

Biochemical Engineering

classmate

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Books

- i) Bailey and Ollis, Biochemical Engineering Fundamentals
- ii) Shuler and Kaagi, Bioprocess Engineering: Basic Concepts.
- iii) Nelson and Cox, Principles of Biochemistry

08/08/2022

- i) What are the reasons for non-eating non-veg?
→ Texture,

- ii) Chemical Engineering Vs Biochemical Engineering?

A) Stability: Chemical
stable for ~~long~~ time
~~longer~~
in Water

A) Biochemical / Biochemical systems
stable for narrow range of condⁿ

e.g. Apple

B) Absence of life force

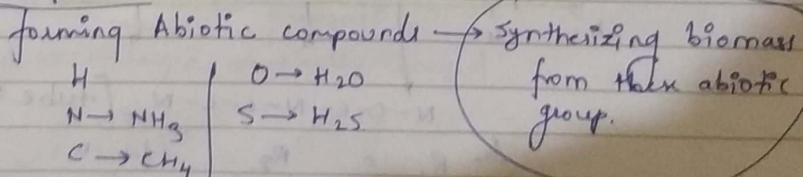
B) Presence of life force.
e.g. growth on its own (Bacteria in water)

- Biochemical Engineering:
- i) Processing Biochemical systems at large scale
 - ii) Standardization of Biochemical manufacturing
on Alcohol / Bread

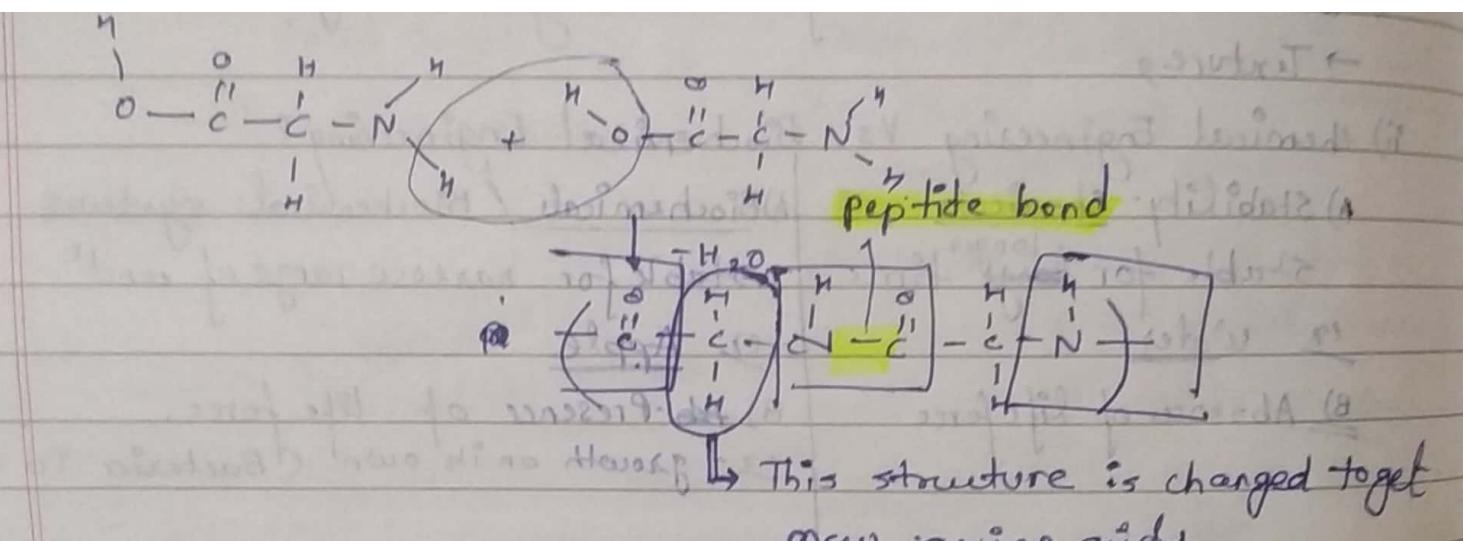
* Autotrophic carbon assimilation.

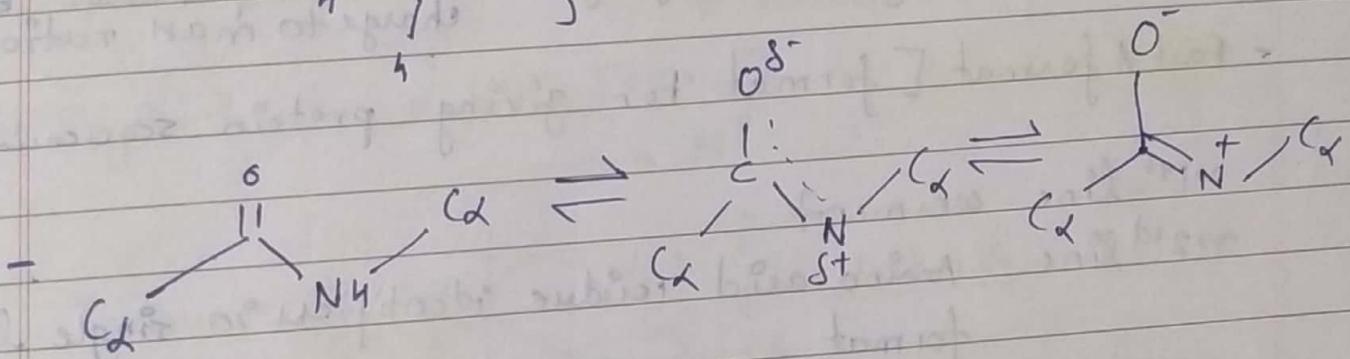
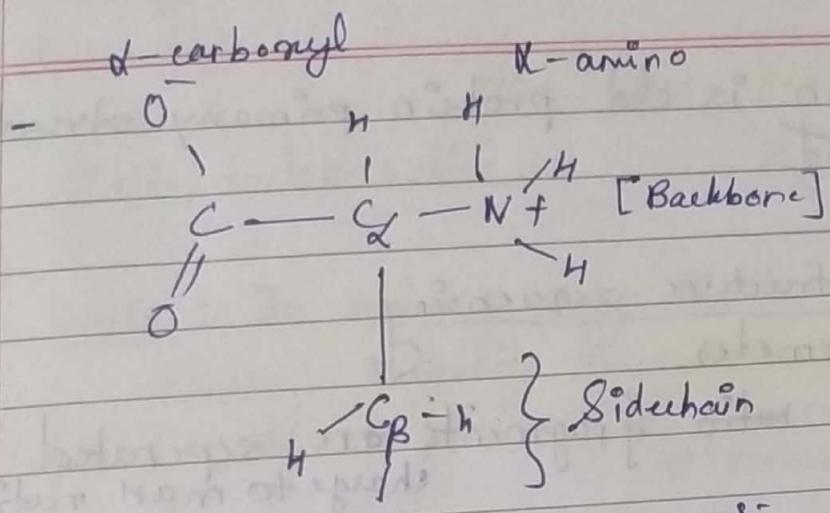
Plants convert CO_2 to biomass

1953: Miller experiment



Primordial soup:





$\frac{1}{2}$ of hydrolysis of peptide bonds ~ 350 years!

CORN rule

- Rule to find optical isomers [Stereoisomers]

L-Alanine
(Levo)(Left)

D-Alanine
(Dextro)(Right)

- 4 types of groups [R]

Nonpolar Polar-uncharged. Electrically charged Aromatic

Edman degradation is old protein primary structure sequencing method.

Modern primary structure sequencing

mass spectrometer

~~mass/charge ratio~~ fragments are separated by charge to mass ratio!

- Fasta format [format for giving protein sequence]

first line - comment

~ 2nd line - Amino acid residue identified in single letter format

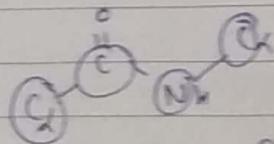
and Each line with a maximum of 80 characters.

- Two questions \Rightarrow

How are the precursor amino acids synthesised?

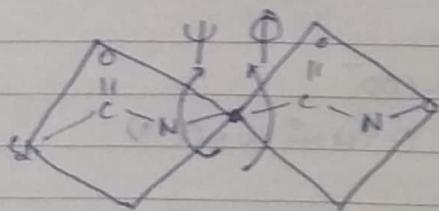
How does their polymerization take place?

How are proteins synthesised in biological system?



