

ENZYMES

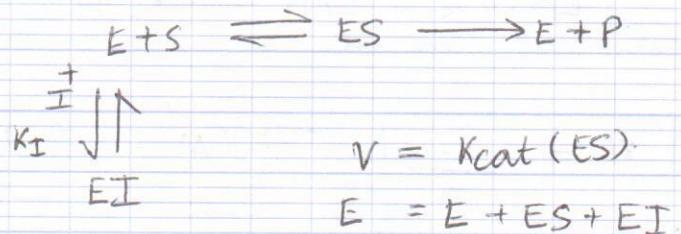
VARUNI SARWAL

2016BB10035

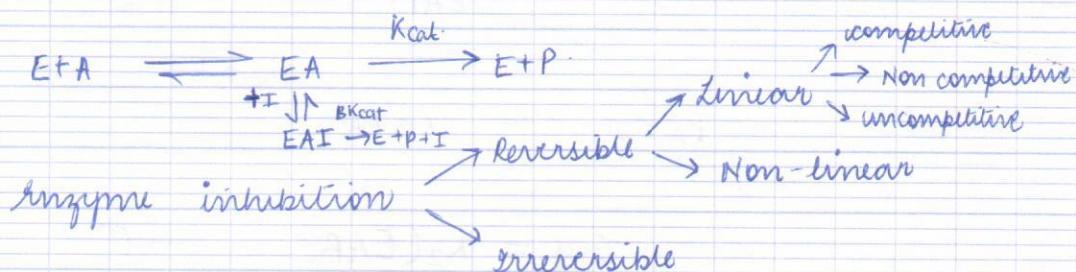
$$\frac{V}{V_0} = \frac{K_2 E A B / K_B' K_A}{E + \frac{E A}{K_A} + \frac{E B}{K_B} + \frac{E A B}{K_A K_B}}$$

$$\boxed{\frac{V}{V_0} = \frac{K_2 \cdot A \cdot B / K_A \cdot K_B'}{1 + \frac{A}{K_A} + \frac{B}{K_B} + \frac{A \cdot B}{K_A K_B}}}$$

Reversible Inhibition → competitive
 → Non-competitive
 → Uncomp.



$$K_S = \frac{E \cdot S}{ES} \Rightarrow K_I = \frac{E \cdot I}{EI}$$



Inhibit process → non linear. Some of the reaction still continues forward.

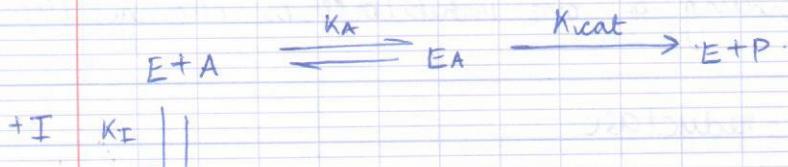


same binding site : comp.

both!

different binding site : Non-competitive (not exactly binds at binds only to E-S complex) : Uncompetitive

① COMPETITIVE (Total amt of E ↓)

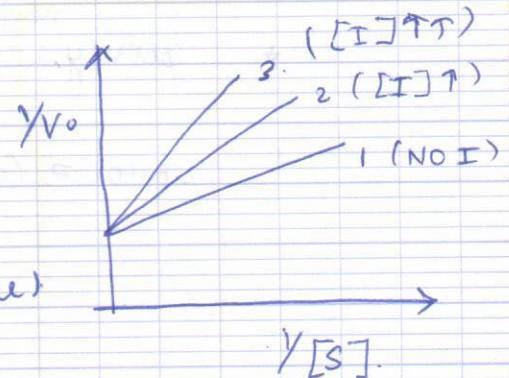


EI (can't react anymore)

$$\eta = k_{cat}(EA)$$

$$E_0 = E + EA + EI$$

$$K_A = \frac{E \cdot A}{EA}, \quad K_I = \frac{E \cdot I}{EI}$$



apparent

$$\frac{K}{V_{max}} \uparrow (K_m T)$$

$$\frac{1}{V_{max}} = \text{same}$$

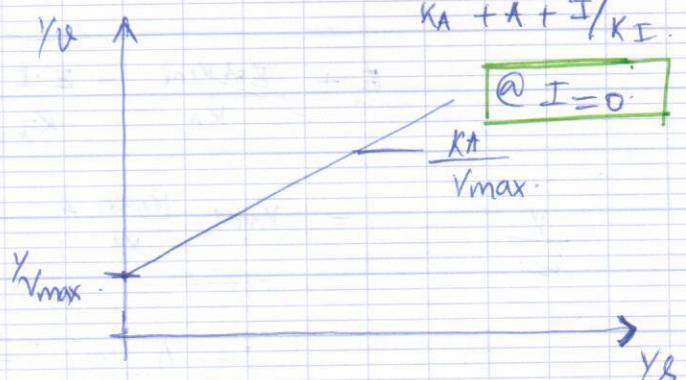
$$\frac{\eta}{E_0} = \frac{k_{cat} \frac{E \cdot A}{K_A}}{E + \frac{E \cdot A}{K_A} + \frac{E \cdot I}{K_I}}$$

$$\frac{\eta}{E_0} = \frac{\frac{k_{cat} \cdot A}{K_A}}{1 + \frac{A}{K_A} + \frac{I}{K_I}}$$

$$\frac{\eta}{E_0} = \frac{V_{max} \cdot A}{K_A + A + \frac{I}{K_I}}$$

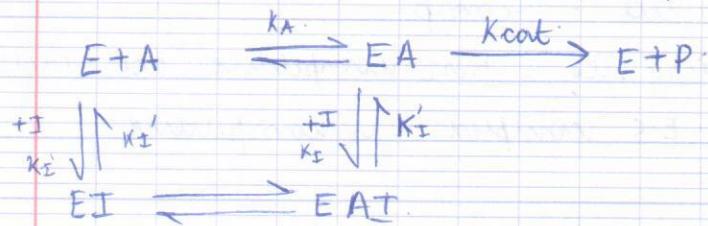
$$\frac{1}{\eta} = \frac{K_A + A + \frac{I}{K_I}}{V_{max} \cdot A}$$

$$\frac{1}{\eta} = \frac{1}{V_{max} \cdot A} (K_A + A + \frac{I}{K_I})$$



Intercept = const always

"Mix of both"
Non-competitive (Total amt of E = same)



$$\boxed{KA = KA'}$$

$$\boxed{KI = KI'}$$

- * noncomp does not change binding of sub
purely as binding of one inhibitor ~~but~~ changes the other

Lipitor \Rightarrow CoA-reductase

$$V = EA \cdot K_{cat}$$

$$E^o = E + EA + EI + EA\bar{I}$$

$$KI = \frac{E \cdot I}{E\bar{I}}, KA = \frac{EA}{K_A}, K_I' = \frac{EA \cdot I}{EA\bar{I}} = \frac{E \cdot A \cdot I}{KA \cdot EA\bar{I}}$$

$$\frac{V}{E^o} = \frac{EA \cdot K_{cat}}{E + EA + EI + EA\bar{I}}$$

$$= \frac{\frac{E \cdot A}{KA} \cdot K_{cat}}{E + \frac{E \cdot A \cdot K_{cat}}{KA} + \frac{E \cdot I}{K_I} + \frac{EA \cdot I}{K_I'}}$$

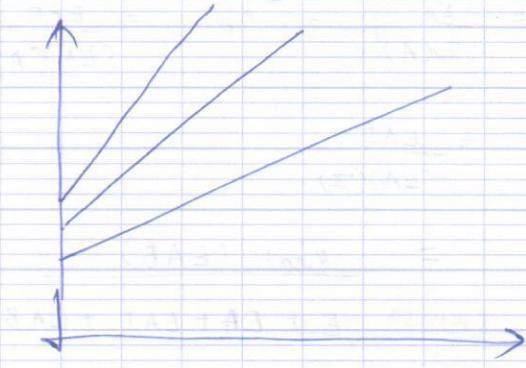
$$\frac{V}{E^o} = \frac{\frac{E \cdot A}{KA} \cdot K_{cat}}{E + \frac{E \cdot A \cdot K_{cat}}{KA} + \frac{E \cdot I}{K_I} + \frac{EA \cdot I}{K_A K_I'}}$$

$$\frac{V}{E^o} = V_{max} \frac{\frac{K_{cat}}{KA} \cdot A}{1 + \frac{A \cdot K_{cat}}{KA} + \frac{I}{K_I} + \frac{A \cdot I}{KA \cdot K_I'}}$$

$$K_I' = K_E$$

$$v = \frac{V_{max} \cdot A}{(K_A + A) \left(1 + \frac{I}{K_I} \right)}$$

$$\frac{1}{v} = \frac{1}{V_{max}} \left(1 + \frac{I}{K_I} \right) + \frac{K_A}{V_{max} A} \left(1 + \frac{I}{K_I} \right)$$

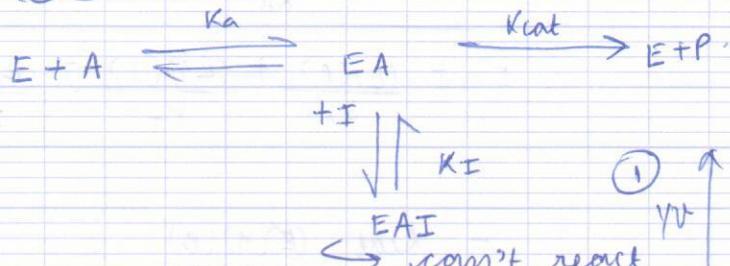


slope = $\frac{K_m}{V_{max}}$ ↑

y-axis = $\frac{1}{V_{max}}$ ↑

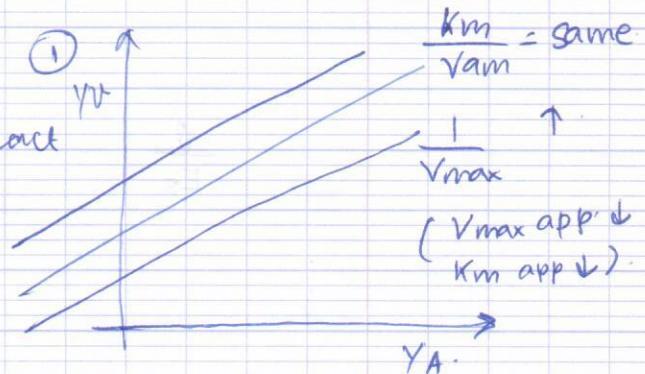
$\Rightarrow V_{max} \downarrow \downarrow$

③ Uncompetitive



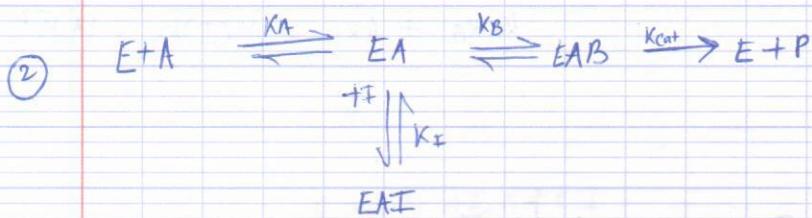
$$v = K_{cat} \cdot EA$$

$$E^o = E + EA + EAI$$

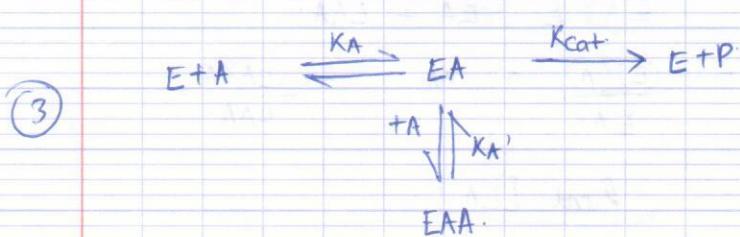


$$\frac{v}{E^o} = \frac{K_{cat} \cdot EA / K_A}{E + \frac{E \cdot A}{K_A} + \frac{E \cdot A \cdot I}{K_A \cdot K_I}}$$

$$\Rightarrow \frac{1}{v} = \frac{K_A}{V_{max}} \left(\frac{1}{A} \right) + \frac{1}{V_{max}} \left(\frac{1+I}{K_I} \right)$$



