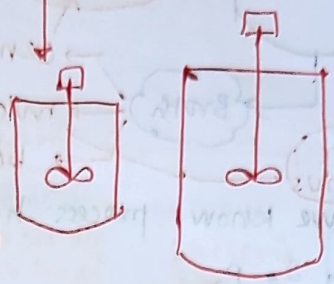
8/1/23

buffer
C source
N source
trace elements

Bioprocess

1. Upstream → prep^n of medium, sterilization, grow microbes, strain selection, innoculum 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)

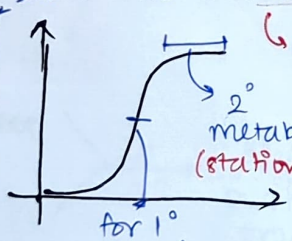
$$\ln \frac{N_o}{N} = kt$$

Design eqn. of steriuzer

N_o = microbes before steriliz^n
N = microbes after steriliz^n
k = death rate const.

3. Inoculum development

time of innocul^n



↳ done in laminar air flow (HEPA filters)
↳ not turbulent to prevent vortex form^n & contamination

$$\ln \frac{x}{x_0} = \mu t$$

eg: enzymes, macromolecules, organic acids

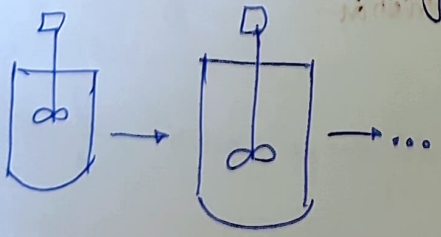
eg: antibiotics, surfactants, polymers

Midstream

process scale-up → geometrical scaling + some const. factors

power drawn / vol. of reactor (most universal)

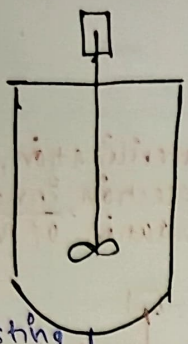
- P/V
- K_La (vol. MT coeff.)
- N



model fitting

- ✓ Monod
- ✓ Modified Monod

Downstream Processing



harvesting of cells

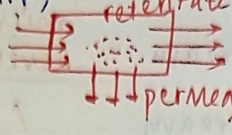
Broth

- centrifugation → continuous / batch
- microfiltration → continuous / batch
- both (if mycelia / flagella present)

Cepa Sherpies

millipore satorius

tangential flow filtration (TFF)

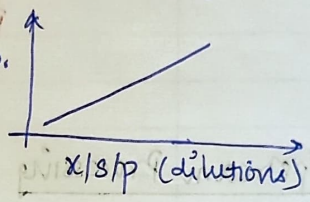


polymer filtration membranes
PVDA, etc.

how do we know process has ended?

x, s, p → standard curves

- x → biomass
- s → substrate
- p → product



Biomass

- processed if product is intracellular
- spent medium (supernatant / filtrate) — if product is extracellular

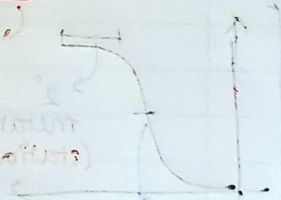
if product is captured in inclusion bodies

- renaturing buffer
- denaturing buffer
- dissolve IBs
- Guanidine, urea

cell disruption

enzymatic

- lysozyme → lyse cellular components (small scale)



physical / mechanical

- high pressure homogenization (large scale)
- ultrasonication
- bead mill / ball mill (very large scale)
  - Dynomill®
  - cells are ruptured by attrition (or) friction

ICP in

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

→ supernatant containing ECP

Ultrafiltn ← membb.-based filtration → classified based on pore size

sterilizing air

microfiltration

5 μ → 3 μ → 1 μ → 0.45 μ to cut off all fungi & yeast → 0.2 μ bacteria & spores

industries ↑ sequential removal of contaminants

to prevent clogging of pores

≤ 0.1 μ ultrafiltn

UF membranes characterized by → mol. wt. cut-off

→ Gel filtn
→ SDS-PAGE
→ MS

eg: insulin 5.5 kD
80 cut-off ~ 5 kD (NMWCO)

but similar MW-cutoff proteins will also be retained

ICP in Buffer
ECP in buffer
→ UF / NF → 80% purification product → chromatography

→ GFC / SEC / GPC
→ IEC
→ HIC
→ IMAC (Ni²⁺ NTA column) ← Nitrilotriacetic acid
↳ directly capture 6X His-tagged proteins