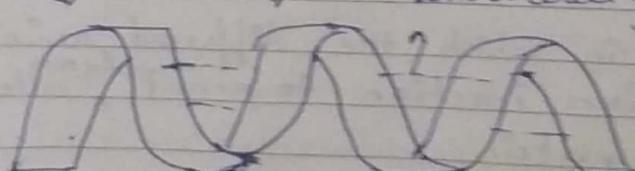
All these four atoms are on same plane.

- Helices



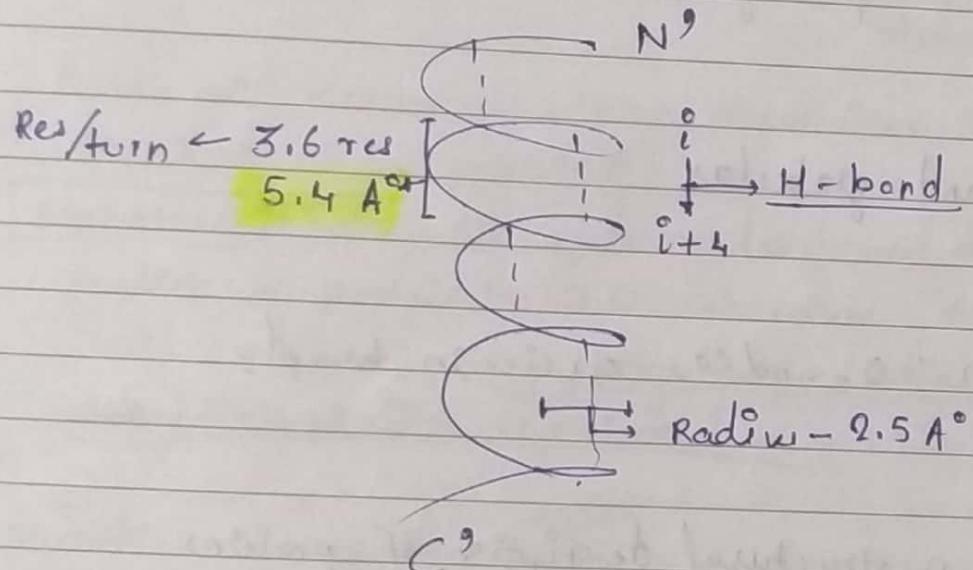
Ψ & $\Phi \rightarrow$ defined structure

van der waals force of attraction



[*amino acids are present in L form except few exceptions*]
D isomers.

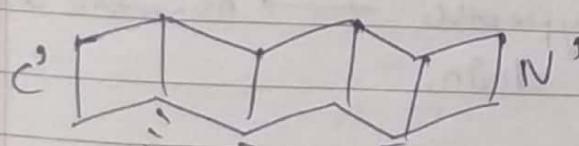
- Right handed α helix [80%]



Other - β_{10} , π , PPIJ

- β sheets

Parallel β sheet.

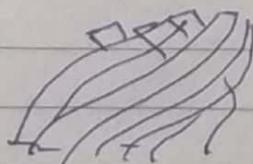


Antiparallel β sheet.

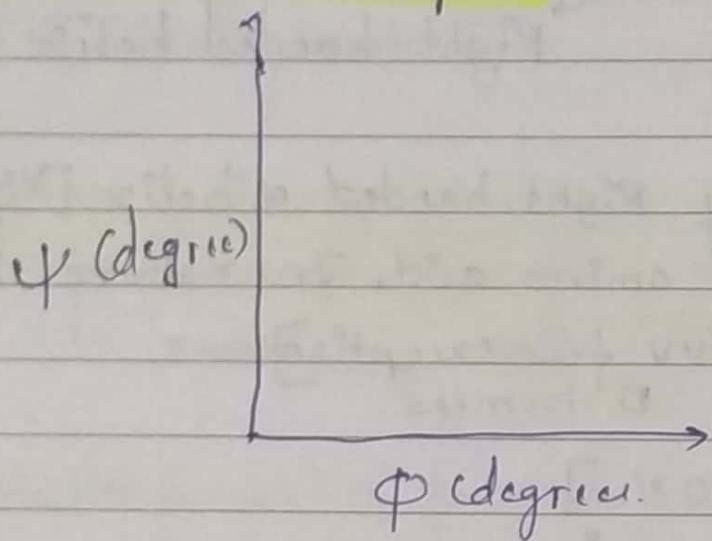


- β barrel

- Loops



Ramachandran plot



Fibrous and globular

$$\Delta E, \Delta H, \Delta A, \Delta G \} \text{ Thermodynamic potentials.}$$

\downarrow \downarrow \downarrow \downarrow
 $S_{\text{G}}V$ S, P $T_{\text{G}}V$ T, P
 $\underline{\underline{T_{\text{G}}V}}$ $\underline{\underline{T, P}}$ $V + P V$

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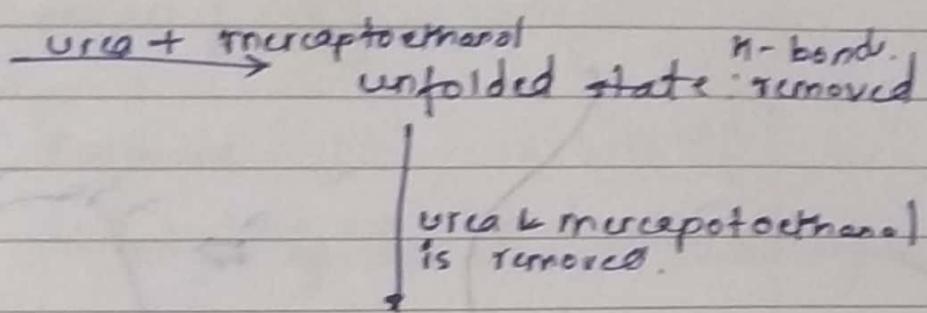
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29/02/2022.

Protein folding: Anfinsen's experiment.

* The folding of primary structure is unique.

Native Ribonuclease



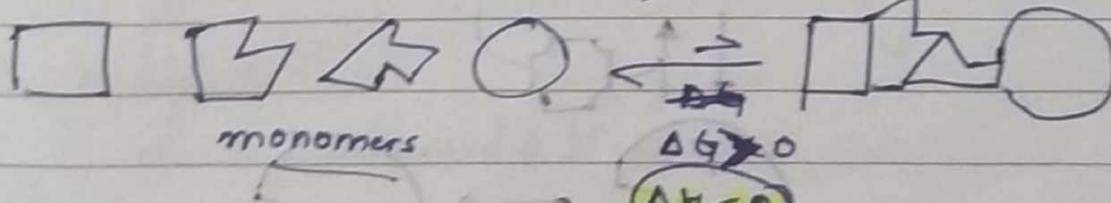
The same structure of ribonuclease is obtained.

Amino acid sequence alone determines the complete 3-D structure of a protein!!!

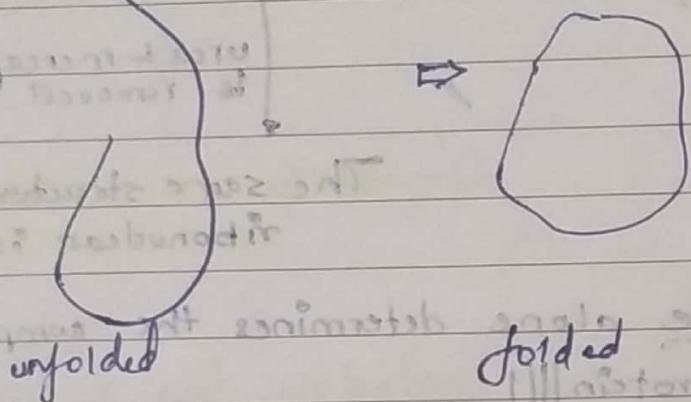
AlphaFold is an AI system developed by DeepMind that predicts a protein's 3D structure from its amino acid sequence. It regularly achieves accuracy competitive with experiment.