① → comp for B
↓
uncomp for A



$$v = k_{cat} [EAB].$$

$$E_0 = E + EA + EAI + EAB$$

$$K_A = \left(\frac{EA}{(E)(A)} \right)^{-1} \Rightarrow K_A = \frac{EA}{(EA)(A)}.$$

$$K_I = \frac{EAI}{(EA)(I)}.$$

$$\frac{v}{E_0} = \frac{k_{cat} (EAB)}{E + EA + EAI + EAB}.$$

$$\frac{v}{E_0} = k_{cat} \frac{(EA)(B)}{K_B}$$

$$\frac{E + \frac{(E)(A)}{K_A} + \frac{(EA)(I)}{K_I} + \frac{(EA)(B)}{K_B}}{K_A K_I K_B}$$

$$\frac{v}{E_0} = k_{cat} \frac{(E)(A)(B)}{K_A K_B}$$

$$\frac{E + \frac{(E)(A)}{K_A} + \frac{(E)(A)(I)}{K_I} + \frac{(E)(A)(B)}{K_B}}{K_A K_I K_B}$$

$$v = \frac{(v_{max})(A)(B)}{K_A K_B + A K_B + (A)(I)K_B + (A)(B)}$$

$$v = k_{cat} [EA].$$

$$E_0 = E + EA + EAA.$$

$$K_A = \frac{(E)(A)}{EA} \Rightarrow K_A' = \frac{(EA)(A)}{EAA}.$$

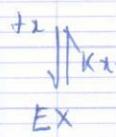
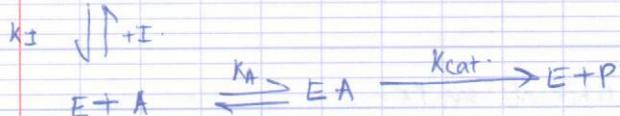
$$\frac{v}{E_0} = \frac{k_{cat} [EA]}{E + EA + EAA}$$

$$= \frac{k_{\text{cat}} \frac{(E)(A)}{K_A}}{E + \frac{(E)(A)}{K_A} + \frac{(EA)(A)}{k_A K_A}}$$

$$\frac{v}{v_0} = \frac{k_{\text{cat}} \frac{(E)(A)}{K_A}}{E + \frac{(E)(A)}{K_A} + \frac{(EA)(A)}{k_A K_A}}$$

$$\vartheta = \frac{v_{\max} \cdot A}{k_A + 1 + \frac{A}{K_A}}$$

$$= EI.$$



$$v = f(k_{\text{cat}})(EA)$$

$$E_0 = E + EA + EX + EI$$

$$\frac{v}{k_I} = \frac{(EX)I}{EI} \Rightarrow k_X = \frac{(EX)X}{EX} \Rightarrow K_A = \frac{(E)(A)}{K_A}$$

$$\frac{v}{v_0} = \frac{(k_{\text{cat}})(EA)}{E + EA + EX + EI}$$

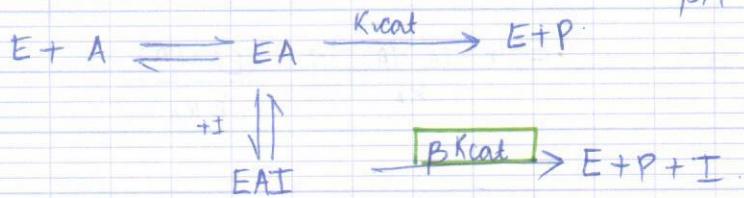
$$= \frac{(v_{\max})(E)(A)}{K_A} \frac{1}{E + \frac{(E)(A)}{K_A} + \frac{(E)(X)}{K_X} + \frac{(E)(I)}{K_I}}$$

$$\vartheta = \frac{v_{\max}(A)}{K_A + 1 + X \frac{KA}{K_X} + \frac{I \cdot KA}{K_I}}$$

Non-linear

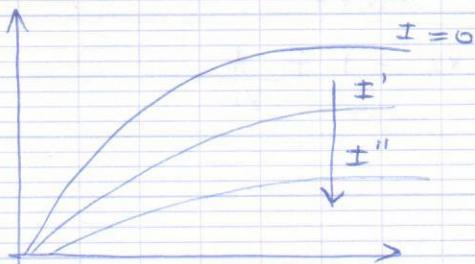
$B < I$: inhibitor

$B > I$: activator



Q K_m ? K_{cat} ? \rightarrow graphically

with an inhibitor



Q $[B] = \text{max} \Rightarrow$ rate depends on k_A .

$[A] = \text{max} \Rightarrow v \propto k_A \propto K_B$.



Not just shape, shape + charge \Rightarrow Enzyme specificity - catalytic efficiency : $K_2 = \frac{V_{max}}{E_T} = K_{cat}$

Slightly destabilized ($v \cdot k_{off} \rightarrow$ won't go forward)
Transition state analog (TSA)

Near attack conf. helps : $ES \rightarrow EX$

Q how can we design an enzyme of our interest
"atomic dance"

NMR / X-ray / crystallography \rightarrow structure of protein / enzyme

low barrier H-bond
energy

shared b/w 2 atoms at = dist.

enzyme keeps atom in near attack conq.

Mechanisms by which enzymes operate

\rightarrow covalent

\rightarrow acid-base

\downarrow metal-ion

Near attack: $d < 3.2 \text{ \AA}$

serine protease \rightarrow can break peptide bonds

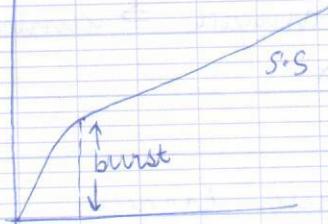
stroke: blood clot in artery / brain

tissue plasminogen activator \rightarrow helps fix the clot

AA: ser, His, aspartic acid \rightarrow 3 AA fixed in serine protease

initial rate of rxn \rightarrow before equilibrium occurs.

Serine protease,

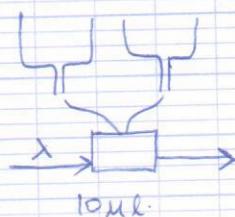


transient kinetics tell us about the # of steps

We want the rxn to start at the same pt \rightarrow rxn must be homo.

5s of mixing occurs \rightarrow every rxn has a 5s lag.

stop-flow measurement.



(so that it's homogeneous)

LBHB: lone barrier H-bond

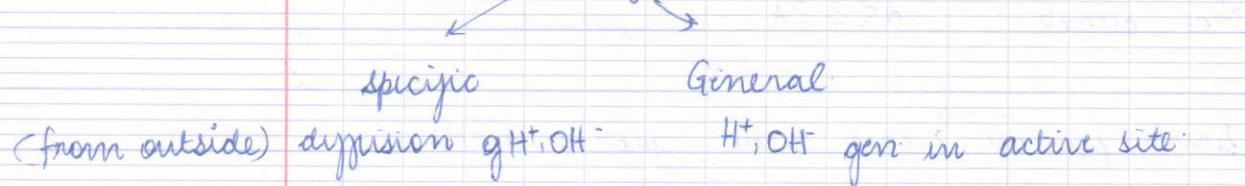
→ energy involved = twice H-bond form'n

diffusion of water into active site

prod. rel. from active site

enzyme funct. at atomic level → goal

④ acid - Base catalysis



pKa diff for 2 AA (depends on envr. condns)

specific pH window \Rightarrow both AA in protonated state

Inhibit enzyme \rightarrow TSA

Idea \rightarrow proof of concept \Rightarrow Mass market \Rightarrow clinical evaluations

difficulty:

x 10x 100x

- TSA can be used as drug targets.

25.1.19 ENZYME SOURCE (where do we get enzymes from?).

(1) animals (catalase from bovine liver).
 (chymosin from 3rd stomach of cow)
 ↳ cheese making

- (2). Plants
- Papain, papaya (proteolytic enzyme, cleaves bonds in the cell, tenderizes meat).
 - Bromelain, isolated from pineapple. polymer breakage hydrolytic property

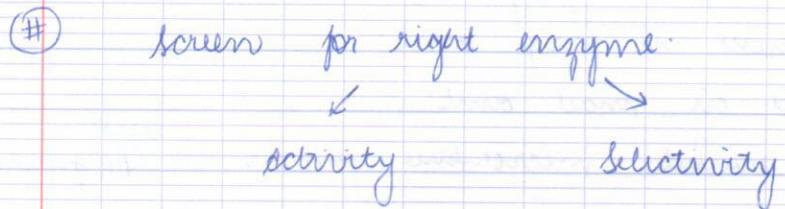
(3) Microbial source

1945: Fermentation to grow microbes. Growth of enzymatic ^{met}

enzymatic/pl: Identify host (may not be expressed well)

Microbes: Many species, ↑ diversity of enzymes → we can easily find what we're looking for.

↓
prod'n control diversity.



(1) activity (nmol sec/mg of enzyme).

$$v = \frac{V_{max} \cdot S}{K_m + S}$$

↑ K_{cat} ↓ K_m (Good enzyme)

⇒ $V_{max} \uparrow \Rightarrow$ faster rxn ⇒ More productivity
 $K_m = f$ (time req to complete rxn)

- (2) selectivity
cleaves at a part DNA seq (restriction enzymes, ECORI)
- (3) Temperature, pH, organic solvents.
tag polymerase → isolated from thermophile (active at 7T)
enzyme in detergent should be stable in pH.

TRADITIONAL SCREENING

- Nx [(1) Isolate the microbe
(large #) [(2) activity with lysate / purified enzyme.

Q what happens if you discover a useful enzyme?

29/1/19 time consuming, laborious, expensive (screening)

Q secondary metabolites of therapeutic use.

Metabolite: product from rxn inside a cell

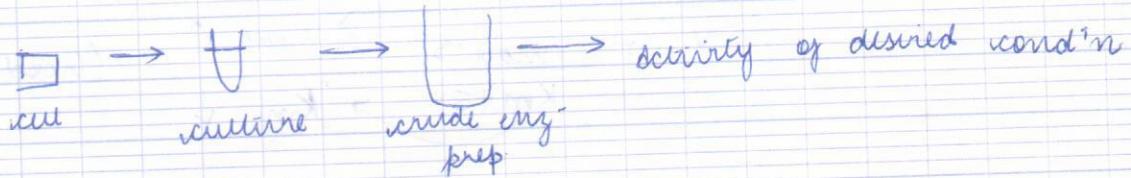
1°: synthesis in large amt.

2°: synthesis in small amt.

e.g.: Taxol: inhibit microtubule growth in cells (polymerization).

in the cell: cancer treatment.

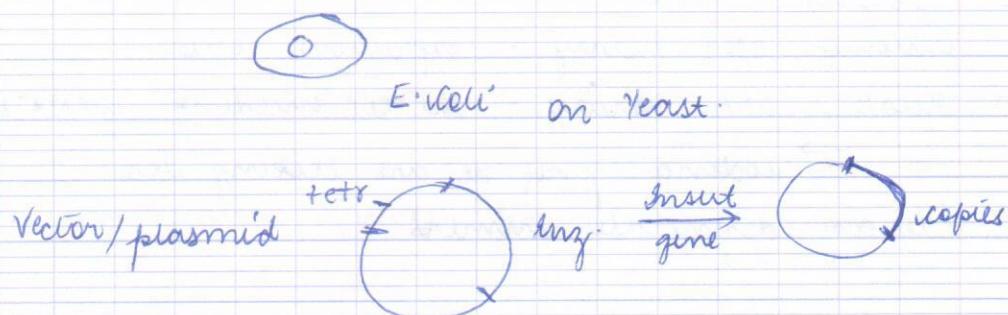
L-dopa (H-C structure) → synthesis via microorg is much easier + cheaper.



NOTE:

we cannot ignore all microorg in the lab: we don't know the metabolites req. by the microorg.

Screening → Production → Purification



amt of enz in broth: 10g/L - happens expensive, was earlier the limiting reagent.

tetr: tetracycline resistant \Rightarrow screen to see if cloning worked.

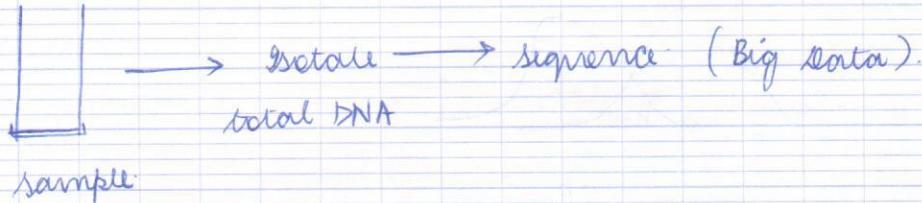
(Green fluorescent protein) GFP: measure if gene is there or not

- (1) sequence
- (2) enzyme assay for purified extract.

New model \rightarrow METAGENOMICS



Q: Learn Oxford nanopore.



Q: If you have a DNA code, how do we know if it has a gene or not?

Blast phosphokinase (we meant one that works at 9°C)

\hookrightarrow we get a very similar seq.

High throughput screening



GOAL: Getting a gene

* Genomic DNA library, expression vector

Isolate cDNA library • Isolate encoding mRNAs

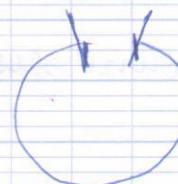
→ coding genes we are looking for

design degenerate primers

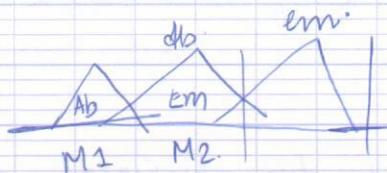
Metagenomics



copies of a gene

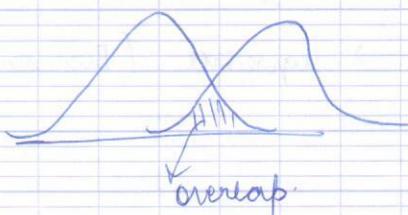


FRET



→ what is a molecular beacon?