Affinity chrom
→ Antigen-antibody
→ Biotin-Streptavidin

Lyophilization

↳ Packaging → Marketing (end of DSP)

17/1/24

Upstream Processes:

Microorganism → Initial isolation → Strain improvement → Production strain

Fermentation raw Materials → media development → propag^n medium / maintenance medium

Production strain → starter culture propagation

Production medium

Starter culture propagation + Production medium → Fermentation (Midstream)

Downstream Processes:

Fermentation (Midstream) → cell separation centrifugation or filtration → Mf

Mf ← based on pore size

cell separation centrifugation or filtration → Harvested cells / Spent medium

Harvested cells → intracellular or periplasmic product → cell disruption → centrifugation or ultrafiltr^n

centrifugation or ultrafiltr^n → cell-free extract / IBs

IBs → cells quarantine proteins (mostly recomb^n proteins) as they are foreign → IBs → denature using guanidine, urea → renaturing buffer

Spent medium → extracellular product → conc. step → UF (based on mol. wt. cutoff (NMWCO))

conc. step → medium concentrate

cell-free extract + medium concentrate → Dialysis, ppt^n, partition, chromatography, UF, distillation, etc.

salting out → amm. sulphate → dialysis

dialysis → get rid of the salt

Dialysis, ppt^n, partition, chromatography, UF, distillation, etc. → ethanol

UF → chromatography

Product purification

polishing/ desalting

crystallization, drying, lyofilization, sterile filtrn, packaging, etc.

finishing processes

supersat. solution → heat → suddenly cool → crystals + impurities (do not crystallize)

finished product

~95% purity → Aff. chrom. eg: IMAC

freeze drying/ spray drying → not suitable for thermolabile products

Choice of recovery:

1. location of product → intracellular / extracellular
2. conc. of product in broth
3. physical & chem. properties of the desired product → pI, activity at a pH, stable with a net charge
4. intended use of product → therapeutical, etc.
5. minimal acceptable standard of purity
6. magn. of biohazard of the product → Hazoc analysis
7. impurities in the fermn broth → load of contamn → higher cutoff memb. to reduce load
8. market prices

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-disrupⁿ
- ✓ stabilizⁿ
- ✓ Pasteurizⁿ
- ✓ flocullⁿ → algal cultures

SEC columns
- sepharose (agarose) 100, 50
- sephacryl → acrylamide
- Sephadex 30, . . → dextran

DAEAE sepharose → +ve → exchanges anions → anion exchanger
↳ diethyl aminoethyl

$-COO^{\ominus}$, $SO_3^{\ominus}$ → cation exchangers
↳ Na^+ exchange with P^+

---

cell harvesting
↓
Disruption of cells → sonication / high-P homogenizⁿ / bead mill
↓
cellular debris sepⁿ
↓
protein pptⁿ → amm. sulphate
↓
Recovery of protein by centrifugation → SDS-PAGE → whether protein is there or not → how dark the band is
↓
Desalting of resuspended protein pellet → gel filtrⁿ
↓
IEC
↓
Dialysis
↓
Lyophilization

Chromatographic steps:
- SEC
- ↓
- IEC → elution → Na^+ is sent to dislodge P^+ → cation exchange
- ↓
- HIC → phenyl agarose column
- ↓
- AC → Ni^{2+} – NTA column (IMAC)

Ppt^{n} — solvent → ethanol, acetone, ethyl acetate, amyl acetate
Ppt^{n} — salt → amm. sulphate

Expanded Bed Adsorption Chromatography :

24/1/24

PRECIPITATION

cell harvesting — centrifugation
cell harvesting — multifiltration → pore size, sequential filtr^{n}

intracellular products → cell disruption
intracellular products → isolation from IBs

Intracellular Products:

significance of cell lysis:

1. intracellular product
   - → most enzymes & proteins
   - → biopolymers (PHAs)
   - → metabolites
   - → IBs (recomb^{n} proteins)
2. membrane proteins
3. periplasmic products

Methods of cell lysis → preferred by industries.

Chemical
- Osmotic shock
- Enzyme digestion → Lysozyme (expensive)
- Solubilisation
- Lipid dissol^{n}
- Alkali treatment

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

Alkali treatment

- Harsh method → does not disrupt only cells → also disrupt intracellular proteins → not preferred much

Disadvantages of enzymes

- works well with cultured cells → may not be effective with tissues
- buffer has to be removed → before DSP (filtration)

- physical disrupⁿ → more efficient in lysis
  - however, protein denaturation & aggregation may occur.

