Heterogeneous phase enzyme reactions

Advantages/disadvatages:

Advantages:

- homogeneity of the system,
- enzyme does not need previous preparation - (over isolation and purification)

Economic disadvatages:

- Enzymes are expensive, 1-10- $/mg
- can be used only once, after reaction they are to be discarded…

Technological disadvatage:

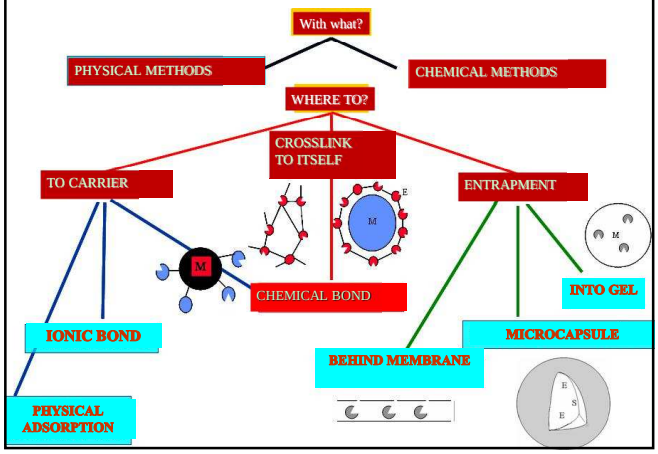
- Proteins contaminate products

CHEMICAL METHODS

Covalent bond between non essential amino acid sidechain(!) and water insoluble matrix with function groups

CARRIERS :

natural polymers: *agar, agarose, chitin, cellulose, collagene,...*,
synthetic polymer: *polyurethane*, *polystyrene, nylon*, ...,
inorganics: *glass, aluminium, silicagel, magnetit*,...



CHEMICAL METHODS

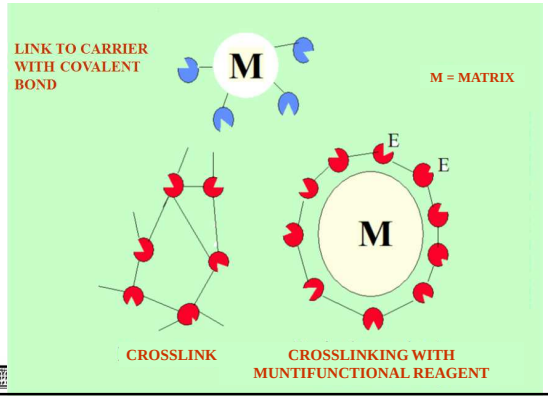
Building of covalent bond:
free α-, β- or γ-COOH , α-, β –NH_2 groups
phenyl-, OH-, SH- imidazole-groups

STEPS:

1. Activation of carrier (arm and reactive X-group),
2. Creating covalent bond between enzyme and activated carrier.

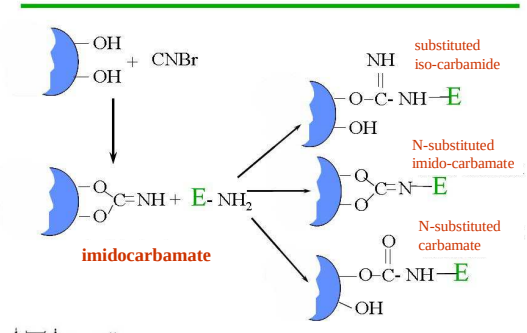
Protection of the active sites: S or analog

CHEMICAL METHODS



MATRIX: vicinal –OH goups like:

cellulose, Sepharose, Sephadex



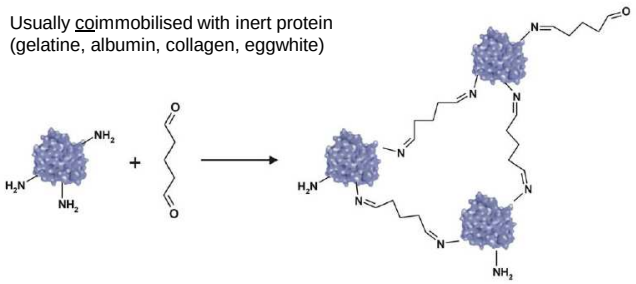
Origin of carbohydrate matrix

Glucose → dextrane → Sephadex®

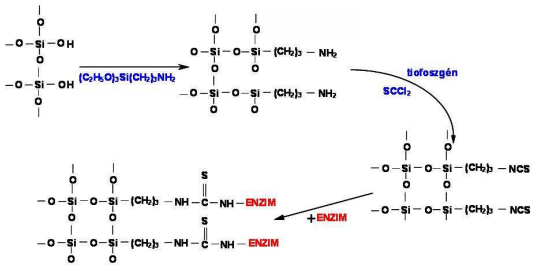
Alga → agar(ose) → Sepharose ®

Chemical methods: crosslinking

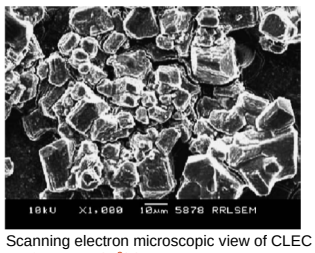
Usually coimmobilised with inert protein
(gelatine, albumin, collagen, eggwhite)



Immobilization onto glass surface



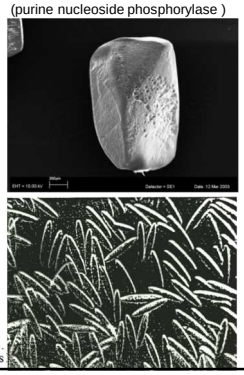
CLEC = Cross-Linked Enzyme Crystals



Scanning electron microscopic view of CLEC laccase
Surface area (m^2/g) 2.456

Preparation and characterization of cross-linked enzyme crystals of laccase, J. J. Roy, T. E. Abraham Journal of Molecular Catalysis B: Enzymatic 38 (2006) 31–36

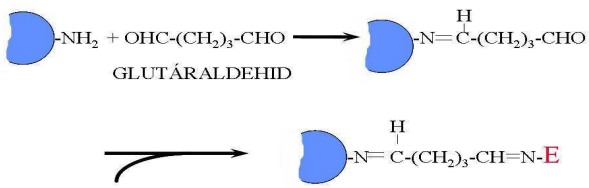
Cross-linked Enzyme crystal of PNP
(purine nucleoside phosphorylase)



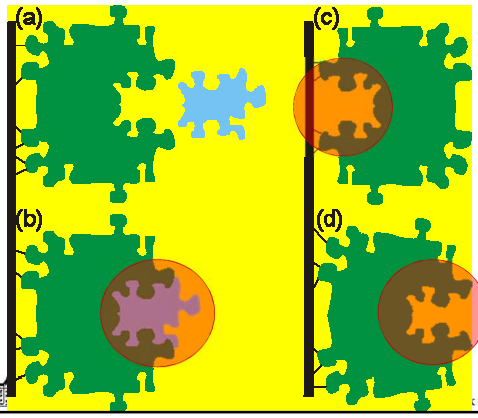
Chemical methods: bifunctional molecules

MATRIX: $-NH_2$ goups like:

AE-cellulose, DEAE-cellulose, collagen, chitin, nylon…



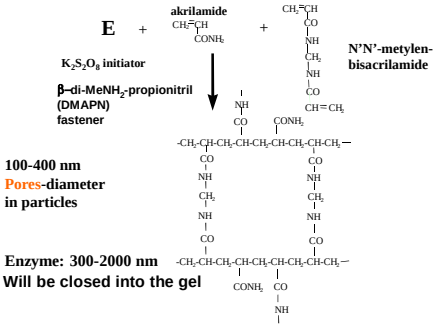
Possible effect of chemical immobilisation: Specific activity loss



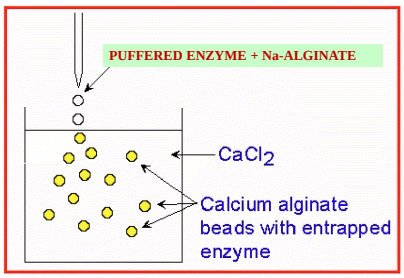
PHYSICAL METHODS

1. Adsorption e.g. on *ionexchanger resins* – nonspecific, easily desorps (pH)
2. Gel entrapment
3. Microencapsulation
4. Closing behind membrane