- * non radiative transfer b/w 2 mol. You excite one, the other emits (when 2 molecules are super close to each other.)



Molecules separated → no FRET!

1 cell in 3 droplets.

electrodes → droplets go either to waste or sorted-

Take only those with a good fluorescence expression

Large scale enzyme production

case study: chymosin (dipeptidyl protease).

(Pro₁₀₅-Met₁₀₆) α -caesin (protein similar to albumin \rightarrow BSA)

- ① Aggregation (clot formation of caesin) \rightarrow bond cleavage
- ② T-stable

disadvantage: can't be obt'd in a \uparrow scale.

pepsin/protease from microbial source.

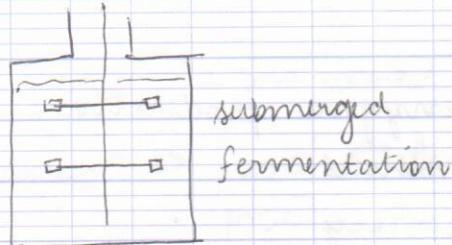
isolate cDNA of chymosin from calf.

- prochymosin
 - cDNA = reverse transcribe mRNA.
- isolate *in vivo* plasmid + put into *E. coli*
- will cDNA from calf express some protein in *E. coli*?

cDNA \longrightarrow *E. coli*
origin host \Rightarrow *Trichoderma*
 \Rightarrow *Saccharomyces cerevisiae*

pyfori \rightarrow secreted out.

grow microbes in a $\uparrow\uparrow$ scale \Rightarrow Biofermentor / Bioreactor



maintain pH, T, O₂

- ① Mixing \rightarrow liquid mixing comp'sn should be uniform
Small *Big* reactor magnet.
N

- ② Nutrients \rightarrow liquid media

Media optimization

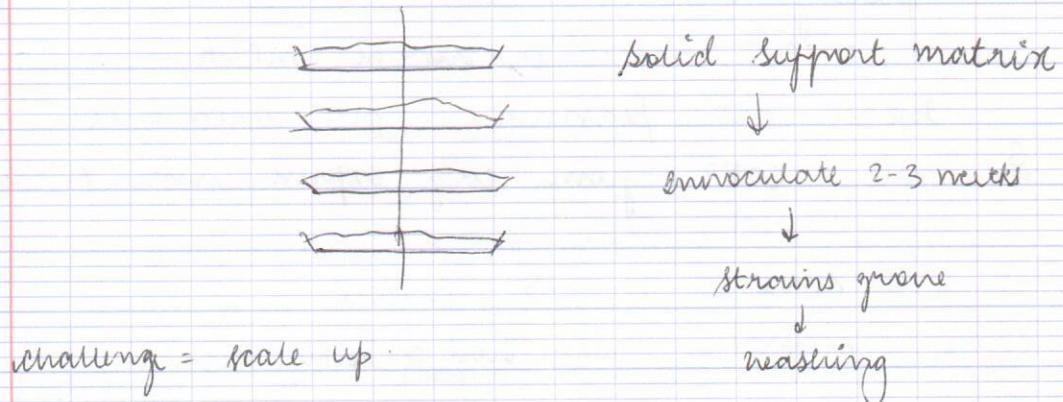
\uparrow volume \Rightarrow eng. challenge!

challenges:

- contamination
- scale-up problem.

Moving: stress and shear. Mammalian cells are fragile, will break.

E. coli: filamentous fungi \rightarrow Need a solid support
Solid state Fermentation.



\uparrow amt microbes \rightarrow need to purify

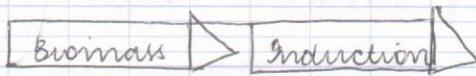
done & downside of \uparrow scale prod'n of enzymes

- 12.2.19
- (+) Large scale microbial - enzyme prod'n
 - (-) complexity

Industrial enzyme production

- (1) Enzyme synthesis
- (2) Enzyme recovery
- (3) Enzyme purification
- (4) Enzyme formulation

Bioreactor ① (Enzyme synthesis)



common promoter : IPTG

inductor \rightarrow induces own for product prod'n

intracellular + extracellular proteins

↓
Need to sap from protein it's bound to \rightarrow prepared!

② Enzyme Recovery

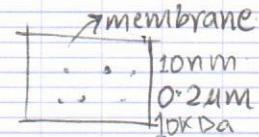
\rightarrow solid - liquid \rightarrow adv: simple.

a) centrifugation \rightarrow (scale up)

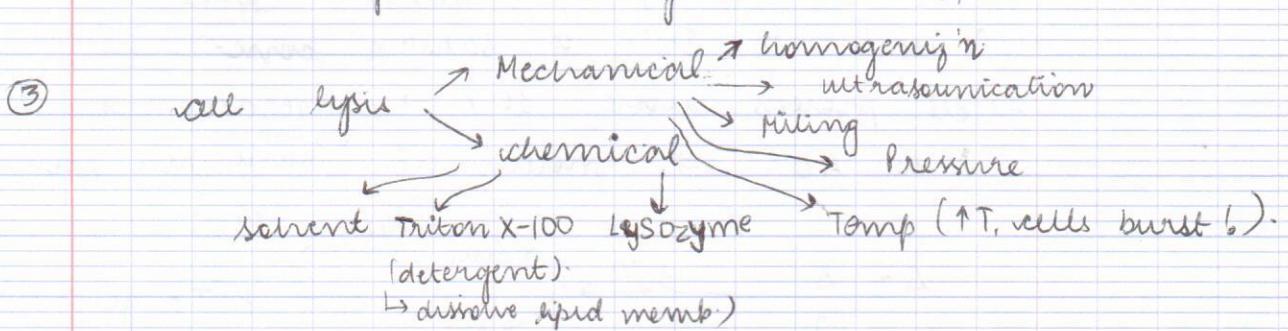
b) gravity size: hard for $\uparrow T$ vol.

c) Filtration (Micro, ultra, nano): pore size

⊕ ⊖



rate, T scale after 1st round of cake forming, $\uparrow P$ to maintain



liquid reduction \Rightarrow 1000:1 ($\downarrow V^+$ liquid)

1000 factor scale down of eq.

(a) ultra filtern 100 kDa, filter = 20 kDa

(b) precipitation / aggregation (salting out, solubility of enzyme)

\rightarrow ionic

\rightarrow surface charge

hydrophobic core



$\Delta pH \rightarrow \Delta$ charge on residues

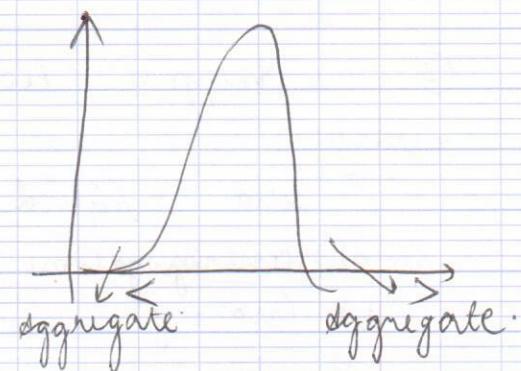
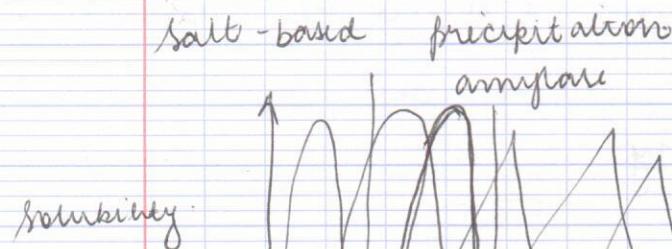
touch water \rightarrow come together + aggregate

ionic strength = # of ionizable molecules in a soln

$$F = \frac{q_1 q_2}{\epsilon r^2}, \quad \epsilon = \text{dielectric const. of medium}$$

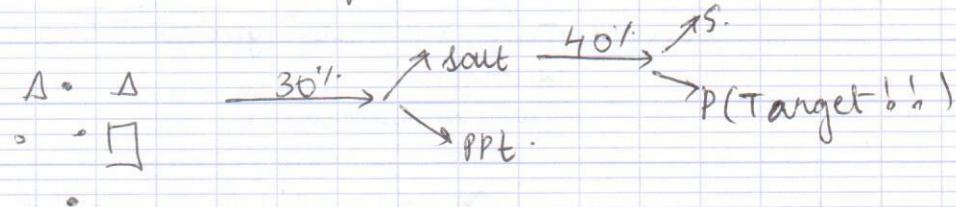


Q → How can we study the denaturation of prot.



salt-cut

- * ammonium sulphate (3M) → 100% solution
- ↑ salt to 30% of satn conc.
- ⇒ all proteins before 30% → aggregate
- ↑ to 40%. ⇒ protein we want will ppt.



Q how can we remove salt from protein.?

All proteins have different solubility curves.

centrifuging aggregates → removal

Solvilization sphere



polyethylene amine

(si) Glass \rightarrow surface charge. Lipids: intrinsic charge.

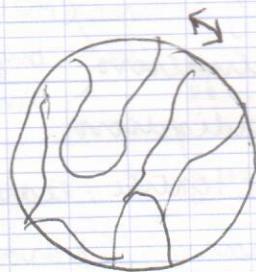
\uparrow degree purification methods.

Ion exchange

gel filtration

hydrophobic interaction

chromatofocusing

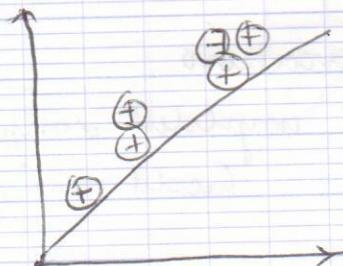


\uparrow molecules come out first (\uparrow pore size, limited diffusion).

determining the isoelectric pt of a protein
retention (binding of proteins to column)

\hookleftarrow -ve interaction.

\uparrow salt conc (ramp \uparrow)



diff proteins have diff pts where linkage is broken. \uparrow selective. \uparrow salt \rightarrow dest. stronger bonds elusion at different times.

Monoclonal antibody \rightarrow produced for 1 specific antigen
G. protein based.
antibody based therapies

WPS

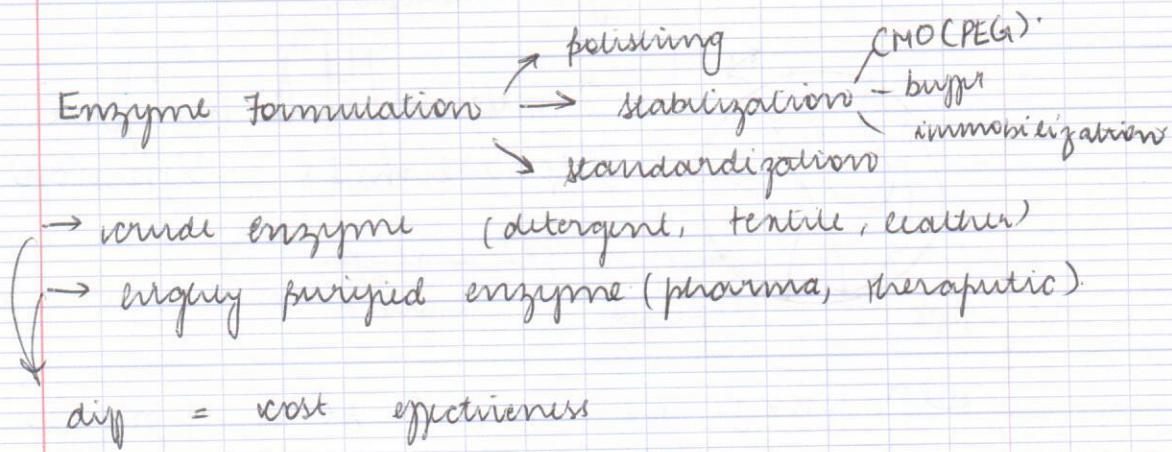
product standardization sheet
operating manual

G-coupled enzymes - treatment
(G_i-protein)

Add tags: N-terminal / C-terminal.

chemical, biological + physical influences

Purified eng. → product (Formulation step)



stabilization: stable for ↑ durations:

specific act: 1U/ml/mg stored for a long period of time ⇒ specific activity changes

↓ in activity: denaturation

folded → unfolded conformation 6 months ↑

Ⓐ Temp ↑ ⇒ unfold (cold storage?) (4°, -20°, -80°, liquid N₂)
Ⓑ ↓ week ↓ month 2 yrs

water sometimes forms ice crystals → damaging

∴ we add cryo preservatives → glycerol, sucrose (viscosity ↑), DMSO

+ limits the entropy lost of side chains

Ⓑ PEG: polyethylene glycol ⇒ limits loss of side chains mimic T effect (amine groups etc)

⑥ Chemical modifications

side chain + O₂ → oxid'n

" + N₂ → amination

⇒ form amine groups in side chains modified

⇒ dest. / loss of activity

⇒ pack sample in N₂ ↑ ⇒ remove oxidn process

DBT/ β-mercaptoethanol → removes ROS (reactive oxygen species).