Osmotic Shock:

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

- π: osmotic transmemb. pressure
- C_i: total solute molarity inside cell
- C_o: total solute molarity outside cell

Enzymes

- Protoplast: removing the cell wall → cell is still intact
- Spheroplast

Lysozyme

Detergents

- use in disruption (Ampipathic / ampiphilic molecules)
- SDS, Tween-80 (anionic) (non-ionic)
- LABS: Linear Alkyl Benzene Sulphonates
- CTAB (cationic)

Chaotropic agent:

- ✓ Guanidine hydrochloride
- ✓ Urea

Alkali treatment:

Physical methods

small-scale

→ mech^{n} homogenizer
↳ polytron

large-scale

→ high P homogeniz^{n}
→ Ball mill
↳ Manton-Gaulin homogenizer

↓

periplasmic prot. released faster

intracellular slow release

memb-bound several passes

- ↑ turbulence & shear
- impingement on the valve seat

high Pressure homogeniz^{n}

$$R = \frac{C_r}{C_r^{max}}$$

C_r → conc. of released prod.

C_r^{max} → max. conc. of prod. that can be released

R → extent of disruption

→ 1st order process

unreleased product → released product

→ rate of release ∝ conc. of unreleased product

$$\frac{dC_r}{dN} = K_h (C_r^{max} - C_r)$$

K_h → rate const.

N → no. of passes thru homogenizer

$$K_h = k P^{\alpha}$$

k → empirical const.

P → applied pressure

↓ integrate

$$C_r = C_r^{max} (1 - e^{-K_h N})$$

$$\ln\left(\frac{C_r^{max} - C_r}{C_r^{max}}\right) = -K_h N$$

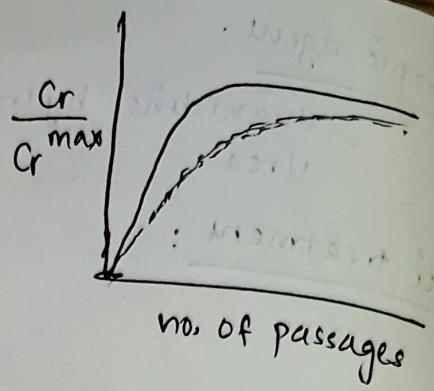
$$\ln(1-R) = -K_h N$$

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

S. cerevisiae (Baker's yeast). $\boxed{n = 2.9}$

$\frac{C_r}{C_r^{max}}$ vs N plot

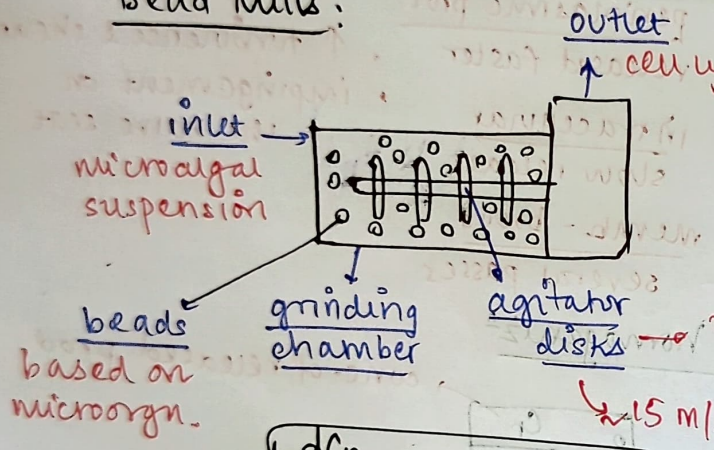
relative release



Ultrasonication:

- ✓ ↑ freq. vibrn (~ 20 kHz)
- ✓ cavitation → formn of vapor cavities

Bead Mills:



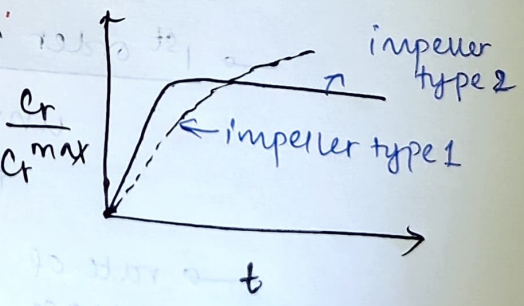
$$\frac{dC_r}{dt} = K_b(C_r^{max} - C_r)$$

processing time

rate const.

similar to HPH but w.r.t time here

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



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

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

equiv. to no. of CSTRs in series