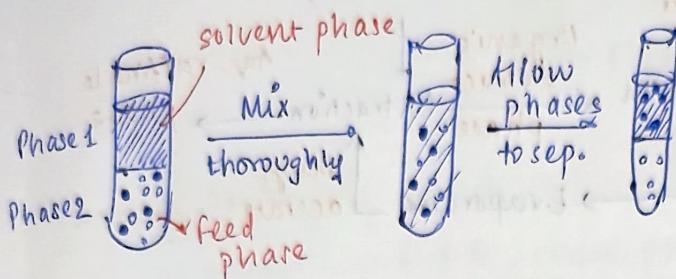
$$n_{out} = \frac{(n-1) n_i}{(n-1) + \frac{1}{j}} n_i$$

7/2/24

Precipitation → Dialysis (Desalting step)

SOLVENT EXTRACTION

sepⁿ of the constituents of a liquid solution by contact with another insoluble liquid.



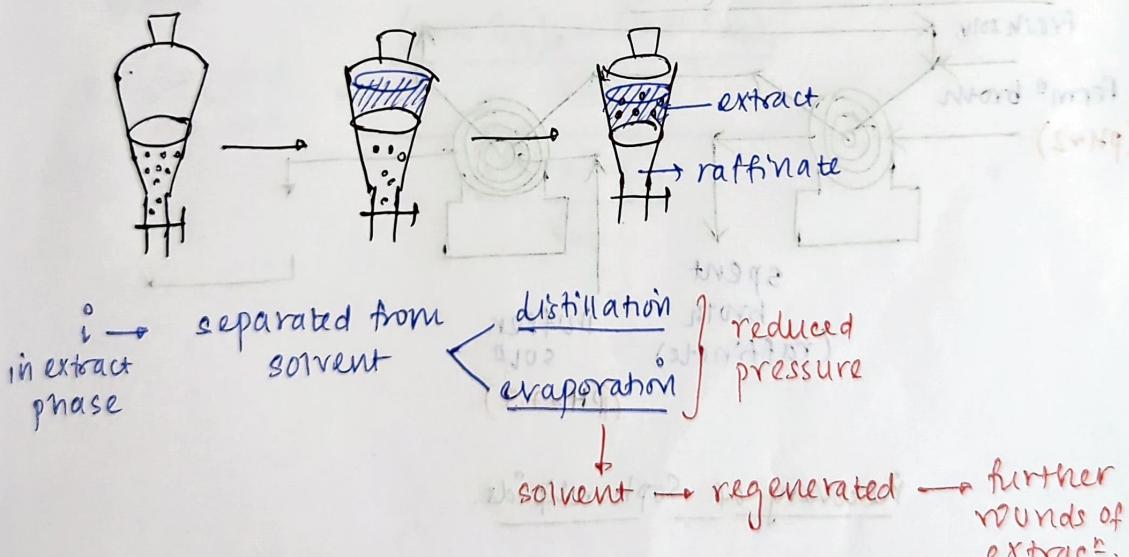
Feed liquid → component i to be removed + solvent → immiscible with feed phase but i is soluble in both phases

✓ some solute (i) transferred from feed phase → solvent phase

Extract layer of solvent + extracted solute

Raffinate layer from which solute has been removed.

✗ Extract may be lighter than raffinate



When is solv. extrⁿ used?

✓ components to be sep. → close B.P.

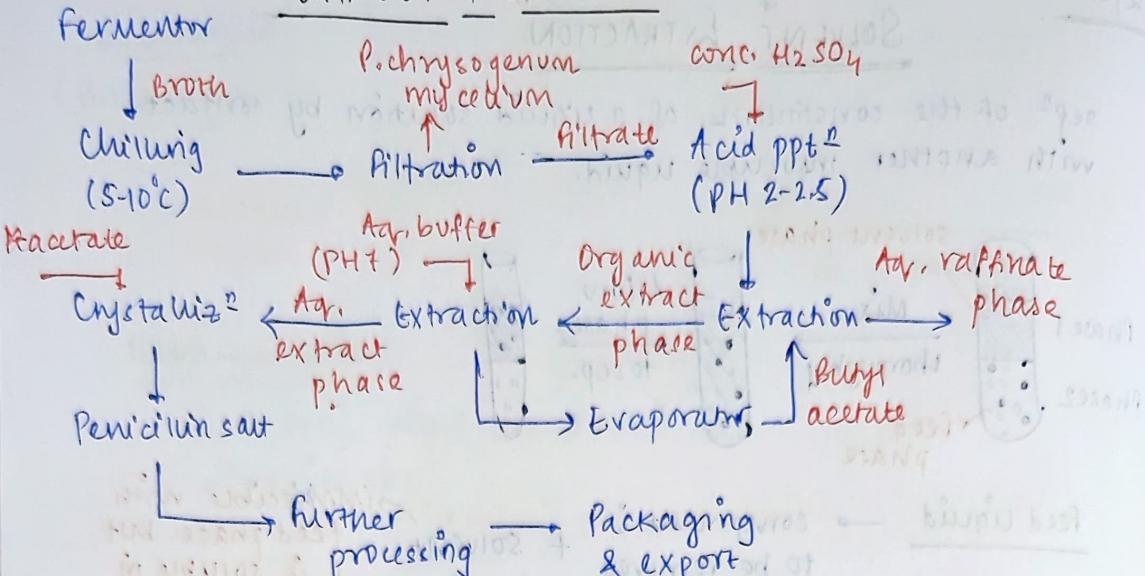
✓ sepⁿ of heat-sensitive materials (antibiotics, vitamins)