⑦ Mechanical : side chain move ⇒ denaturation

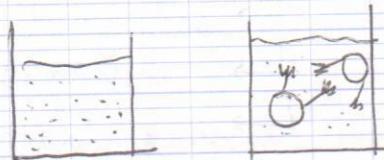
⇒ loss of activity

Add Ca²⁺ ions, pH so that the protein remains soluble (pH, coenz., ionic st → bigger takes care)

20/2/19

IMMOBILIZATION: enzymes confined to a physical matrix / location

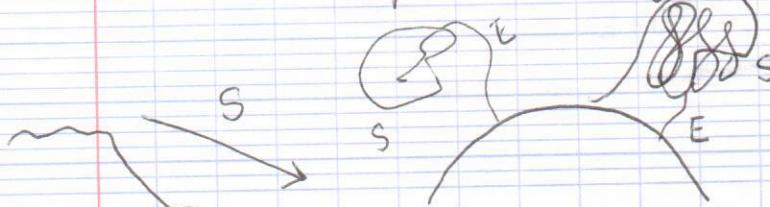
① less damage → Re-usability, K_m, k_{cat}, Specificity



Most soluble enzymes are single use.

immobilized: ↓ # enzymes/unit volume. (won't be free to move around & mix uniformly like free enzymes)

② low enzyme loading / volume, diffusion restriction / limitation



orient'n
random immobility loss of activity
put in 2 waters \rightarrow more ordered, well oriented
immobilization

sometimes, the 3D space struct gets changed during immob
 \Rightarrow change in how it binds.
 specificity also changes

gradient b/w S conc. &nt reaching surface \Rightarrow if
 earlier diffusion had no role, it would directly
 come in contact.

confinement

Immobilization
Methods

- entrapment - poly acrylamide
- ↳ covalent
- carrier bound
- ↳ ionic
- No reactivity / inert
- Mechanically stable
- No toxic
- ↳ cross linked enz agg.
- ↳ carrier loss
- ↳ across n enz crystals

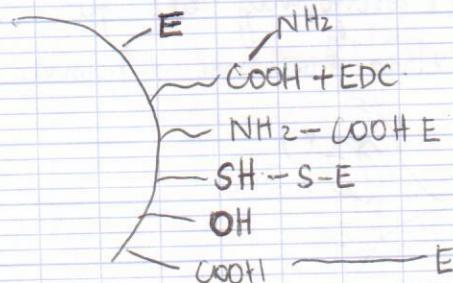
carrier: surface / matrix where immobiliz'n takes place.

Inorganic imm \rightarrow silica beads / alumina

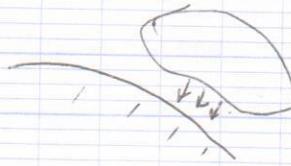
- ↳ acrylamide
- ↳ collagen
- ↳ alginate

porous particle: amt of available area ↑↑

Linking \Rightarrow covalent based carrier bound immobiliz'n \rightarrow robust, bonding
 does not get disrupted by spit etc.



IONIC BONDING

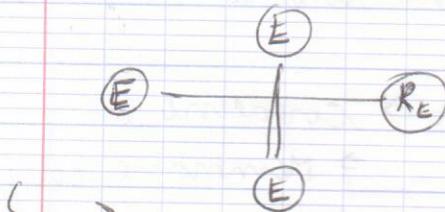


Δ ionic charge → ions absorbed on carrier
very random → loss of activity

Crude eng prep.

+ NH_4SO_4 to precipitate

+ cross linked glutaraldehyde



More susceptible to mechanical damage

protein crystal: NMR/X-Ray

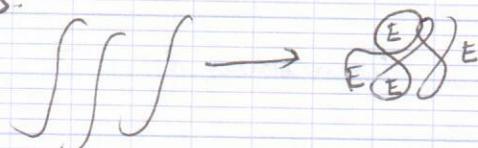
→ ordered molecule

Add cross linkers

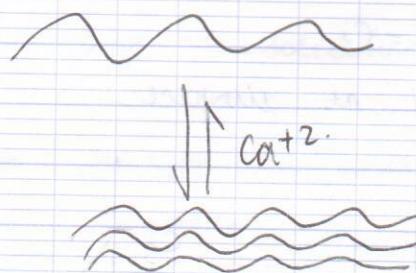
(-) : Need ↑↑ purified eng.

(IV)

Intralipament : poly acrylamide
long polymers.

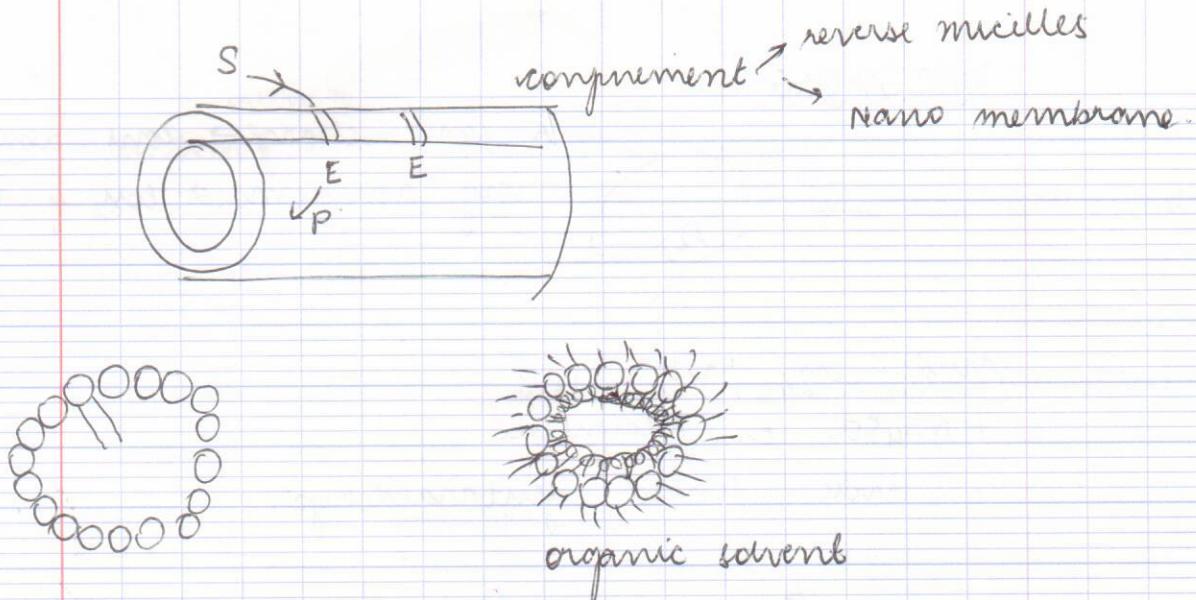


enzyme trapped b/w acrylamide



eng. trapped in solid matrix

to add & dial \Rightarrow submit



Most mechanically robust \Rightarrow covalent

\Rightarrow Membrane engg. (Nano membr.)

22/2/19 Immobilization Performance.

$$\text{imm. yield} = \frac{E_1}{E_0}$$

$$\text{enzyme loading} = 10/\text{g carrier}$$

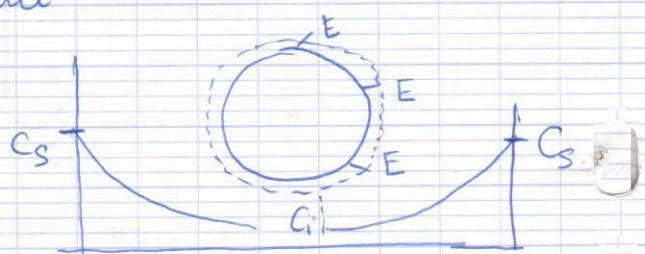
\rightarrow activity retained by the enzyme

$$\text{enzyme immobilized} = \text{mg/g carrier}$$

challenge -2: diffusional restriction \nearrow external
 \searrow internal

$$\text{bulk subs conc} = C_s$$

\hookrightarrow consumed at surface



→ conc available to enzyme

$$c_i < K_m$$

we want the rxn to be fast \rightarrow not true for $c_i < K_m$.

we want $c_i \approx c_s$ (as \uparrow as possible)

keep very \uparrow mixing, decrease viscosity

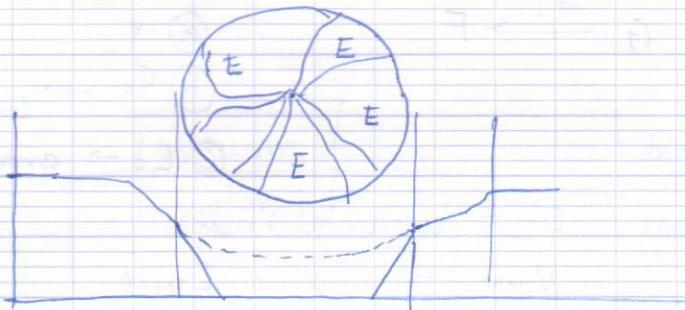
? reduce boundary layer, subs = bulk subs.

subs / carrier charge: ability of diffusion to subs.

same charge \rightarrow bad. H ion displacement.

diffusion \downarrow , pH gradient.

② internal diffusion restriction



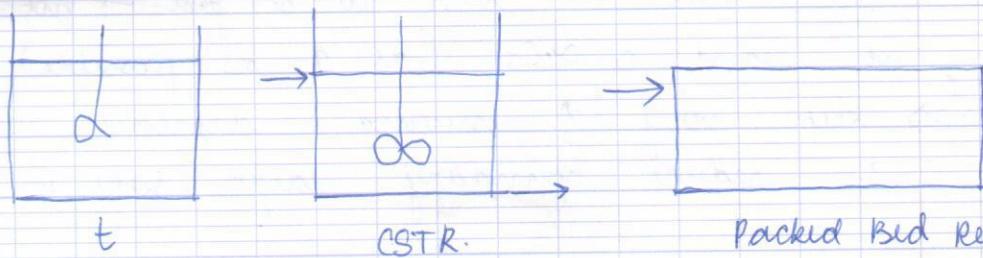
symm \rightarrow enzyme loading = uniform

rate of consumpt'n $>$ diffusion $\rightarrow c_i = 0 \rightarrow$ large internal region that does not get subs

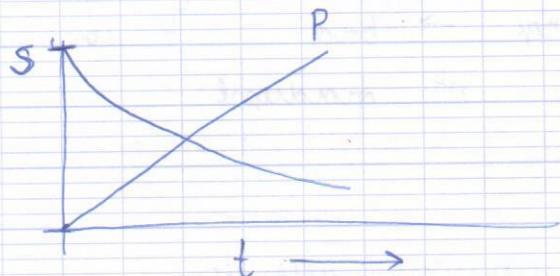
\downarrow particle size \rightarrow solution

$$\eta = \frac{\text{activity of immobilized}}{\text{activity of soluble}}$$

REACTORS



Batch reactor



$\rightarrow S$ come to prod.
 \rightarrow conversion factor
 $x = \frac{S_0 - S}{S_0}$

90%, 99%



(a) x

(b) $S_0 \rightarrow$ subs added.

(c) $E_0 \rightarrow$ amt of enzyme added

change $E_0 \Rightarrow v_{\text{change}} V_{\text{max}}$

$$V_{\text{max}} = K_{\text{cat}} E_{\text{total}}$$

$$\frac{ds}{dt} - \frac{S_0}{K_m + S} - \gamma_s = \frac{ds}{dt} \Rightarrow \frac{ds}{dt} = -\gamma_s$$

$$\frac{ds}{dt} = -\gamma_s = -\frac{V_{\text{max}} \cdot S}{K_m + S}$$

$$\int_{S_0}^S \frac{ds}{(K_m + S)} = -V_{\text{max}} \int_0^t dt$$

$$V_{\text{max}} t = \ln(S_0/S) K_m + S_0 - S$$

reversible enzyme

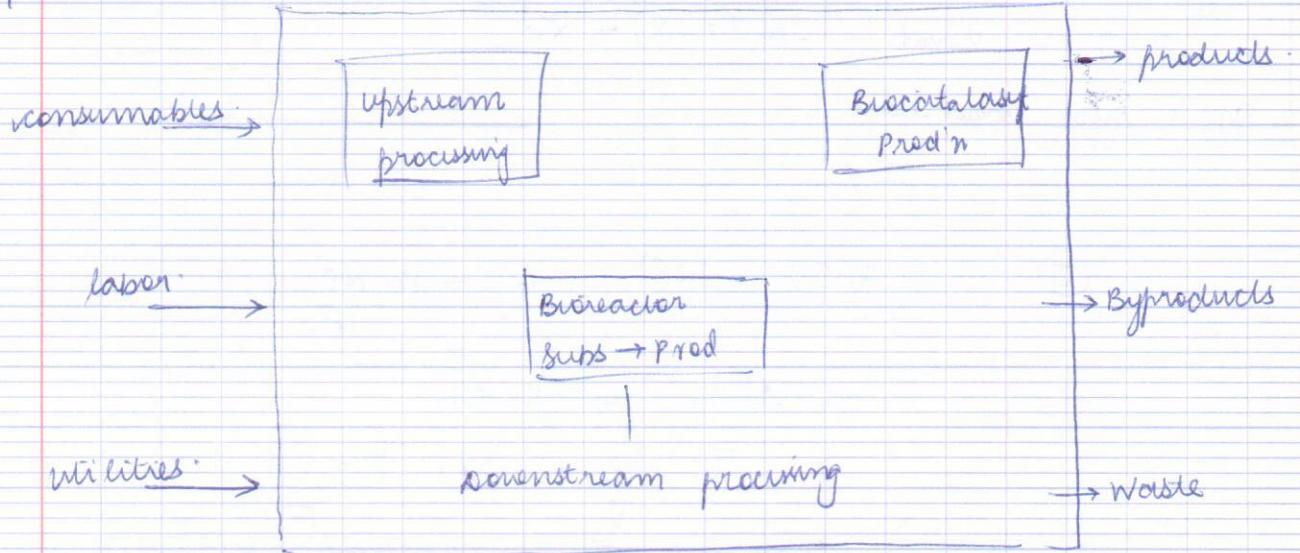
enzyme inhibition

enzyme degradat'n ($E^\circ \neq \text{const.}$)

- * Set up large scale \rightarrow how can you assess the productivity?
1. cost of enzyme
 2. t to carry out batch run
 3. # of batches / yr (batch \rightarrow recovery \rightarrow batch...)