Mutation → change in ~~amino~~ acid, The change in one ~~amino~~ acid will change the whole protein structure.

$$\Delta G = \Delta H - T \Delta S$$



* Protein folding thermodynamics : funnel shaped energy landscape



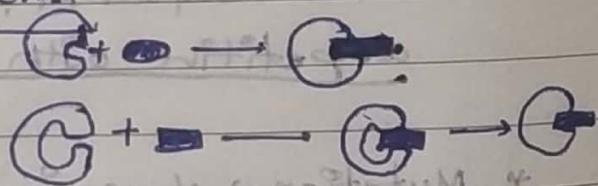
$$\Delta G_{\text{folding}} = G_{\text{folded}} - G_{\text{unfolded}} < 0$$

* Protein-substrate interactions

→ Lock and key mechanism

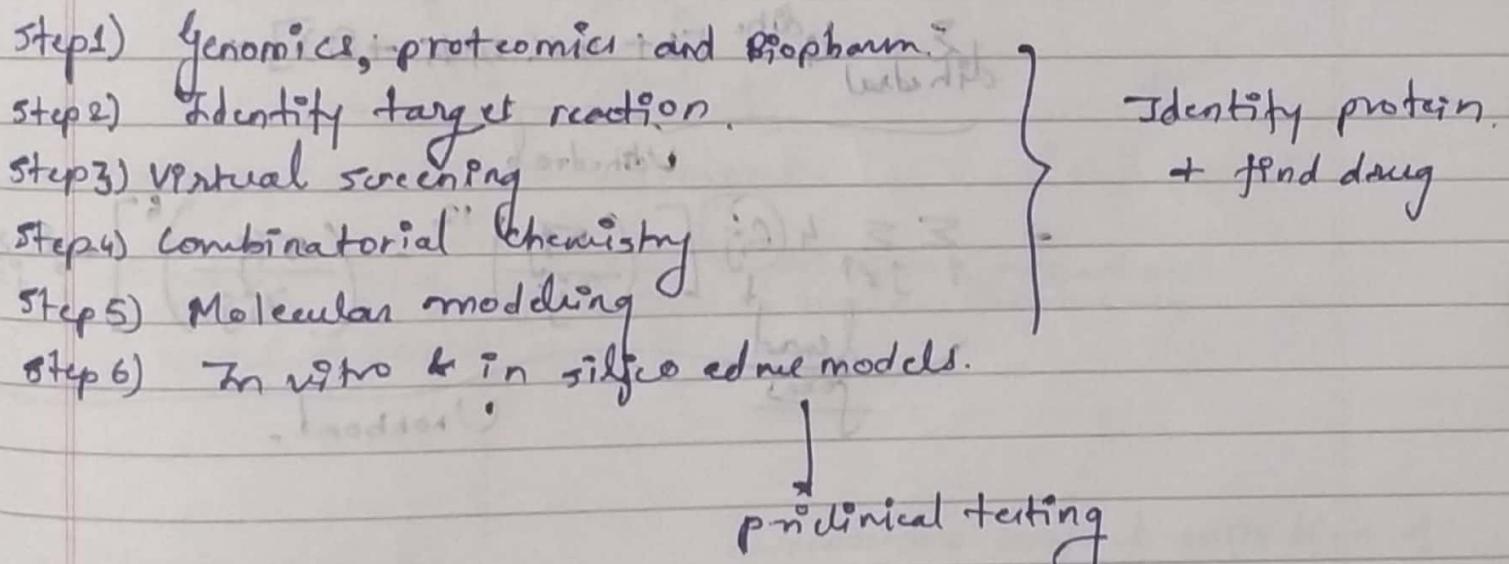
→ Induced-fit mechanism

→ Conformational selection mechanism



* Specificity : Enzymes are more specific than catalyst.

Promiscuity on the level of substrate means how many substrates can the enzyme react with.



* Calculation of energy required to heat an object

$$U(\vec{R}) = \sum_{\text{bonds}} k_i^{\text{bond}} (r_i - r_0)^2 + \sum_{\text{angles}} k_i^{\text{angle}} (\theta_i - \theta_0)^2 +$$

U_{bond} U_{angle}

$$\begin{aligned}
 & \sum_{\text{dihedral}} k_i^{\text{dih}} [1 + \cos(\eta_i \phi_i + \delta_i)] + \\
 & \quad \text{dihedrals} \\
 & + \sum_{i} \sum_{j \neq i} 4 \left(\epsilon_{ij} \right) \left[\left(\frac{\sigma_{ij}}{r_{ij}} \right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}} \right)^6 \right] + \sum_{i} \sum_{j \neq i} \epsilon_{ij}^{\text{nonbond}} \\
 & \quad \text{Lennard-Jones} \quad \text{Coulomb} \\
 & \quad \text{nonbond} \quad \text{nonbond}
 \end{aligned}$$

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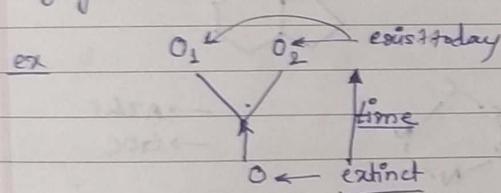
* Analysis of DNA and RNA

Phenotype : Governs the physical appearance of organism.
ex: stripes of tigers
Physical appearance: arrangements of proteins.

Classification of Living Organism

photo-s

Phylogenetic tree

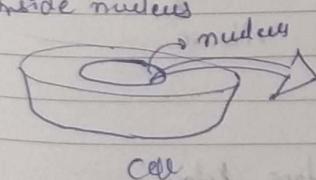


* Prokaryotes vs Eukaryotes

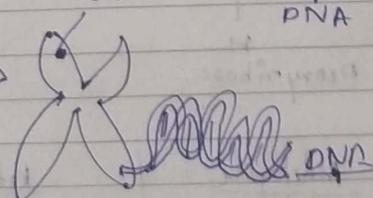
simple, unicellular,
absence of
nuclear membrane

complex, multicellular organisms
well-defined nuclear boundary

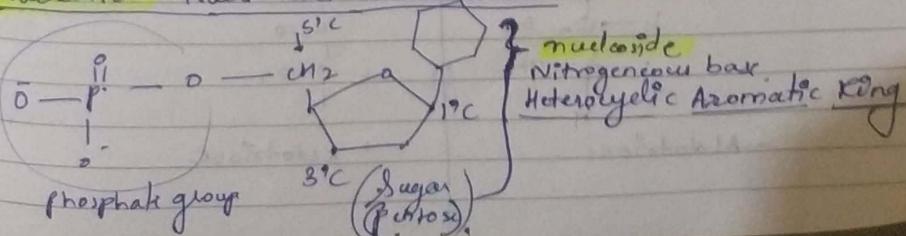
Inside nucleus



chromosome: compact collection of
DNA and RNA



* Nucleotide: Nucleic acid monomeric unit



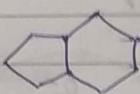
- * Nucleic acid → polymer
- Nucleotide → phosphate + sugar + base
- Nucleoside → sugar + N-base
- Nucleobase → N-base

* Nucleobases

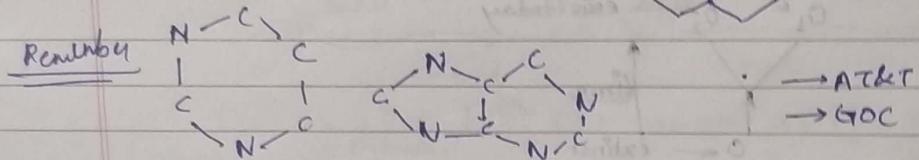
Pyrimidines

Cytosine Thymine Uracil
(C) (T, in DNA) (U, in RNA)

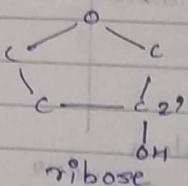
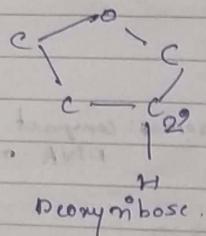
Purines (A) (G)



Adenine (A) Guanine (G)



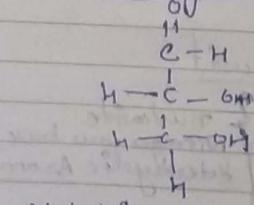
- * Pentose: ribose and deoxyribose.