✓ non-volatile solutes

✓ very dilute solution

e.g. Penicillin → from fermentⁿ broth by n-Butyl acetate, n-amyl acetate → solvent

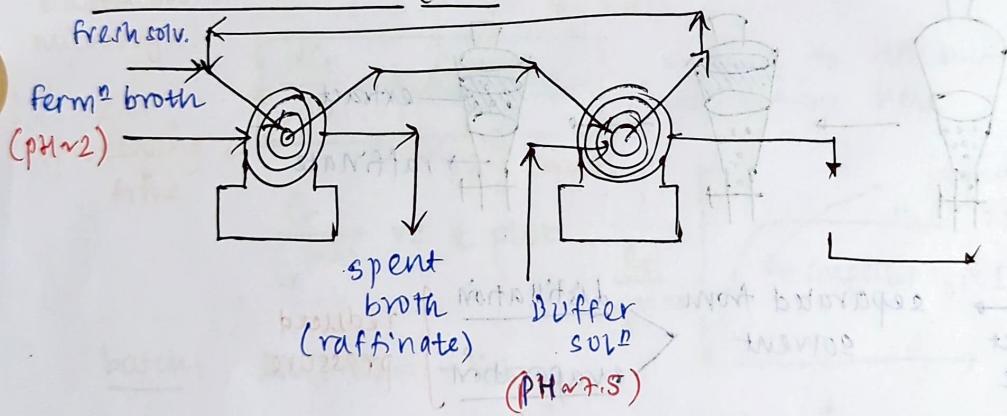
Purification of Penicillin



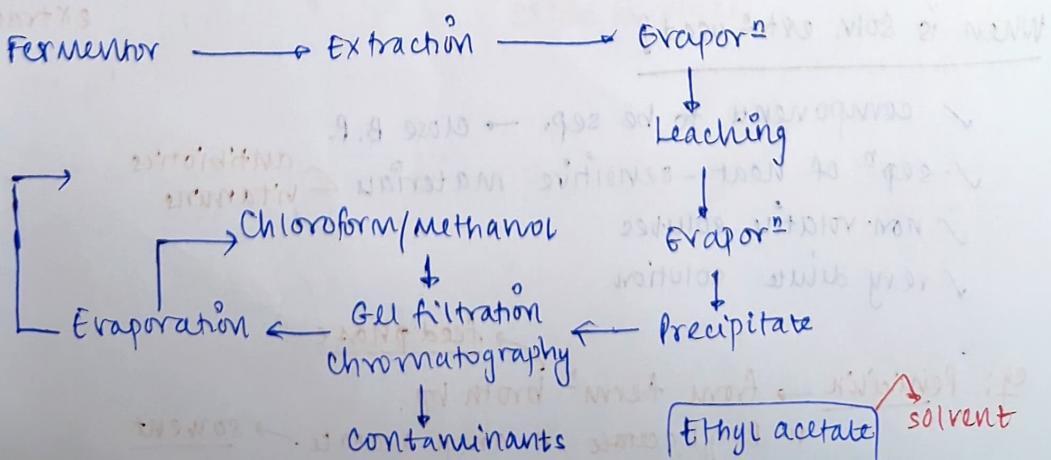
- ✓ organic extract phase → butyl acetate + penicillin
- ✓ aqueous " " → BA + water + penicillin
- ✓ aqueous raffinate phase → water + penicillin

0 discharge of solvent → govt. regulation

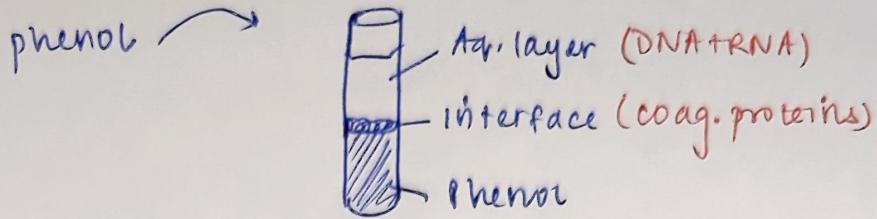
Podbieliuk extractor (Pod):



Recovery of Sophonolipids.



Purificⁿ of Bacterial DNA :



Partition Coefficient :

✓ ease of extraction

at eqbm b/w E & R, chem. potⁿ of solute becomes equal and const. in both phases

$$\text{i.e., } \mu(E) = \mu(R)$$

$$\mu^\circ(E) + RT \ln(y) = \mu^\circ(R) + RT \ln(x)$$

↓ solute conc.
in E phase

↓ solute conc.
in R phase

$$\boxed{\frac{y}{x} = K = \exp\left(\frac{\mu^\circ(R) - \mu^\circ(E)}{RT}\right)}.$$

DEFINITION

$$K = \frac{\text{conc. in E}}{\text{conc. in R}} = \frac{\frac{(mol \text{ Sag})_0 - (mol \text{ Sag})_1}{V_{org}}}{\frac{(mol \text{ Sag})_1}{V_{aq.}}}$$