Normally, K_{cat} , $K_m \approx \text{const}$
 change $[E]$ on surf \rightarrow change boundary layer condn
 we want rate of consumption balanced with
 rate of diffusion

26/2/19



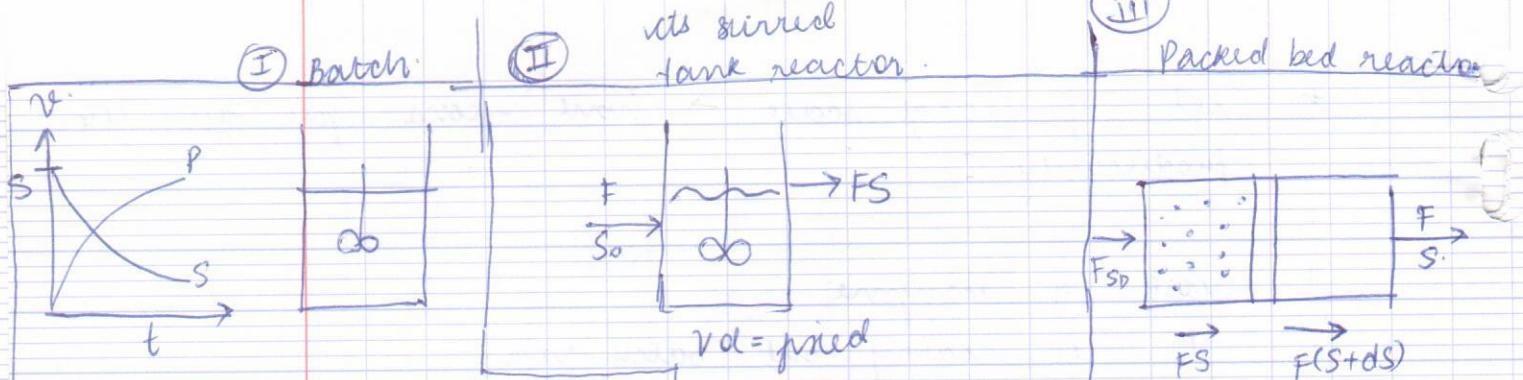
~~boxed~~ Parameters

① Market demand

20% return / year \rightarrow good
 $FD \approx 9\%$.

Mutual Fund: 15-20%.

Q Role of Km in batch, CSTR & PBR?



$$X = \frac{S_0 - S}{S_0}$$

$$t=0, X=0, S=S_0$$

max conv, $X=1$

$$\text{Sum} - S_{\text{out}} - V_s = \frac{dS}{dt}$$

$$\frac{dS}{dt} = -V_s = -\frac{V_{\max} S}{K_m + S}$$

$$\int_{S_0}^S \frac{ds}{(K_m + S)} = -V_{\max} \int_0^t dt$$

$$V_{\max} t = \ln\left(\frac{S_0}{S}\right) K_m + S_0 - S$$

$$-\ln\left(\frac{S}{S_0}\right)$$

$$-\ln\left(\frac{S-S_0}{S_0} + 1\right)$$

$$V_{\max} t = -\ln(1-X) + \frac{X S_0}{K_m}$$

$S_0 \uparrow \uparrow \rightarrow$ subs exhaustion

$$\frac{dF(S)}{dt} = \frac{V dS}{dt}$$

$$F(S_0) - F(S) - \gamma V_{\max} = V_{\max} \frac{ds}{dt}$$

$$dF(S) dt = F dV$$

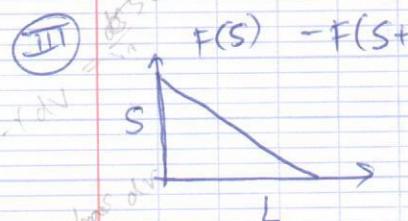
$$\gamma V_{\max} = \frac{V_{\max} S}{K_m + S}$$

$$\gamma(V_{\max}) = F(S_0 - S)$$

$$\gamma = \frac{F}{V} (S_0 - S); \quad \gamma = \frac{V_{\max} \cdot S}{F}$$

$$\boxed{\gamma = \frac{S_0 - S}{V}, \quad \frac{V_{\max} \cdot S}{K_m + S} = \frac{S_0 - S}{\gamma}}$$

$$\frac{S_0 - S}{S_0} = x \quad -ds = dx$$



$$F(S) - F(S+ds) - \gamma dV = \frac{dV ds}{dt}$$

$$-F ds = \gamma dV$$

$$\frac{S_0 - S}{S_0} = x$$

$$\boxed{F ds = -S_0 dx}$$

$$\text{OSTR: } V_{\max} = \left(\frac{S - S_0}{x} \right) \left(\frac{x_m + S + S^2/K_1}{S} \right) = x S_0 \left[\frac{x_m}{S_0(1-x)} + 1 + \frac{S_0(1-x)K_1}{S} \right]$$

$$\int_0^V g dv = -F ds$$

$$ds = -S_0 dx$$

$$\int_0^V g dv = \int_0^x F S_0 dx$$

$$\gamma = \frac{V_{\max} S}{Km + S} = \int_0^V V_{\max} dv = \int_0^x F S_0 \frac{(Km + S)}{S} dx$$

$$\frac{V_{\max} S}{F S_0} = \int_0^x \frac{Km + S}{S} dx$$

$$V_{\max} \cdot \gamma = \int_0^x \left(\frac{Km S_0}{S} + S_0 \right) dx$$

$$x = \frac{S_0 - S}{S_0}$$

$$V_{\max} \gamma = S_0 x - \ln(1-x) km$$

$$\text{Solve: } \gamma = \frac{V_{\max} S}{Km + S + S^2/K_1} = \frac{S_0 x - \ln(1-x) km}{S_0 x - \ln(1-x) km}$$

$$\int_0^V V_{\max} dv = \int_0^x F S_0 \left(\frac{Km + S + S^2/K_1}{S} \right) dx$$

$$\int_0^V V_{\max} dv = \int_0^x -\frac{F S_0}{S_0} \left(\frac{Km}{S} + 1 + \frac{S}{K_1} \right) ds$$

$$-Km \ln(1-x) (-x) ds$$

$$\left(x - \frac{x^2}{2} \right) \left(\frac{1}{K_1} \right)$$

$$-\frac{V_{\max}}{F} (x) = -\int \left(Km \ln S + S + \frac{S^2}{2K_1} \right) ds$$

$$\int_0^V v_m x dV = \int_0^x F S_0$$

$$\frac{ds}{dt} = - \frac{K_2 E_0}{\frac{x_m}{s} + 1 + \frac{x_m \cdot p}{K_i s}}$$

$$\int_0^h n dV = \int_0^x F S_0 dx$$

$$\int ds \frac{x_m}{s} + 1 + \frac{x_m \cdot p}{K_i s} = -K_2 E_0 C$$

$$x_m \ln s + s + \frac{x_m s_0 \ln(s)}{K_i} - \frac{x_m \cdot s}{K_i} \\ = K_2 E_0 C$$

$$\int_0^V \frac{K_2 E_0}{\frac{x_m}{s} + 1 + \frac{x_m \cdot p}{K_i s}} dv$$

$$= \int_0^x F S_0 dx$$

$$\int_0^h \frac{K_2 E_0}{F S_0} dv = \int_0^x \left(\frac{x_m}{s} + 1 + \frac{x_m \cdot p}{K_i s} \right) dx$$

$$x = \frac{s_0 \cdot s}{s_0} \Rightarrow s = s_0 \left(\frac{1-x}{x} \right)$$

$$x s_0 = s_0 \cdot s$$

$$s = s_0 (1-x)$$

$$\frac{K_2 E_0}{F S_0} \int_0^h dv = \int_0^{x_1} \left(\frac{x_m}{s_0 (1-x)} + 1 + \frac{x_m \cdot p}{K_i s_0 (1-x)} \right) dx$$

$$p = s_0 \cdot s$$

$$\frac{K_2 E_0}{F S_0} = - \frac{x_m}{s_0} \ln(1-x) + x + - \frac{x_m p}{K_i s_0} \ln(1-x)$$

$$x = \frac{s_0 \cdot s}{s_0}$$

$$- \ln(1-x) \left[- \frac{x_m}{s_0} - \frac{x_m p}{K_i s_0} \right] + x$$

$$\int x_m \frac{x_m (s_0 - s)}{K_i s} dx$$

$$\frac{x_m s_0}{K_i} \left(\frac{1}{1-x} - 1 \right) dx$$

$$\frac{K_2 E_0}{F S_0} \int_0^h dv \quad \left(- \frac{x_m}{s_0} \ln(1-x) + \frac{x_m s_0 \cdot \ln(1-x)}{K_i} - x \frac{x_m}{K_i} \right)$$

$$(\text{CSTR}) \quad V_{\max} \gamma = (S_0 - S)$$

$$K_2 E_0 C = S_0 X \left(1 - \frac{K_m}{K_i} \right) - K_m \ln(1-X) \left(1 - \frac{S_0}{K_i} \right)$$

$$\frac{dS}{dt} = \frac{-K_2 E_0}{\frac{K_m}{S} + 1 + \frac{K_m}{K_i} \frac{P}{S}} \quad P = S_0 - S$$

$$dS \left(\frac{K_m}{S} + 1 + \frac{K_m}{K_i} \left(\frac{S_0}{S} - 1 \right) \right) = -K_2 E_0 C.$$

$$\int dS \left(\frac{1}{S} K_m \left(1 + \frac{S_0}{K_i} \right) + 1 - \frac{K_m}{K_i} \right) = -K_2 E_0 C.$$

$$\begin{aligned} & \ln(S) K_m \left(1 + \frac{S_0}{K_i} \right) + S - \frac{S K_m}{K_i} \\ &= \ln(S_0(1-X)) K_m \left(1 - \frac{S_0}{K_i} \right) + S_0(1-X) \left(1 - \frac{K_m}{K_i} \right). \end{aligned}$$

$\therefore S_0 = 0.0179 \text{ mol/L}, \quad X = 0.98, \quad K_m = 9.9 \times 10^{-5} \text{ mol/L}.$

$V_{\max} = 2.35 \times 10^7 \text{ mol/L-sec}, \quad F = 7.786 \text{ L/Ah}.$

$V_{\text{CSTR}} = ??$

$$\begin{aligned} -V_{\max} \gamma &= S_0 X - \ln(1-X) K_m. \\ (-2.35 \times 10^7) \left(\frac{\text{vol}}{\frac{7.786}{3600}} \right) &= (0.0179)(0.98) - \ln(0.02) 9.9 \times 10^{-5} \\ V &= \frac{0.0175 \times 7.786}{2.35 \times 10^7} \end{aligned}$$

$$V = 0.0579 \times 10^7$$

$$\frac{-0.2084 \times 10^7}{208} \quad \boxed{208}$$

Subs inhib'n
prod inhib'n } IBR.

$$\text{CSTR: } K_2 E_0 C = S_0 X + \frac{K_m}{1-X} \left(\frac{X}{1-X} \right) + \frac{K_i}{K_i} \left(\frac{S_0 X^2}{1-X} \right).$$

Q2

$$V_{\max} = 250 \text{ mmole/m}^3 \text{ s}$$

$$K_m = 5 \text{ mM}, \quad S_0 = 1000 \text{ mM}$$

calculate τ Batch, $X = 0.8, x = 0.95$

$$V_{\max} \cdot t = S \cdot X - m(1-X)K_m$$

$$\left(\frac{250 \times 10^3 \text{ mole}}{\text{m}^3 \cdot \text{s}} \right) (t) = (1000 \times 10^3)(0.8) - m(0.2) \cancel{5 \times 10^3}$$

$$t = \frac{800 - m(0.2) \times 5}{250}$$

$$t = 3.2 \times 10^3$$

$$(250)(t) = \frac{950 - m(0.05)(5)}{250}$$

$$= 3.85 \times 10^3 \text{ s.}$$

$$V_{\text{ESTR}} \Rightarrow R = \frac{S - S_0}{t}$$

$$\frac{V_{\max} \cdot S}{K_m + S} = \frac{(S - S_0) \cdot F}{V}$$

$$x = \frac{S_0 - S}{S_0}$$

$$x = \frac{0.0179 - S}{0.0179}$$

$$F = \left(\frac{V_{\max} S}{K_m + S} \right) \left(\frac{V}{S - S_0} \right)$$

$$V_{\text{ESTR}} = 165 \text{ L}$$

TAKE HOME

Q1 An enzyme is used to convert substrate to a commercial product in a 1600L batch reactor.

$$V_{max} = 0.9 \text{ g/L·h}$$

$$K_m = 1.5 \text{ g/L}$$

$$S_0 = 3 \text{ g/L, stoichiometric } (S-P = \frac{1.2 \text{ g prod}}{1 \text{ g subs}})$$

$$\text{cost of labor/energy} = 4800 \text{ \$/day}$$

$$\text{cost of downstream processing: } C = 155 - (0.33X)$$

$$\$/\text{kg. } X = \text{% substrate}$$

$$\text{Market price} = 750 \text{ \$/kg.}$$

You can run the batch to 70% conv./90% conversion which one is economical? Assume much material

$$V_{maxt} = -\ln(1-X) K_m + X S_0$$

$$0.99 = -\ln(1-X) (1.5) + (X)(3)$$

$$\frac{0.99}{1.5} = -\ln(1-X) + 2X$$

→ begin solving calculators.
→ tut sheet on the website

• Before monday.