* Sugars

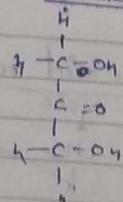
Aldose

Aldehyde



→ Aldotetroses

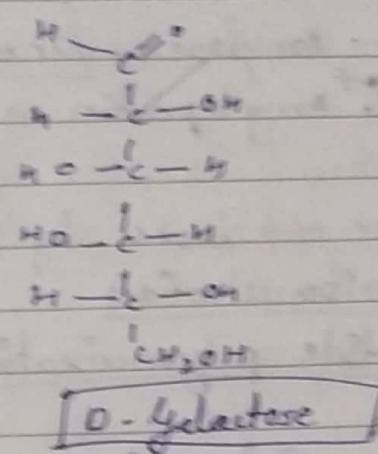
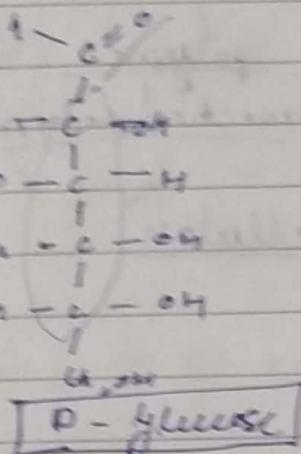
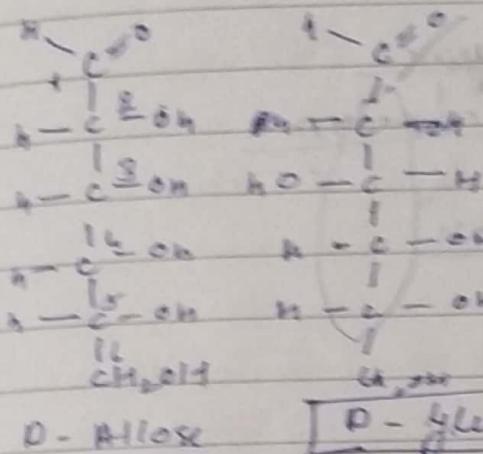
Ketone Ketose



→ Ketotetroses

six carbon

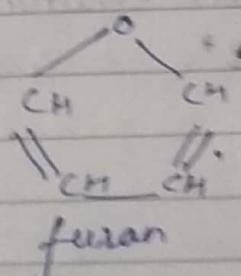
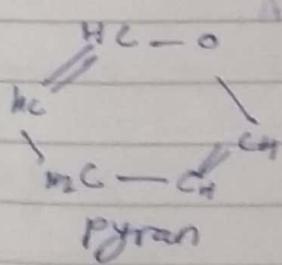
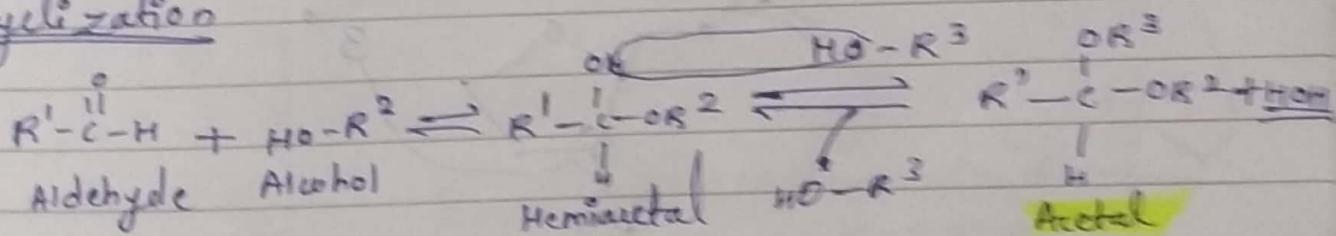
Monosaccharide



Fruitose → sweetest naturally occurring sweet

Sucrose → disaccharide of
glucose + fruitose

cyclization



DNA → furanose

Lactose intolerance

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* Starch - Polysaccharide

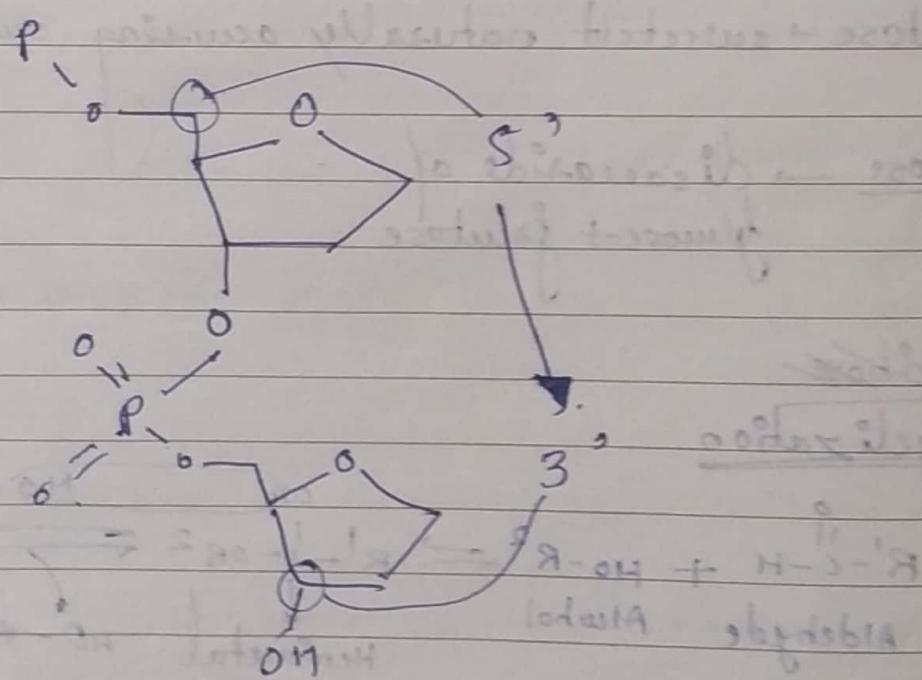
Starch → Amylose → linear polymer
→ Amylopectin → branched polymer

* Cellulose → broken by cellulase

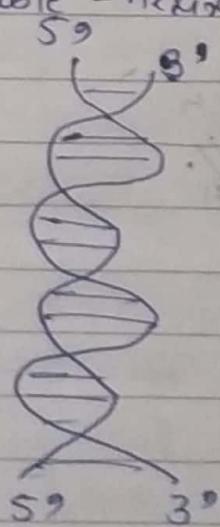
* Deoxyribonucleotides

* Nucleotide polymerization

$5' \rightarrow 3'$



* The double-helix structure of DNA.

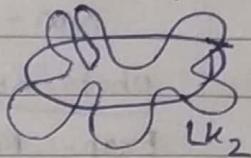
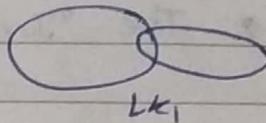


* DNA supercoiling

superhelical density: $\sigma = \frac{\Delta Lk}{Lk_0}$

$$Lk = Tw + Wr$$

linkage no. twisting writhing

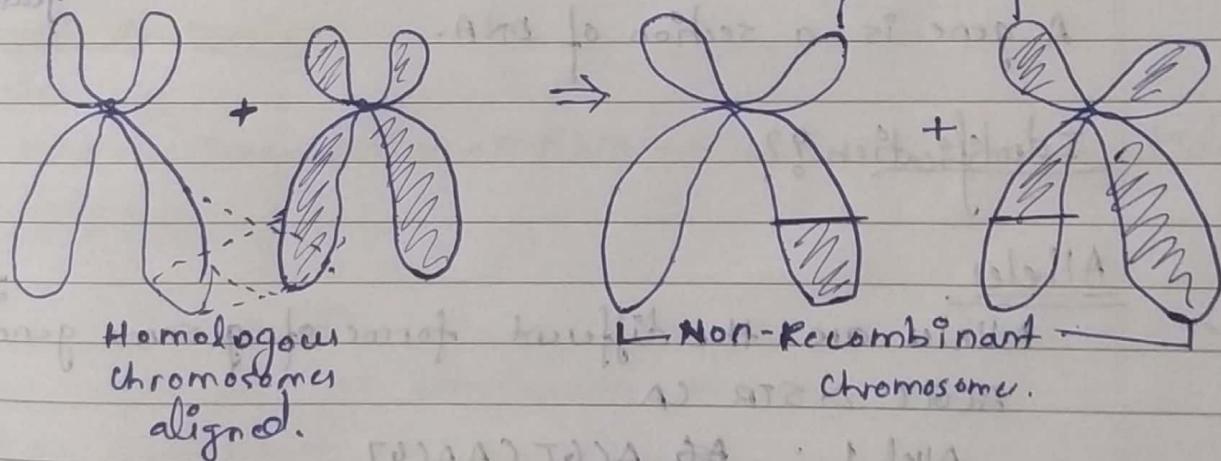


To make a protein of length n you need $3n$ length of DNA.

Maximize linkage no. for compaction.