Poly-acrylamide gel entrapment



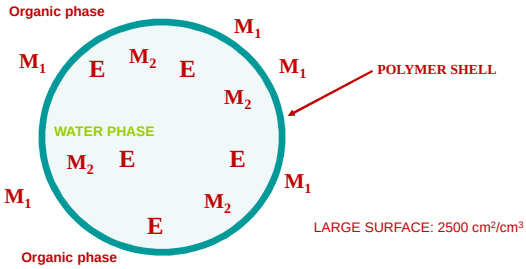
ALGINATE GEL ENTRAPMENT



ALGINATE: poly-β D-mannuronic acid (1→ 4), …..-guluronic acid
Hydrophyl colloid, linear polymer ***Macrocystis pyrifera***

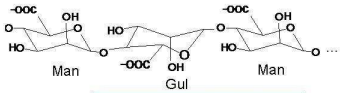
Physical methods: microencapsulation

stable polymeric membranes



Gel forming polysaccharides

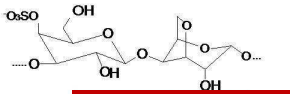
Alginate: heteropolymer of mannuronic acid and guluronic acid, 1,4-bonds



polyanionic

Solvent: water gel:Ca^{++}, Zn^{++}, Al^{3+}

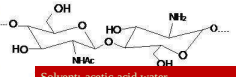
κ-carragenan: helical bio-polymer of 3,6 anhydro-galactose



polyanionic

Solvent: water gel: Ca^{++}, K^{+}

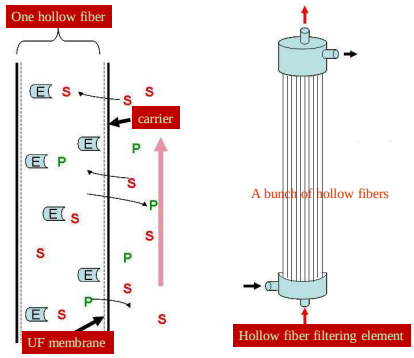
chitosan: partially deacylated N-acetyl-glucoseamin polymer



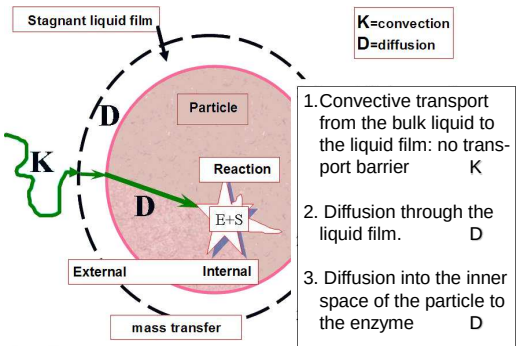
polycationic

Solvent: acetic acid,water gel:ploy phosphates, pH-change

Ultrafiltration membrane



Kinetics of immobilised enzymes



1. Convective transport from the bulk liquid to the liquid film: no transport barrier K
2. Diffusion through the liquid film. D
3. Diffusion into the inner space of the particle to the enzyme D

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Pros/cons about immobilised enzymes

Immobilised enzymes

Advantages
- No contamination of product
- Easily separable
- Possible reuse
- Also continuous technologies
- Easy termination
- Increasing stability

Disadvantages
- Expensine preparation need
- Loss in enzyme activity
- Diffusion barrier

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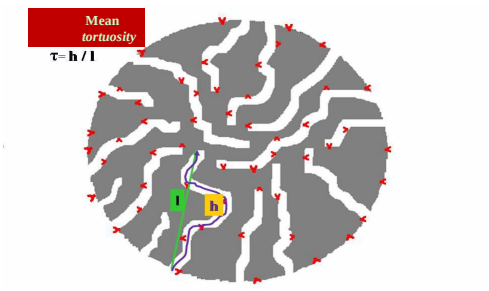
Tortuosity



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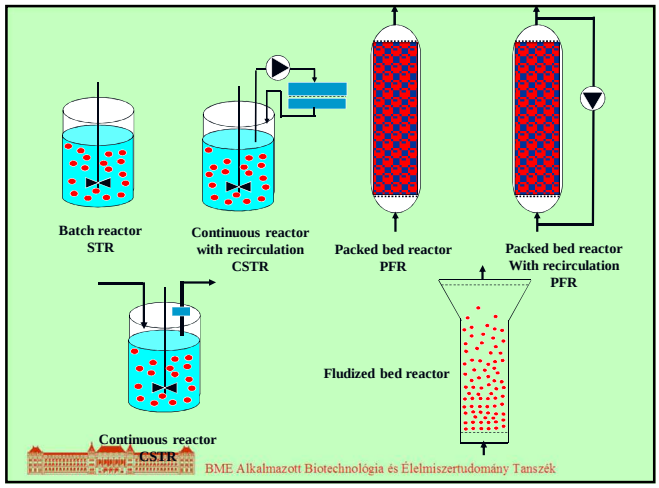