BATCH VS CSTR VS PBR

CSTR
 ○ pumps, stirrer must be automated, hard manag.
 ○ 11 vol.

subs inhibition: batch ×

Variables (batch vs CSTR)

→ Volumetric

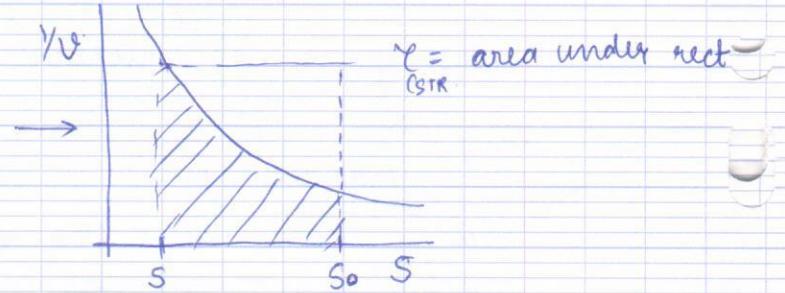
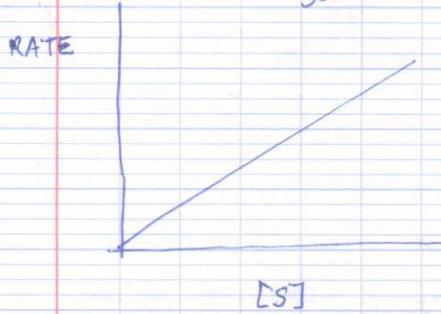
→ inhibition kinetics

CSTR vs PBR

- vol is the same
- E_0 is the same (enzyme loaded)
- Resident time : $\tau_{CSTR} = \frac{S_0 - S}{\dot{V}}$

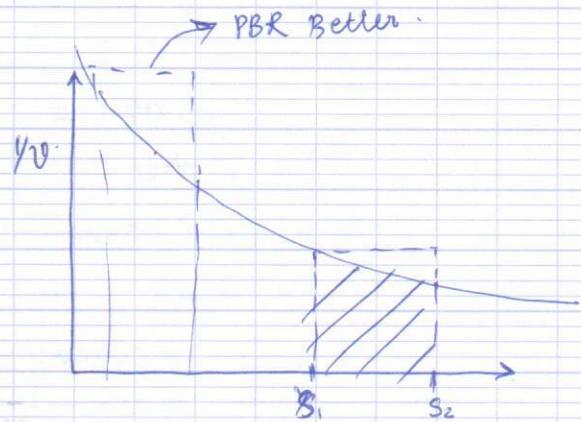
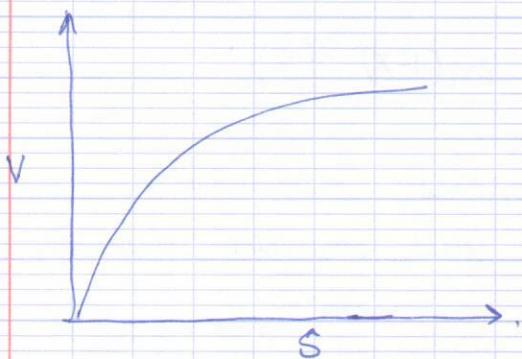
(I) rate @ rxn proceeding = s .

$$\tau_{PBR} = \int_{S_0}^S \frac{ds}{\dot{V}}$$



rate \propto [conc] \Rightarrow PBR is better (resident time)

(Case II) Michaelis-Menten

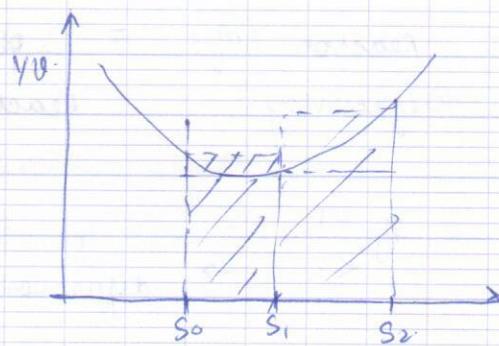
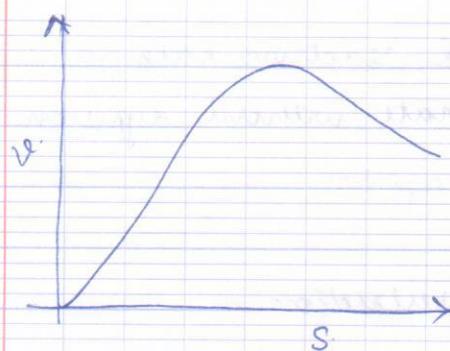


residence time / performance shift ≈ 0 b/w CSTR + PBR.

$\therefore PBR \text{ perf} \geq CSTR \text{ perf}$

PbO

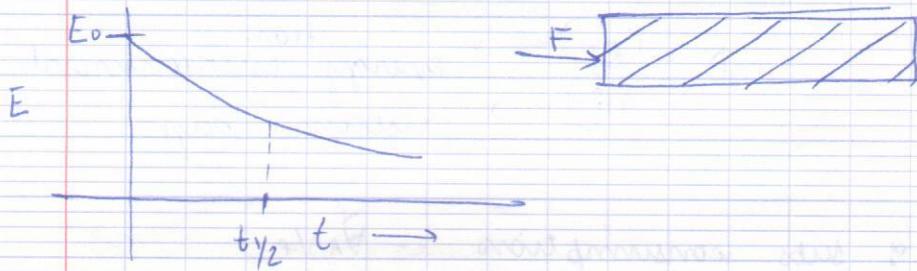
case III



PSTR better in subs inhibition zone!

$[E] \neq \text{const.}$

$t_{1/2} \Rightarrow$ half life time

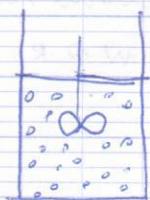
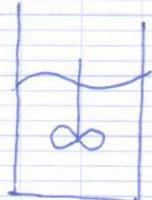


Ex, rate of conversion = const \Rightarrow change flow rate!!

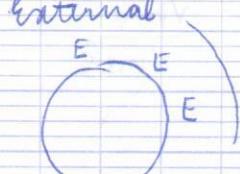
we want to re-use the catalyst

immobilize it on a solid substrate

* difference b/w immobilized + solid substrat / soluble enz



soluble \rightarrow immobilized \rightarrow enz have diffusional restriction
diff b/w [bulk subs] + [available]

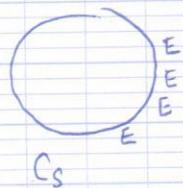


$$\frac{1000 \times V \times D}{U} = \frac{2 \times 10^3 \times U}{10^2 \times 0.01} = \frac{1000}{V}$$

reactor (η_f) = observed reaction rate
efficiency reaction rate without diffusion restriction

$\eta_f = 1$
 \leftrightarrow diffusional restriction

External diffusion restriction



$$C_b \text{ flux by diffusion} \sim (C_b - C_s) a = k_s (C_b - C_s) a$$

FLUX!



static + dynamic O transfer

$$D = \frac{RT}{mf} \rightarrow \begin{array}{l} \text{energy from environment} \\ \text{frictional way} \end{array}$$

$$\text{Rate of subs consumption} = r_A \cdot V_p$$

r_A = rate of enzyme rxn/unit particle

$$r_A \cdot V_p = k_s (C_b - C_s) a$$

case I : zero-order

$$r_A = K_0 \quad \text{if } C_s > 0$$

$$\eta_f = 1 = \frac{\text{observed rate}}{R \cdot W \cdot D \cdot R} = \frac{K_0}{K_0}$$

case II first order

$$r_A = K_1 C_s$$

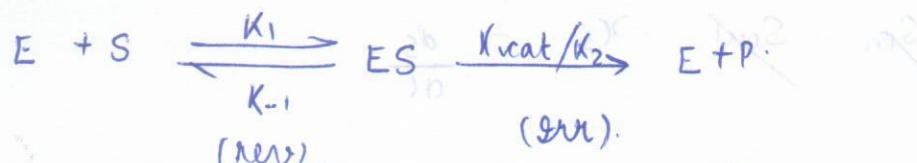
$$\eta_f = \frac{C_s}{C_b}$$

$$\text{case III (MM)} \quad r_A = \frac{V_{max} C_s}{K_m + C_s} = \frac{C_s}{C_b} \frac{K_m + C_b}{K_m + C_s}$$

ENZYMES FORMULA SHEET

① This entire course is about "enzymes" stuff that we add to reactions to make them go faster remember the transition state diagram. Fed batch etc are simple ones, that we care about is those ones + enzymes!!

② Basic overview



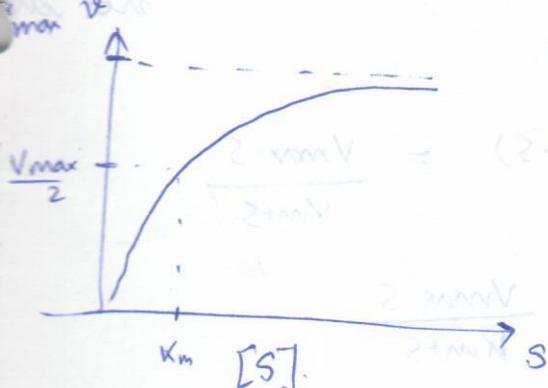
rate constant = K_{cat} , $a = K_{cat}(ES)$.

considering steady state & $E_T = \text{const}$,

$$(K_2)(ES) = \frac{K_2(E_T)S}{S + \frac{K_1 + K_2}{K_1}}$$

when $V = V_{\max}$, $ES = E_T$

$$V_{\max} = \frac{V_{\max} \cdot S}{S + K_m} \rightarrow K_m = \frac{K_1 + K_2}{K_1}$$



$$@ [S] = K_m, V = \frac{V_{\max}}{2}$$

$K_{cat} \uparrow$

$$K_1 = V_{\max} - V_{\max}$$

① zero order \Rightarrow rate ($= K_{cat}$) = independent of subs. $[S] \ggg K_m$

② first order \Rightarrow rate = $S \cdot V_{\max}/K_m$ ($[S] \lll K_m$)

③ Middle case \Rightarrow $V = V_{\max} S / (K_m + S)$

$$m = \frac{K_m}{V_{\max}}$$

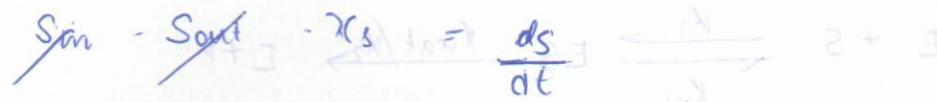
$s = 1/m$

INHIBITION.