④ 11/09/2021

* Chromosome cross over

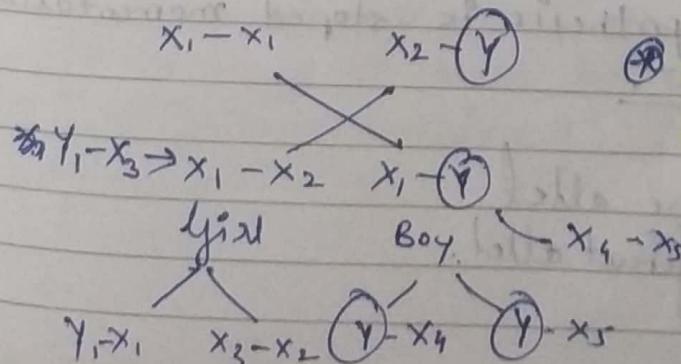


⑤ X chromosome is 3 times larger than Y chromosome.

↓
900 genes

↓
55 genes

⑥ Y chromosome only continues in the family.



⑦ Hence we can trace the ancestry of boy not girl.

Non-coding DNA or satellite DNA or "Junk" DNA.

photo-Ruthi

Exon - Responsible for synthesis of proteins.

Intron - Introns

RVN

Short tandem repeats - STR [2-9 base pair/repeat]

Variable number of tandem repeats (VNTR) [10-100 base pair/repeat]

→ These repeating units don't do anything in existing organism but can do something in future / Responsible for stability / Responsible for structure

photo-Ruthi

* Genes

A gene is a section of DNA.

Identification ?

Alleles

Alleles are the different forms of a same gene. It is a variation of gene.

ACGT & STR CA

Allel 1 : ACGT CAACGT

Allel 2 : ACGT CACA ACGT

Allel 3 : ACGT CAC A CAA CGT

Allel 4 : ACGT, CAC A CAA CGT
exon intron

The protein synthesis is stopped momentarily when CA is encountered.

- Homozygous → same allele

- Heterozygous → different allele.

Neandertal

* Protein Synthesis

Photo-Rahi

DNA → Transcription → mRNA → Translation → polypeptide

Transcription

Step 1 : DNA uncoils

Step 2 : Polymerizes the complementary strand of RNA

Step 3: formation of RNA

Translation

codon - (3 base pairs collection) → ~~particular~~ specific to particular nucleic acid.

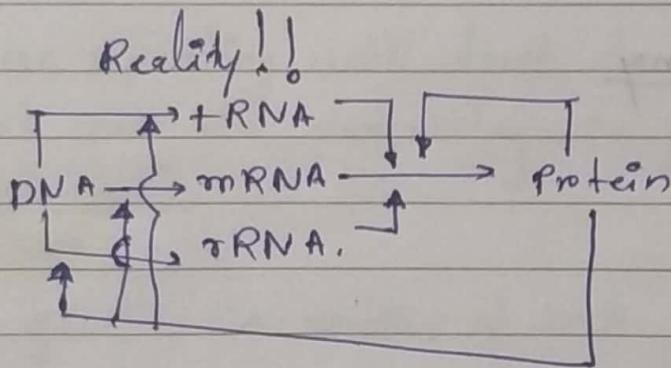
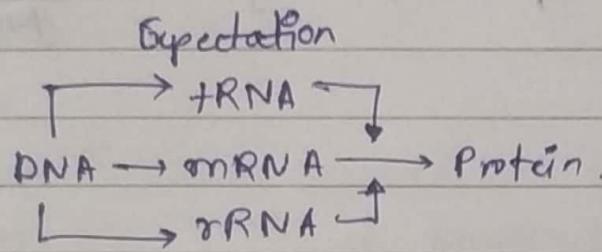
* Viral infections and mRNA vaccines.

Why it is easy to treat bacteria not virus?

Working of mRNA vaccine?

→ Polymerase chain reaction - PCR.

DNA - protein relation = A recursive problem



* Measurement of RTD

→ Pulse input:

$$E(t) = \frac{C(t)}{\int_0^\infty C(t) dt}$$

The quantity $E(t)$ is called the residence-time distribution function. It is the function that describes quantitative manner how much time spent in reactor.

→ fraction of material

leaving the reactor

that has resided in reactor

for time between t_1 & t_2

$$= \int_{t_1}^{t_2} E(t) dt$$

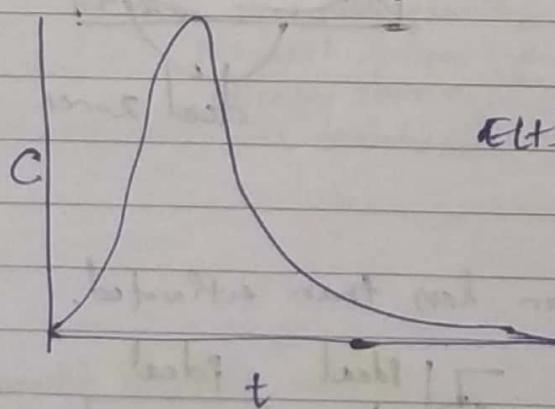
→ mean residence time =

$$\frac{\int_0^t E(t') dt'}{\sum E(t') \Delta t} = \frac{\sum t' \frac{E(t')}{\Delta t}}{\sum \frac{E(t')}{\Delta t}} = \frac{\sum t' G}{\sum G}$$

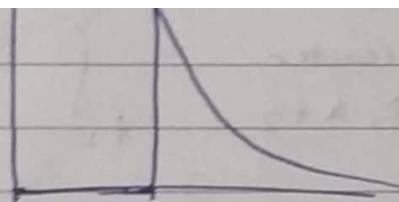
→ Example

Pulse Experiment

t (min)	0	1	2	3	4	5	6	7	8	9	10
Clg/m ³)	0	1	5	8	10	8	6	4	3	2.2	1.5

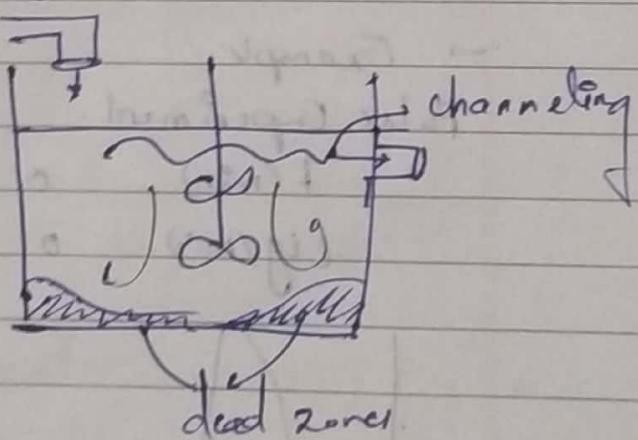
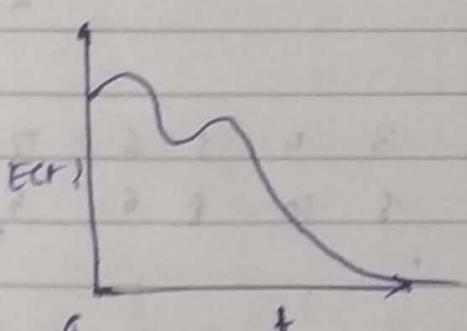


$$E(t) = \frac{C(t)}{\int_0^\infty C(t) dt} = \frac{C(t)}{50 \text{ g-min/m}^3}$$



PFR followed by CSTR

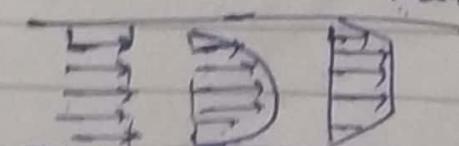
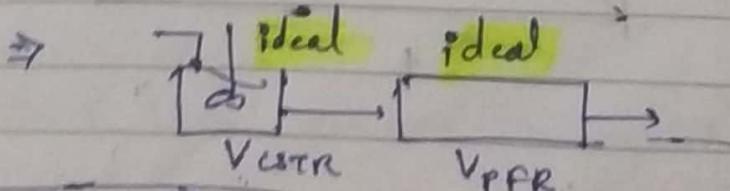
④ CSTR with short-circuiting flow