Pros/cons about immobilised enzymes

Dissoved enzymes

Advantages
- homogeneous system
- no preparation needed
- no mass transfer limitation

Disadvantages
- expensive (1-10-50 $/mg)
- discarded after use
- contamination of product
- only batch technology

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Industrial application of immobilised enzymes

Aminoacilase	resolvation of D,L-amino acids
Glucose-isomerase	conversion of glucose to glucose+fructose 1:1 mixture
Penicillin-amidase	preparation of 6-amino-penicilloic acid
β-galactosidase	hydrolysis of lactose to glucose+galactose
Lipase	hydrolysis and transesterification of lipids
Thermolysin	Preparation of aspartame

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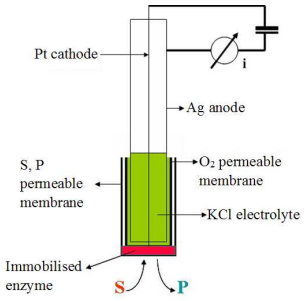
Enzyme electrode

Based on an amperometric electrode for dissolved oxygen measurement. It is covered with an enzyme producing or consuming oxygen.
Eg. glucose oxydase + catalase.
The electrode reaction:

Ag anode: $4Ag + 4Cl^- \rightarrow 4AgCl + 4e^-$

Pt cathode: $O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$



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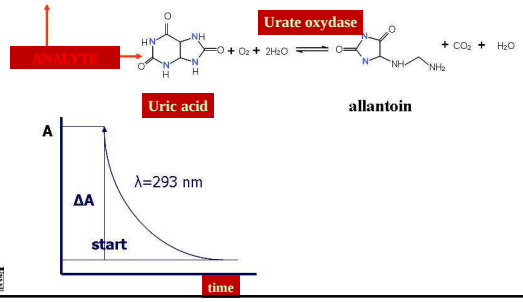
End-point measurement of substrate

The whole amount of substrate is converted – change is measured

$S + E \rightarrow P + E$



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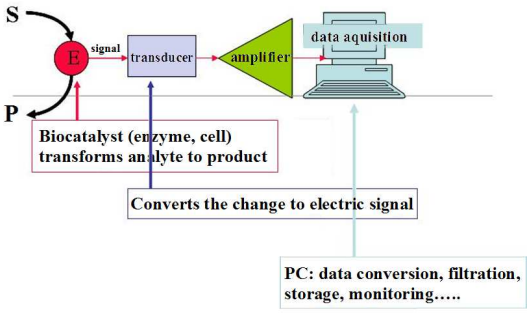
BIOSENSOR



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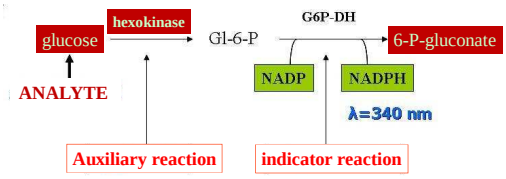
Indicator reaction

If S and P are not observable → an enzymatic indicator reaction makes it measurable.



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Analytical enzyme applications

In these cases not the activity of enzyme is measured but the concentration of an analyt molecule.

1. Determination of S
2. Determination of I
3. Marker reactions (eg. in immunoassays)
   Enzyme Linked Immunosorbent Assay (ELISA)
   diagnostical, research purposes

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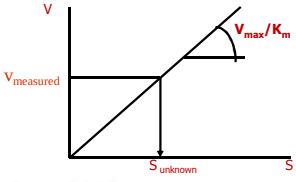
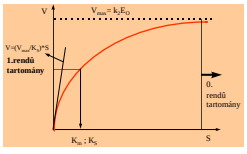
Kinetic measurement of S

At small substrate concentrations the reaction rate changes linearly with S concentration (M-M kinetics).

If $S \ll K_m$ → $V \sim V_{max}/K_m \cdot S$ → $-dS/dt$, dP/dt





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