- comp \Rightarrow same intercept \Rightarrow K_m app \uparrow (bad)
- uncomp \Rightarrow II lines \Rightarrow $v \downarrow$ (bad); K_m app \downarrow (good)
- Non-comp \Rightarrow intercept + slope = diff \Rightarrow $v \downarrow$ (bad)

REACTORS: (Goal: $S \rightarrow P$)

① Batch Reactor:



(ME)

$$-\gamma = \text{rate} = -\frac{V_{max} \cdot s}{K_m + s} = \frac{ds}{dt}. \quad (\text{NO accumul'n})$$

$$(E)_{\text{total}} = \gamma, \text{ total} = \text{initial}$$

$$V_{max} \cdot t = \ln(S_0/S) K_m + (S_0 - S)$$

$$V_{max} = K_{cat} \cdot E_{\text{total}}$$

$$\Rightarrow v_{\text{change}} = E_{\text{total}}, \quad v_{\text{change}} = V_{max}.$$

$$V_{max} \cdot t = -\ln(1-x) K_m + x S_0.$$

② CSTR: Flow rates

$$\frac{F(S_0) - F(s) - \gamma(V)}{\gamma(V) \cdot \text{vol}} = \left(\frac{ds}{dt} \right)_{(\text{vol})}. \quad ([S] \text{ does not change})$$

$$\gamma = \frac{F(S_0 - s)}{V} = \frac{V_{max} \cdot s}{K_m + s}$$

$$\gamma = \frac{S_0 - s}{t} = \frac{V_{max} \cdot s}{K_m + s}$$

③ PBR (volume, subs \neq const.)

$$F(s) - F(s+ds) - \gamma(dV) = \frac{ds}{dt} = \frac{dV}{[2]}$$

$$\Rightarrow F ds = +\gamma dV$$

$$-F ds = -V_{max} \cdot s \cdot dV$$

$$\frac{V_{max} \cdot V}{F S_0} = - \int_0^x \frac{K_m S}{S} dx$$

$\Rightarrow V_{max} \cdot V =$

$$V_{max} \cdot C = S_0 x - \ln(1-x) K_m$$

$$\star x = \frac{S_0 - S}{S_0}$$

$$\text{Reactor efficiency } (\eta) = \frac{\text{observed arm rate}}{\text{Rate after cons. pro diffusion restriction}}$$

↓
entire enzyme is exposed to subs concns.

C_b = bulk subs concn

C_s = subs concn exposed

$$\bullet \text{ zero order} = \frac{R_o [C_s]^0}{k_o [C_b]^0} = 1$$

$$\bullet \text{ First order} = \frac{C_s}{C_b}$$

• MM

$$= \frac{C_s}{C_b} \frac{(K_m + C_b)}{(K_m + C_s)}$$

internal diffusion restrictions

$$\text{Flux by diffusion} \propto (C_b - C_s)^{\frac{1}{2}} = K_b(C_b - C_s) \cdot a$$

$$\text{Rate of subs. consumption} = g_A \cdot V_p$$

rate/particle volume \rightarrow Vol/g particle

$$D_A \Rightarrow \text{Monod} \# = \frac{\text{Max arm rate}}{\text{Max flux/unit area}} = \frac{V_{max}}{K_b \cdot C_b}$$

$D_A \gg 1 \Rightarrow$ too much free enzyme

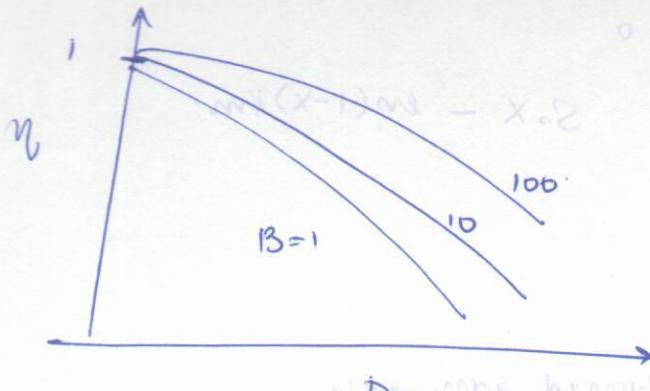
\Rightarrow unchanged V_{max}

$D_A \ll 1 \Rightarrow$ enzyme doesn't have enough flux

$$\beta = \frac{C_b + S}{K_m}$$

$$D(S_0 - S) = \dot{V} A$$

$$= \frac{\gamma V_{max} \cdot S}{K_m + S}$$



internal diffusion restriction

transport of substrate to enzyme active site

$$D_{eff} = \frac{D \cdot E \cdot H}{2}$$

C = porosity

E = porosity

↑ enzyme activity \Rightarrow C = 0 @ R = r (dead zone).

Assumptions

→ Isothermal

→ Particle homogeneous

→ Mass transfer only by diffusion

→ S+S.

maintained monolayer law



$$D_{eff} \cdot \frac{dc}{dr} \Big|_{r+\Delta r} \cdot 4\pi r^2 = in \cdot$$

$$D_{eff} \cdot \frac{dc}{dr} \Big|_r \cdot 4\pi r^2 = out \cdot$$

out-gross + consumption = 0

$$D_{eff} \cdot \frac{r^2 \frac{dC_A}{dr}}{r+\Delta r} \Big|_{r+\Delta r} - \frac{r^2 \frac{dC_A}{dr}}{r} \Big|_r = r^2 V_A$$

V_A · 4πr^2 Δr = consumption

$$\Delta r \rightarrow 0 \Rightarrow$$

$$D_{eff} \left(\frac{d}{dr} \left(r^2 \frac{dC_A}{dr} \right) \right) - r^2 V_A = 0$$

① zero order

$$V = R_0.$$

$$C_A = C_S \text{ @ } \alpha = R.$$

$$\frac{dC_A}{d\alpha} = 0 \text{ @ } \alpha = R_0.$$

R_0

$$C_A = C_S + \frac{K_0 R^2}{6 \text{Dep}} \left(\frac{\alpha L}{R^2} - 1 + \frac{2 R_0^3}{3 \alpha R^2} - \frac{2 R_0^3}{R^3} \right).$$

$$R_0 = 0 \Rightarrow$$

$$(C_{\text{concn}} = \text{const. at } R=0) \Rightarrow C_A = C_S + \frac{K_0 (\alpha^2 - R^2)}{6 \text{Dep}}$$

Max size for no dead zone $\Rightarrow C_A = 0 @ \alpha = 0.$

$$K_{\max} = \sqrt{\frac{6 \text{Dep} \cdot C_S}{K_0}}$$

② First order

$$\alpha = k_1 C_A.$$

$$C_A = C_S @ \alpha = R.$$

$$\frac{dC_A}{dS} = 0 @ \alpha = 0$$

$$C_A = C_S \cdot \frac{k}{\pi} \left[\frac{\sin(\alpha \sqrt{k_1 / \text{Dep}})}{\sinh(\alpha \sqrt{k_1 / \text{Dep}})} \right] \quad (\text{Min } \alpha = \frac{e^x - e^{-x}}{2})$$

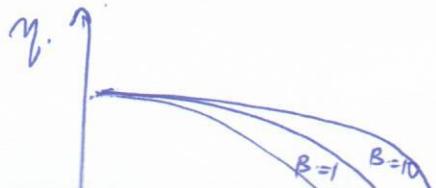
③ MM

$$\alpha_A = \frac{V_{\max} \cdot C_A}{K_m + C_A}. \quad (\text{No eqn of } C_A).$$

 we calculate C_A graphically.

three modulus (ϕ):

$$\phi = \frac{V_p}{Sx} \frac{\alpha_A / C_S}{\sqrt{2}} \int_{C_A \text{ eq}}^{C_A} \sqrt{\text{Dep} \cdot \alpha_A dC_A}$$



$\beta = 100$

$$\text{poreholder #} = \frac{\text{max run rate}}{\text{max feed throughput}} = \frac{V_{\text{max}}}{K_S \cdot C_b}$$

flux/area.

- if $dA \gg 1 \Rightarrow$ rate of run \gg diff. restriction
- \Rightarrow too much enzyme
- \Rightarrow change V_{max}
- $dA \ll 1$
- \Rightarrow enzymes don't have enough flux
- \Rightarrow \uparrow bulk concn \Rightarrow \uparrow surface concn

$$\beta = \frac{C_b}{X_m}$$

$[S] \ll K_m$: 1st order

$[S] \gg K_m$: 0 order

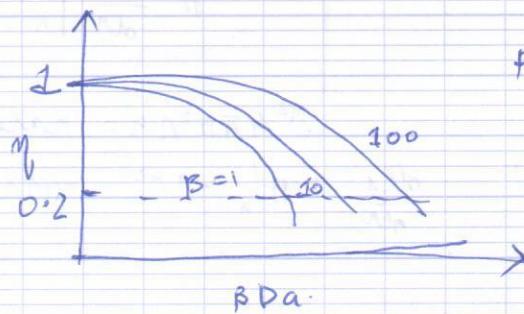
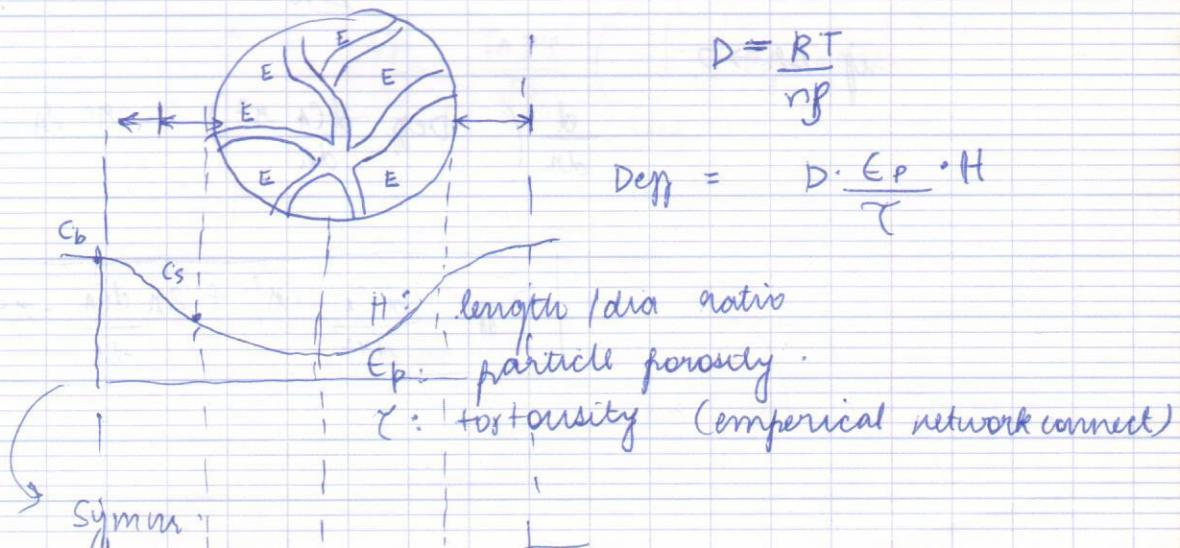


Fig 2.9 - Clark Blanch

$$\begin{aligned} D(S_0 - S) &= \gamma_A \\ &= \frac{\eta V_{\text{max}} \cdot S}{K_m + S} \end{aligned}$$

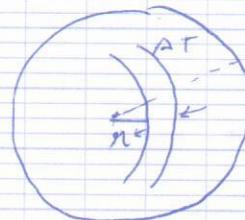
Internal diffusion restriction



Q How can we determine the D_{eff} for sucrose in immobilized bead? (experimental determination $\Rightarrow ?$)

- availability of enzyme
- $\eta \approx 1$

- (a) Isothermal
- (b) Particle homogeneous
- (c) Mass transfer only by diffusion (Pict lane)
- (d) Steady state



$$D_{eff} \frac{dC_A}{dr} \Big|_{r=r_0} \frac{4\pi r^2}{r+r_0} = \text{out}$$

$$D_{eff} \frac{dC_A}{dr} \Big|_{r=r_0} \frac{4\pi r^2}{r} = \text{out}$$

$\pi^2 4\pi r^2 \Delta r = \text{rate of consumption}$

$$\frac{dC_A}{dr} D_{eff} \Big|_{r=r_0} 4\pi r^2 - \frac{dC_A}{dr} D_{eff} \Big|_{r=r_0} \frac{4\pi r^2}{r} - 4\pi r^2 \Delta r V_A = 0$$

$$\frac{-4\pi \Delta r \quad D_{eff} \pi^2 \frac{dC_A}{dr} \Big|_{r=r_0} - \frac{dC_A}{dr} \pi^2 D_{eff} \Big|_{r=r_0} - \pi^2 V_A}{\Delta r} = 0$$

if $\Delta r \rightarrow 0$

$$\frac{d}{dr} \left(D_{eff} \frac{dC_A}{dr} \pi^2 \right) - \pi^2 V_A = 0'$$

$$\boxed{D_{eff} \frac{d^2 C_A}{dr^2} \pi^2 + 2\pi \frac{dC_A}{dr} - \pi^2 V_A = 0'}$$

case 1: zero order

$$V_A = K_0 \quad (\text{if } C_A > 0)$$

Boundary cond'n

$$C_A = C_S \quad @ \quad r=R$$

$$\frac{dC_A}{dr} = 0 \quad @ \quad r=R_0$$

$$\frac{d}{dr}$$

$$C_A = C_S + \frac{K_0 R^2}{6 D_{eff}} \left(\frac{r^2 - 1 + 2R_0^3}{R^2} - \frac{3R^2 r}{2R_0^3/R^3} \right)$$

$$R_0 = 0$$

$$C_A = C_S + \frac{K_0 (r^2 - R^2)}{6 D_{eff}}$$

case 2: first order

$$V_A = K_1 C_A$$

$$\text{B.C.} \quad C_A = C_S @ \quad r=R$$

$$\frac{dC_A}{dr} = 0 @ \quad r=0$$

$$C_A = C_S \frac{r \sinh(a\sqrt{K_1/D_{eff}}r)}{\sinh(R\sqrt{K_1/D_{eff}}r)}$$

$$\sinh u = \frac{e^u - e^{-u}}{2}$$

case 3: MM.