Graph

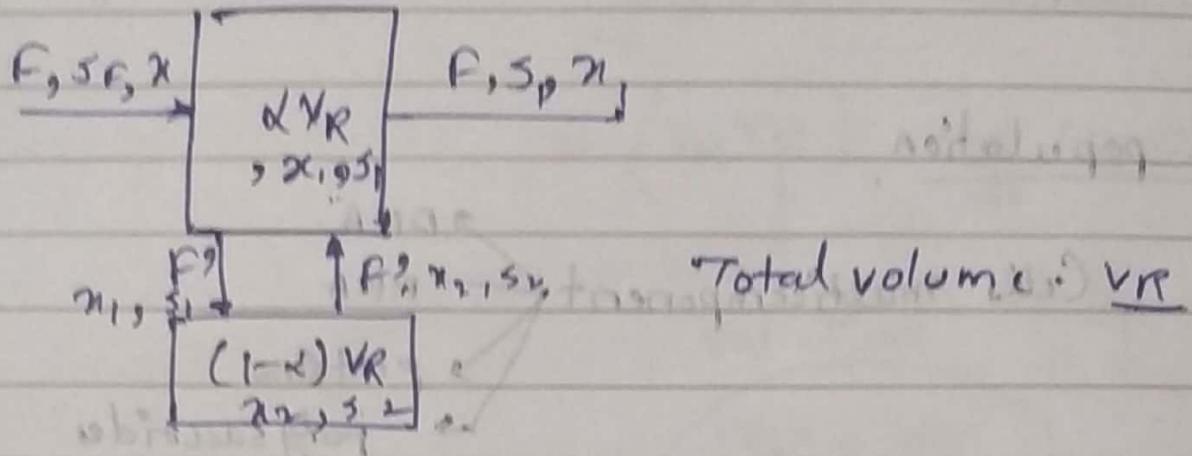
Real PFR → conversion less than estimated.

non-plug
behavior
at entrance



plug flow laminar, turbulent .

cSTR (chemostat) with a deadzone (real)
→ non ideal, real reactor.



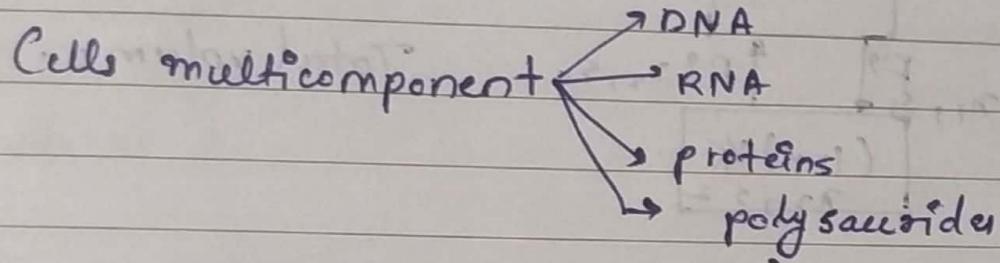
How do you view cell population?

Treat cell as a single component → unstructured.

II multicomponent → structured.

Treat population of ^{cells} discrete → segregated

II homogeneous → unsegregated



Growing cell population \rightarrow cell to cell heterogeneity

- differ in age
- differ in chemical activity.

Consider

viewpoint:

Cell population as one single component \rightarrow unstructured

cell population as multicomponent \rightarrow structured.

Cell s - as discrete hetero. entity \rightarrow segregated
 - as homogeneous entity \rightarrow unsegregated.

Well mixed bioreactors for kinetic measurement

(Ideal)

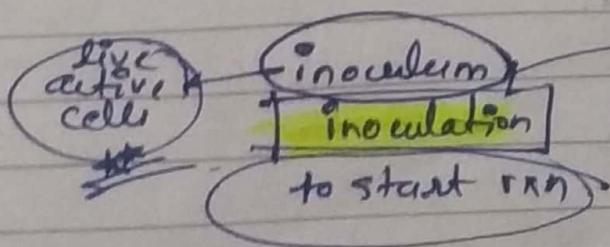
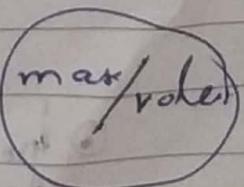
- Avoid spatially non-uniform conditions

- ① Batch reactors (unsteady)
- ② CSTR/ chemostat (steady)

Batch

C_i \rightarrow concentration of i^{th} component

VR \rightarrow volume



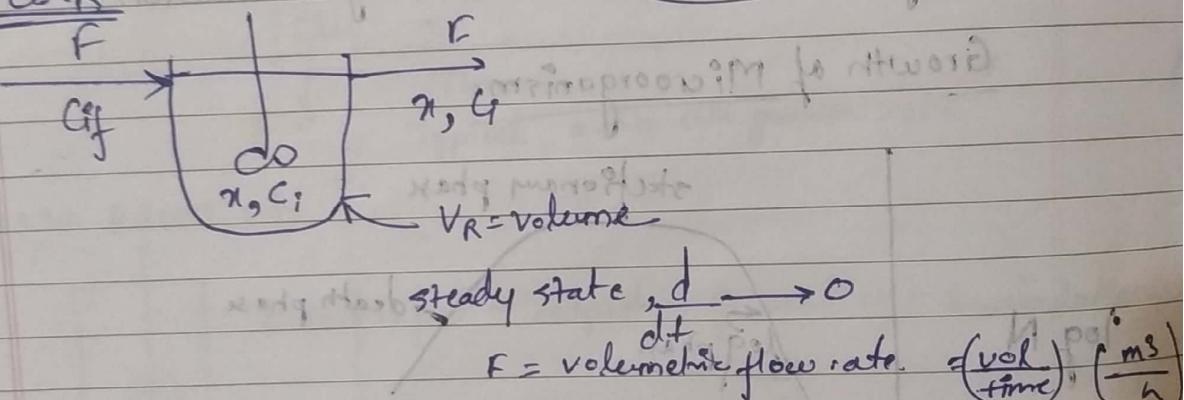
(dil + substrate \rightarrow more cells + product)

flow rate - flow rate + rate of formation = rate of accumulation.

$$\Rightarrow (V_R r_f^o) = \frac{d}{dt} (V_R i)$$

$\frac{\text{mass}}{\text{vol. time}}$ if $V_R = \text{constant}$

$$r_f^o = \frac{dc_i}{dt}$$

CSTR

steady state, $\frac{d}{dt} \rightarrow 0$

$F = \text{volumetric flow rate. } \left(\frac{\text{vol}}{\text{time}} \right) \cdot \left(\frac{\text{m}^3}{\text{h}} \right)$

$$F(C_{if} - C_i) + V_R r_f^o = 0$$

$$\text{CSTR} \Leftrightarrow r_f^o = \left(\frac{F}{V_R} \right) (C_i - C_{if})$$

$\Rightarrow D = \text{dilution rate}$

\Rightarrow Assumption about cell population (structured, unstructured environment \Rightarrow limiting growth rate subs. $\frac{dx}{dt}$)
 \Rightarrow Regulated $T, pH..$

$$k_C C(1-x)$$

- * Rate of microbial growth is characterized by specific growth rate, μ

$$\mu = \frac{1}{x} \frac{dx}{dt} = \frac{1}{N} \frac{dN}{dt}$$

$x = \frac{\text{cell mass conc}}{\text{total volume}} = \frac{\text{mass}}{\text{volume}}$

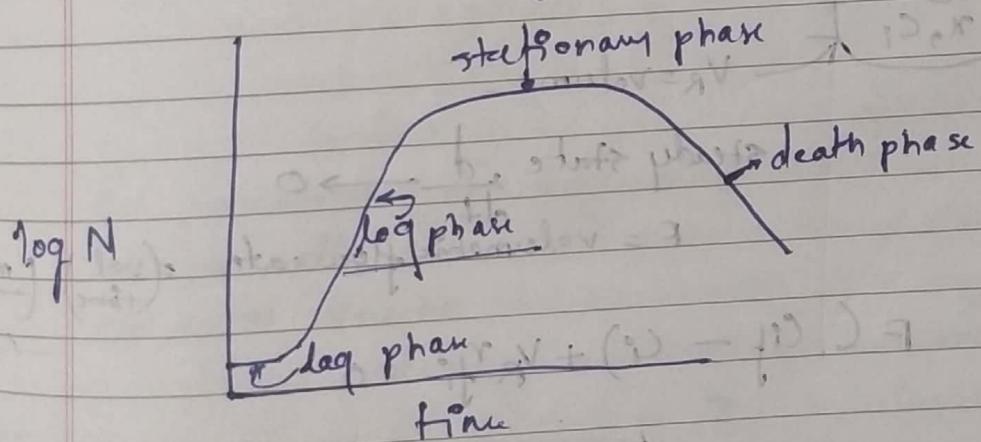
$$\frac{dx}{dt} = \mu x$$

$\approx N_x \approx \frac{dN}{dt}$

$t = \text{time}$
 $\mu = \text{specific growth rate, } \frac{1}{\text{time}}$

Cell growth \Rightarrow 1st order autocatalytic reaction

Growth of Microorganisms



06/09/2022

Microbial growth can be characterized

by specific growth rate, μ

$$\mu = \frac{1}{x} \frac{dx}{dt}$$

$x \rightarrow \text{cell mass conc } \left(\frac{\text{mass}}{\text{vol}} \right)$