$$V_A = \frac{V_{max} \cdot C_A}{C_A + K_m}$$

we want C_A is never zero inside the particle

\Rightarrow max. size s.t. no dead zone $\Rightarrow C=0 @ \quad r=0$.

$$r=0 @ \quad C=0$$

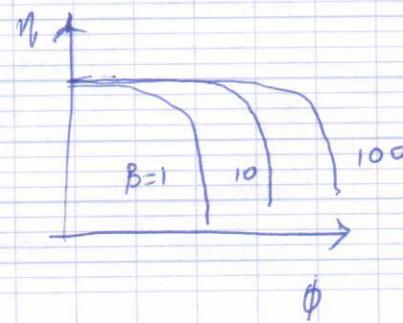
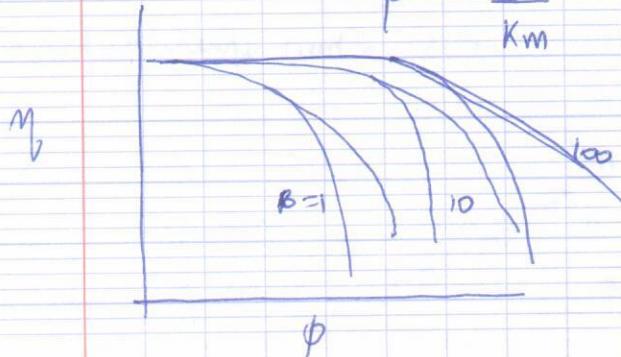
$$R = \sqrt{\frac{6 D_{eff} C_S}{K_0}}$$

$$\boxed{D_{eff} \left(\frac{d^2 C_A}{dr^2} r^2 + 2r \frac{dC_A}{dr} \right) - r^2 V_A = 0}$$

Third modulus:

$$\phi = \frac{V_p}{Sx} \frac{r_A C_S}{\sqrt{2}} \left(\int_{C_{eq}}^{C_A} \sqrt{D_{eff} r_A d(C_A)} \right).$$

$$\beta = \frac{C_S}{K_m}$$



observable Thiele modulus (case: NO Km, Vmax of immobilized enzyme)

$$\phi' = \left(\frac{V_p}{Sx}\right)^2 \frac{\eta_A ds}{\text{Deg CAS}}$$

1st $\phi' = \phi^2 \eta_0$

zero $\phi' = 2\phi_0^2 \eta_0$

MM $\phi' = 2\phi_m^2 \eta_m (1+\beta) \left[1 + \beta \ln\left(\frac{\beta}{1+\beta}\right) \right]$

Generalized Thiele modulus

$$\phi_1 = \frac{R}{3} \sqrt{\frac{K_1}{\text{Deg}}}$$

$$\phi_0 = \frac{R}{3\sqrt{2}} \sqrt{\frac{K_0}{\text{Deg} \eta \text{CAS}}}$$

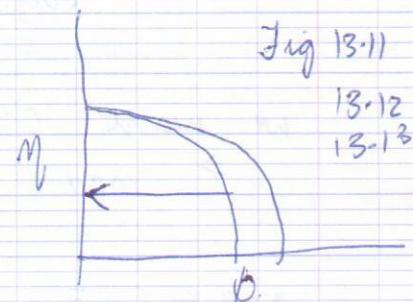


Fig 13.11

13.12
13.13

$$\phi_m = \frac{R}{3\sqrt{2}} \sqrt{\frac{V_{max}}{\text{Deg} \eta \text{CAS}}} \left(\frac{1}{1+\beta} \right) \left[1 + \beta \ln\left(\frac{\beta}{1+\beta}\right) \right]^{-1/2}$$

$$\beta = \frac{K_m}{C_{AS}}$$

$[S] \gg K_m$; 0 order

$[S] \ll K_m$; 1st order

- Immobilized (STR)

- Mushroom tyrosinase enz; 2 mm spherical bead

- $K_m = 2 \text{ g mol/m}^3$

$S_0 = 15 \text{ g mol/m}^3$

$V_{max} = 1.5 \times 10^{-2} \text{ g moles per m}^3 \text{ bead}$

enzyme loading = $0.25 \text{ m}^3 / \text{m}^3 \text{ g reactor volume}$

$$D_{\text{eff}} = 7 \times 10^{-10} \text{ m}^2/\text{s}$$

external diff restriction is negligible
 $X = 0.99$

$$F = 18 \text{ m}^3/\text{day}$$

Volume of reactor = ?

(a) V_{reactor} assuming no diffusion restriction

$$F(S_0) - F(S) - \gamma_{\text{per vol}} \cdot V_{\text{reactor}} = 0$$

$$\downarrow \\ \eta \circlearrowleft \\ \downarrow \\ \text{per vol g mmol}$$

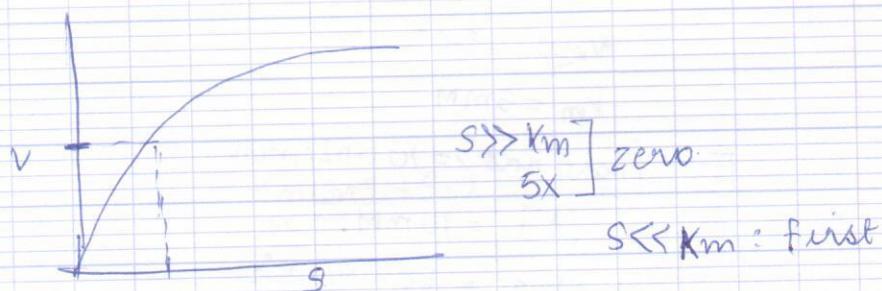
$$X = \frac{S_0 - S}{S_0} = \frac{15 - S}{15} = 0.99$$

$$15(0.01) = S \Rightarrow 0.15$$

$$\frac{18 \text{ m}^3}{\text{day}} \left(0.99 \times 15 \frac{\text{g mmol}}{\text{m}^3} \right) - \gamma V_A = 0$$

$[S] \ll K_m \Rightarrow$ here we take S , not S_0 !

$$V = 11.05 \text{ m}^3$$

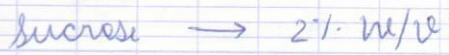


$$V_i = \frac{V_{\text{max}}}{K_m + S}$$

$S \ll K_m$: first

ENZYME

Q1 d batch reactor

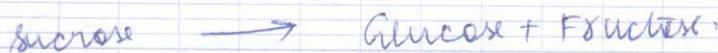


Mol wt \rightarrow 342 (Sucrose) $\rightarrow G+F$

$$V_{\max} = 2 \text{ mmol/min I}^{-1}$$

$$K_m = 5 \text{ mM}$$

% conversion after 40 mins



$$S_0 - S$$

$$X = \frac{S_0 - S}{S_0} \alpha \quad \alpha$$

$$S_0 - \frac{2}{100} \times \frac{1}{342} \text{ w/v}$$

100ml \rightarrow 2g

$$V_{\max} \cdot t = -\ln(1-X) K_m + X S_0$$

$$(2 \times 10^{-3})(40) = -\ln(1-X)(5 \times 10^{-3}) + X \left(\frac{2}{100} \times \frac{1}{342} \right)$$

Q2

degradation

(a) CSTR

(b) PBR

$$V_{\max} = 500$$

$$K_m = 5 \text{ mM}$$

$$F = \text{flow rate} = 1000 \text{ L/min}$$

$$[A]_0 = 10 \text{ mM} \rightarrow S_0$$

$$U_{\max} = 50 \text{ mM/min}$$

99% conversion

$$\text{total enzyme} = 10^{-3} \text{ mM}$$

$$(500)(t) = -\ln(1-0.99)(5 \times 10^{-3}) + (0.99)$$

$$V_d = \frac{(S_0 - S)(K_m + S) \times F}{V_{\max} \cdot S} = \frac{(0.99)(15)(1000)}{(500)(10)}$$

$$\tau = \frac{V_{\text{vol}}}{F}$$

(STR, PBR,
1000, 658)

$$V_{\text{max}} \tau = S_0 X - \ln(1-X) K_m$$

$$V_{\text{vol}} = \frac{(10 \times 10^3)(0.99) - \ln(0.01) 5 \times 10^{-3} \times 1000}{500}$$

$$\text{case 2} \Rightarrow V = V_{\text{max}} \frac{110}{\text{max.}}$$

$$= - \frac{dE}{dt} = k_o E^2 \quad \therefore \text{2nd order kinetics}$$

- fraction conversion
- time
- enzyme \rightarrow Batch reactor

$$-\int \frac{dE}{k_o E^2} = \int dt \quad V_{\text{max}} = k_{\text{cat}} \cdot E$$

$$(E)^{-2} \frac{dE}{dt} = t \quad \frac{S_0 - S_0 e^{-k_o t} - V_s}{S_0} = \frac{ds}{dt} = -V_s = \frac{V_{\text{max}} s}{K_m + s}$$

$$E = \frac{1}{k_{\text{cat}}} \left[\frac{1}{E} \right]_{E_0}^E$$

$$\int ds \cdot \frac{(K_m + s)}{s} = \int k_{\text{cat}} \cdot E dt$$

$$\frac{1}{E_0} - \frac{1}{E} = k_{\text{cat}} t$$

$$K_m(\ln s + s) = \frac{k_{\text{cat}} t}{k_o}$$

$$\frac{1}{E_0} - \frac{1}{E} = \frac{1}{k_{\text{cat}} t}$$

$$E = \frac{1}{k_{\text{cat}} t + E_0}$$

$$-k_{\text{cat}} \log \left(\frac{E_0}{E} \cdot \frac{R_o}{R} \right)$$

- Numerical
- Guis +
- 5 min via

S11



$$d = 1.6 \text{ mm}$$

$$S = 0.1 \text{ } \mu\text{mol enzyme/g}$$

$$\text{effective diff} = 1.3 \times 10^{-11} \text{ m}^4/\text{s}$$

$$[S] = 0.85 \text{ kg/m}^3$$

$$\text{conversion rate} = 1.25 \times 10^{-3} \text{ kgS}^{-1} \text{ m}^{-3}$$

$$K_m = 3.5 \text{ kg/m}^3$$

$$\eta = ?$$

29/3/19

Minor 1.

application ^{savory}
textile

Functional nature + kinetics inhibition

enzyme production

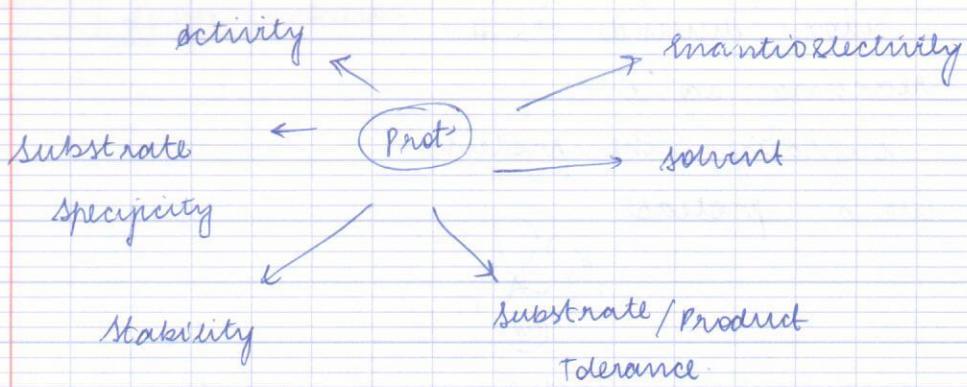
Minor 2

- enzyme immobilization
- reactor mass balance

Major

- enzyme eng.
- Non-aqueous enzymology
- 3 sectors in detail
- Techno-commercial aspects

PROTEIN ENG.



Always want $\xrightarrow{\text{activity}}$ more product/enzyme/t.

- substrate specificity \Rightarrow high recognition to a particular chemical signature.
Atomic orientations recognize subs & make it suitable for binding
uase \rightarrow used in an organic solvent (oil).
- recombinant enzymes: is it possible?
ep PRR \rightarrow methodology for modification

Improving thermal st

- \rightarrow add disulphide bonds
- \rightarrow replacing labile A-A (V-reactive) \Rightarrow harmful effect.
- \rightarrow reduce # free SH groups. (S-H dimerize to cause loss of act)
Prot. thermal st can be \uparrow by creating molecules that do not fold

adding disulphide bonds \rightarrow stabilize native st.
 Table 8.2 \rightarrow makes the molecule more thermally stable.
 $T_m = 41.9^\circ\text{C}$

ENZYM