$t \rightarrow \text{Time}$

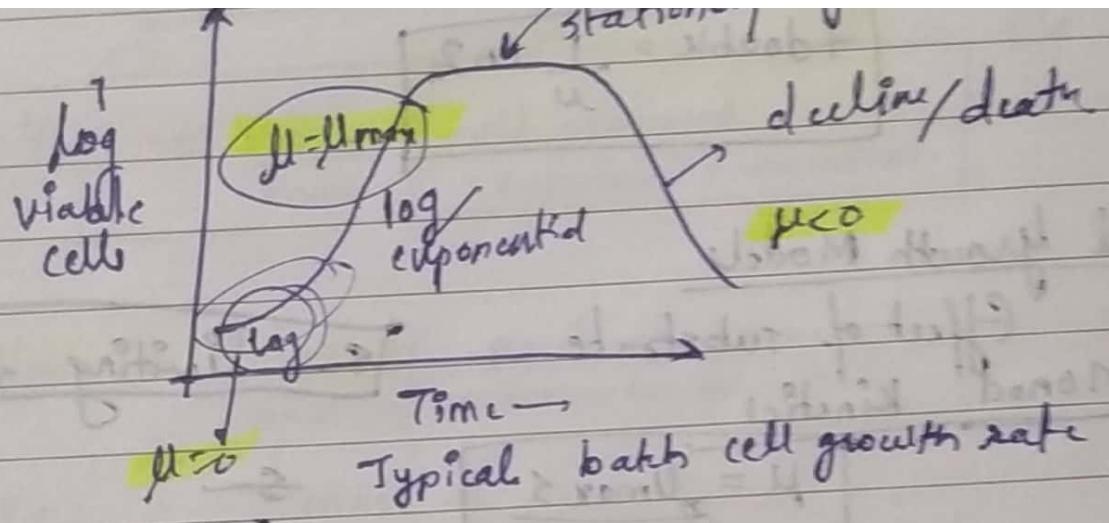
$$\mu \rightarrow \frac{1}{\text{time}}$$

In terms of no. of cell.

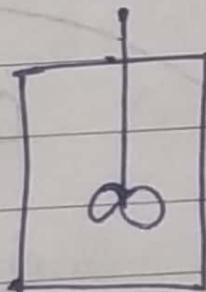
$$v = \frac{1}{N} \frac{dN}{dt}$$

$N \rightarrow \text{no. of cells}$

$\mu = v$ only for balanced growth cond^m



Mass Balance rate



Rate of input - output + generation = Accumulation.

$$0 - 0 + \tau x v = \frac{d(xv)}{dt}$$

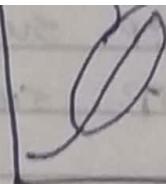
$$\mu(xv) = \frac{d(xv)}{dt}$$

$$-k_d(xv).$$

$$\mu(xv) - k_d(xv) = \frac{d(xv)}{dt}$$

$$At t=0, X=X_0 \quad \Rightarrow \quad X = X_0 e^{\mu t} \quad \checkmark$$

exponential growth (X)



Double time

At what time t have $X = 2X_0$?

$$2X_0 = X_0 e^{\mu t_{\text{double}}}$$

$$t_{\text{double}} = \frac{1}{\mu} \ln 2$$

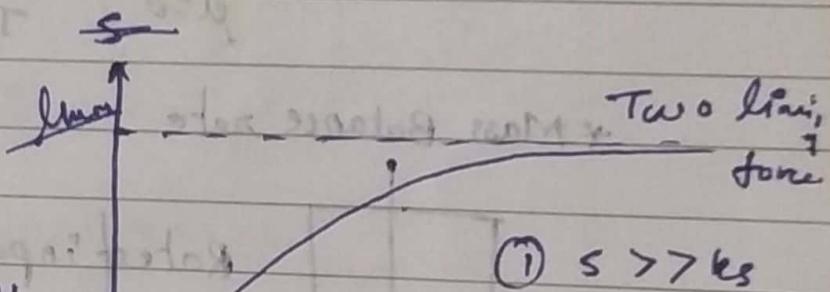
Cell Growth Models

Effect of substrate
Monod Kinetics

$s \rightarrow$ limiting substrate conc.

$$\mu = \frac{\mu_{\max} s}{K_s + s}$$

Same units.



$$\textcircled{1} \quad s \gg K_s$$

mass of substrate consumed.

$$Y_{P/S} = \frac{\text{mass of product formed}}{\text{mass of substrate consumed}}$$

$$Y_{P/X} = \frac{\text{mass of product}}{\text{increase in cell mass}}$$

$$Y_{X/S} = \frac{\text{mass of cell mass}}{\text{mass of } O_2 \text{ consumed}}$$

* Production kinetics in cell culture.

Fermentation products can be classified according to the relationship b/w product synthesis and energy generation

13/09/2022

$$Y_{X/S} = \frac{x - x_0}{S_0 - S} = \frac{\text{mass of cell produced}}{\text{mass of substrate consumed}}$$

Instantaneous yield

$$Y_{J/K} : \lim_{\Delta K \rightarrow 0} \frac{-\Delta J}{\Delta K} = \frac{-dJ}{dk} = \frac{-dJ/dt}{dK/dt} = \frac{r_J}{r_K}$$

$$Y_{J/X} = \frac{r_J}{r_K}$$

for example, $Y_{X/S}$ at a particular instant in time is defined

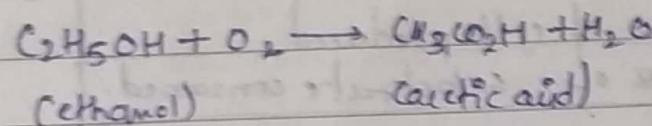
$$Y_{X/S} = \frac{x}{S} = \frac{\text{growth rate}}{\text{substrate consumption rate}}$$

Theoretical and observed yields.

$$\text{Y}_{xs} \text{, observed yield} = \frac{-\Delta X}{\Delta S} = \frac{-\Delta X}{\Delta S_G + \Delta S_P}$$

\downarrow cell growth \downarrow other metabolic activities.

photo-soham



$$\text{Overall yield} / \text{observed} = \frac{-\Delta X}{\Delta S} = \frac{x - x_0}{S_{0-S}} = \frac{9.5}{10^{-2}} = 0.94 \text{ g.g}^{-1}$$

$$\text{Theoretical} = \text{calculate from stoichiometry} = \frac{1 \text{ mol of acetic acid}}{1 \text{ mol of ethanol}}$$
$$= \frac{60 \text{ g}}{46 \text{ g}} = 1.3 \text{ g.g}^{-1}$$

Product formation kinetics

The specific growth rate of the cell (μ) is:

$$\mu = \frac{1}{x} \frac{dx}{dt}$$

The specific substrate consumption rate is:

$$q_S = \frac{1}{x} \frac{ds}{dt}$$

The specific product formation rate is:

$$q_P = \frac{1}{x} \frac{dp}{dt}$$

photo-soham 3.31 fm

$$r_p = q_P x$$

r_p = the volumetric rate of product formation

x = biomass concentration (mass/volume)

q_P = the specific rate of product formation.

photo

Sho Soman 31.86

- * Product formation Directly Coupled with energy metabolism
Kinetic expression for product formtⁿ i) growth ii) maintenance

$$\dot{r}_p = Y_{px} \dot{r}_x + m_p x$$

$$\dot{r}_x = \mu x$$

$$\dot{r}_p = (Y_{px}\mu + m_p)x$$

$$[\dot{r}_p = q_p x]$$

- * Substrate Uptake kinetics

In the absence of extracellular product synthesis, we assume that all substrate entering the cell is used for growth and maintenance functions.

The

substrate Uptake with Extracellular Product Formation:

→ Product formation directly coupled to energy metabolism

(in presence of product)

- Product formation
- directly coupled with energy metabolism $\Rightarrow r_s = \left(\frac{\mu}{Y_{xs}} + m_s \right) x$ maintenance coefficient
 - indirectly coupled with energy metabolism $\Rightarrow r_s = \left(\frac{\mu}{Y_{xs}} + \frac{q_p}{Y_{ps}} + m_s \right) P$
- Product formation
- $$q_p = \frac{1}{x} \frac{dp}{dt}$$
- $$r_p = q_p x$$

- Product formation is directly coupled to energy metabolism

$$r_p = (Y_{px} r_x + m_p x)$$

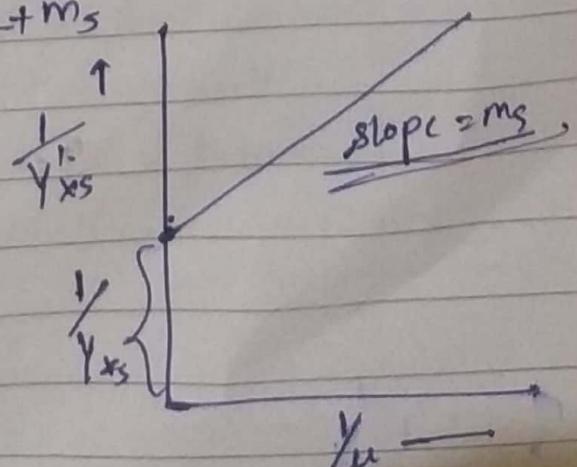
$$\Rightarrow r_p = (Y_{px} \mu + m_p) x$$

specific rate of product formation due to maintenance

Observed yield [directly coupled with energy metabolism]

$$Y'_{xs} = \frac{r_x}{r_s} = \frac{r_x}{\left(\frac{\mu}{Y_{xs}} + m_s \right) x} = \frac{\mu}{\frac{\mu}{Y_{xs}} + m_s}$$

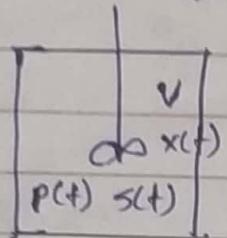
$$Y'_{xs} = \frac{\mu}{\mu + m_s}$$



$$\frac{1}{Y'_{xs}} = \frac{1}{Y_{xs}} + \frac{m_s}{\mu}$$

* Batch Reactor

well mixed, constant volume (V)



Cell

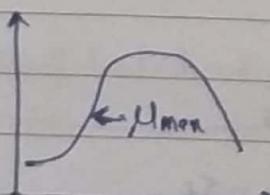
$$\frac{d}{dt}(xV) = \mu(xV) \rightarrow \frac{dx}{dt} = \mu x$$

substrate

$$\frac{d}{dt}(SV) = r_s V = -q_s(xV), \rightarrow \frac{ds}{dt} = \underline{\underline{-q_s x}}$$

Product

$$\frac{d}{dt}(PV) = q_p(xV) \Rightarrow \frac{dp}{dt} = q_p x$$



Monod's model

$$\mu = \frac{\mu_{max} s}{K_s + s}$$

$$K_s \ll s \Rightarrow (\mu = \mu_{max})$$

let us consider, $\mu \rightarrow \mu_{max}$

Batch culture time for cells to grow from $x_0 (t=0)$ to x_p (at t_b)

given model for cell growth (a)

$\int dt$

→ How much time is required for substrate conc. to go from $s_0 \rightarrow s_f$

$q_s \rightarrow \text{constant}$

$$\frac{ds}{dt} = -q_s x = -q_s x_0 e^{\mu_{max} t}$$

$$s_f - s_0 = -q_s x_0 \int_0^t e^{\mu_{max} t} dt$$

$$(s_0 - s_f) = q_s x_0 \left[\frac{e^{\mu_{max} t} - 1}{\mu_{max}} \right]$$

$$\frac{1}{\mu_{max}} \ln \left[\left(\frac{s_0 - s_f}{q_s x_0} + \frac{1}{\mu_{max}} \right) \cdot \frac{\mu_{max}}{q_s x_0} \right] = t_b$$

$$t_b = \frac{1}{\mu_{max}} \ln \left[1 + \frac{s_0 - s_f}{\frac{q_s x_0}{\mu_{max}}} \right]$$

in directly
For energy coupled case

$$q_s = \frac{\mu_{max} + m_s + \frac{q_p}{Y_{ps}}}{Y_{xs}}$$

$$t_b = \frac{1}{\mu_{max}} \ln \left[1 + \frac{\frac{s_0 - s_f}{(1 + \frac{q_p}{Y_{xs}} + \frac{m_s}{Y_{ps} \mu_{max}}) x_0}}{\frac{q_s x_0}{\mu_{max}}} \right]$$

if q_p & m_s not given neglect

→ for product

find batch time for production of given quantity of product P_f

$$\int_{P_0}^{P_f} dP = q_p \chi_0 \int_0^t e^{\mu_{max} t} dt$$

$$P_f - P_0 = q_p \chi_0 \frac{e^{\mu_{max} t} - 1}{\mu_{max}}$$

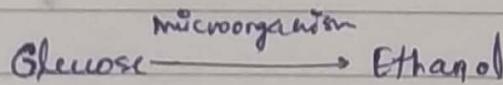
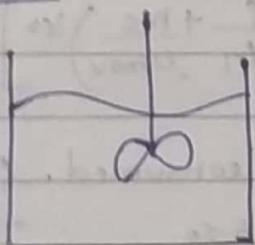
$$t_b = \frac{1}{\mu_{max}} \ln \left[1 + \frac{P_f - P_0}{q_p \chi_0} \right]$$

for directly energy coupled

$$q_p = (Y_{px} \mu_{max} + m_p)$$

put $q_p = 0$ if the product is not formed
directly coupled energy

Problem



$$Y_{xs} = 0.06 \text{ g/g}$$

$$Y_{px} = 7.7 \text{ g/g}$$

$$m_s = 2.2 \text{ g g}^{-1} \text{ h}^{-1} \quad s_0 = 12 \text{ g/L}$$

$$\mu_{max} = 0.3 \text{ h}^{-1} \quad m_p = 1.1 \text{ h}^{-1}$$

Batch fermentation
well mixed

$$\text{Vol. of Fermentor} = 50 \text{ L}$$

~~Initial inoculum = 5 gm~~ $\Rightarrow \chi_0 = \frac{5}{50} = 0.1 \text{ g/L}$

Find batch time for

(a) producing 10g biomass

$$x_f = 0.3 \text{ g/L} \quad \chi_0 = 0.1 \text{ g/L}$$

$$t_b = \frac{1}{\mu_{max}} \ln \frac{x_f}{\chi_0} = \frac{1}{0.3} \ln 2$$

$$= \frac{1}{0.3} \ln \frac{(15)}{50}$$

$$= \frac{1}{0.3} \ln 3$$

b) 90% conversion of substrate.

$$t_b = \frac{1}{\mu_{max}} \ln \left[1 + \frac{s_f - s_0}{\left(\frac{1}{Y_{XS}} + \frac{m_s}{\mu_{max}} \right) x_0} \right]$$

0.950

$$= \frac{1}{0.3} \ln \left[1 + \frac{0.9 \times 12}{\left(\frac{1}{0.06} + \frac{0.2}{0.3} \right) 0.1} \right]$$

0.95

c) producing 100g ethanol $\rightarrow P_e = 0$

$$P_f = \frac{100}{P_0} = 2 \text{ g/L}$$

~~q_p = ?~~

$$q_p = (Y_{PE} K_p + m_p)$$

d) How much time required for reaching stationary number of cell?

$$t_b = \frac{s_f - s_0}{\mu_{max}} \ln \left[1 + \frac{s_0}{\left(\frac{1}{Y_{XS}} + \frac{m_s}{\mu_{max}} \right) x_0} \right]$$

s_f = 0

e) stop when 75% of substrate is consumed. (cell mass?)

$$t_b = \frac{1}{\mu_{max}} \ln \left[1 + \frac{0.75 s_0}{\left(\frac{1}{Y_{max}} + \frac{m_s}{\mu_{max}} \right) x_0} \right]$$

s_f = 0.25

$$x = x_0 e^{\mu_{max} t}$$

f) Question: Cell mass production only

98% conversion of substrate | downtime between batches
 $= 10h$

\Rightarrow Annual production = ?

\Rightarrow Reactor volume for a given amount of annual product
 $?_r$