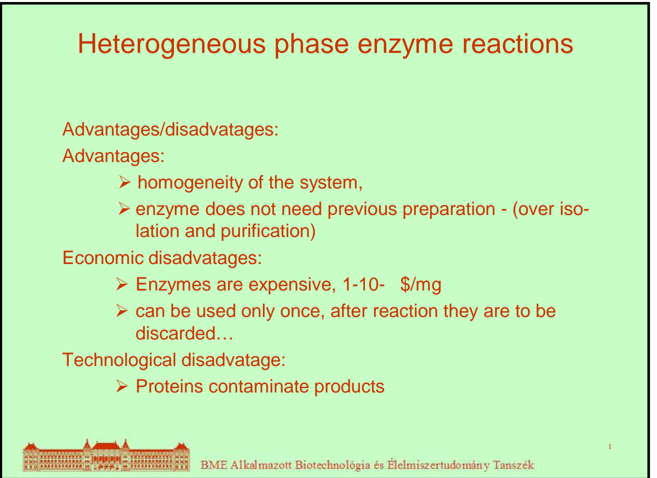
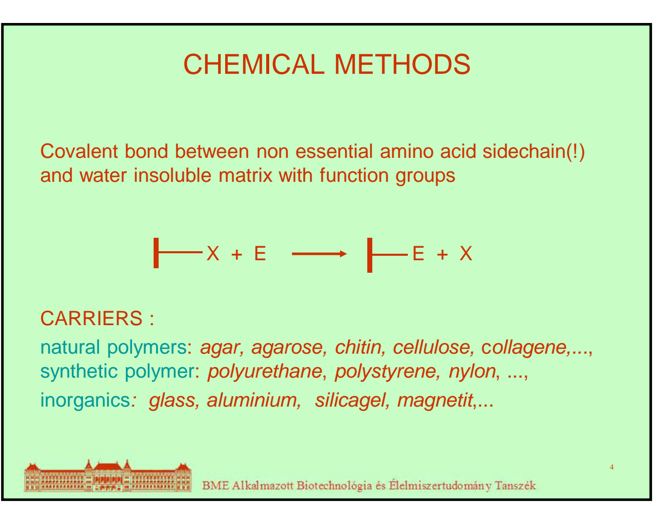

Heterogeneous phase enzyme reactions
Advantages/disadvatages:
Advantages:
homogeneity of the system,
enzyme does not need previous preparation - (over iso-lation and purification)
Economic disadvatages:
Enzymes are expensive, 1-10- $/mg
can be used only once, after reaction they are to be discarded…
Technological disadvatage:
Proteins contaminate products
BME Alkalmazott Biotechnológia és Élelmiszertudomány Tanszék


CHEMICAL METHODS
Covalent bond between non essential amino acid sidechain(!) and water insoluble matrix with function groups
X + E → E + X
CARRIERS :
natural polymers: agar, agarose, chitin, cellulose, collagene,...,
synthetic polymer: polyurethane, polystyrene, nylon, ...,
inorganics: glass, aluminium, silicagel, magnetit,...
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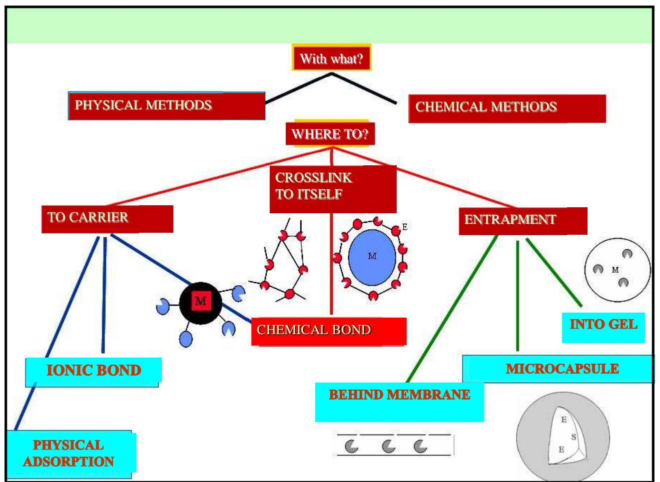
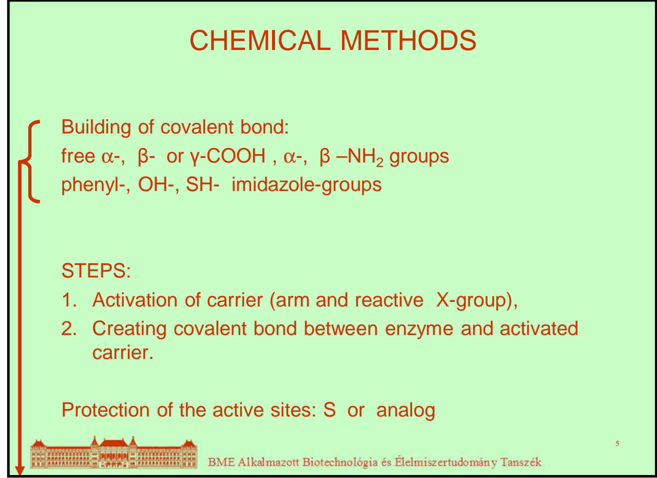
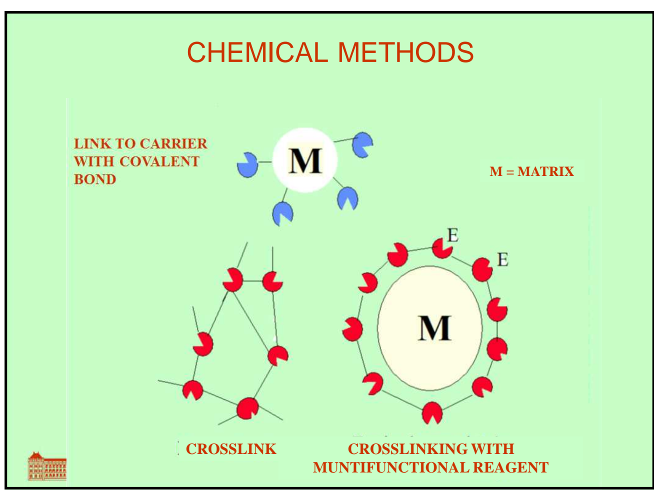
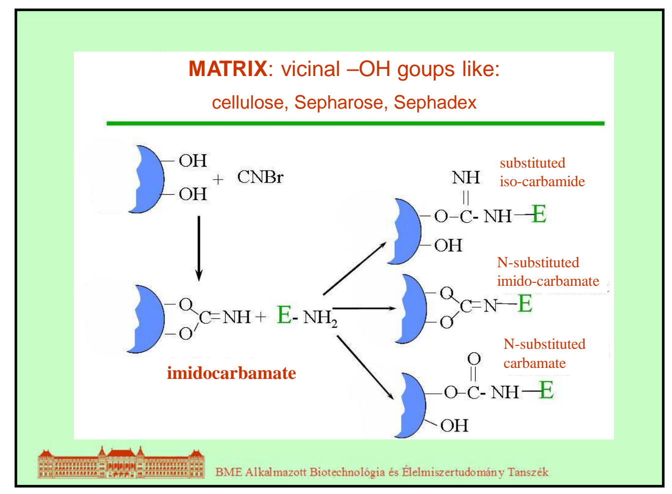
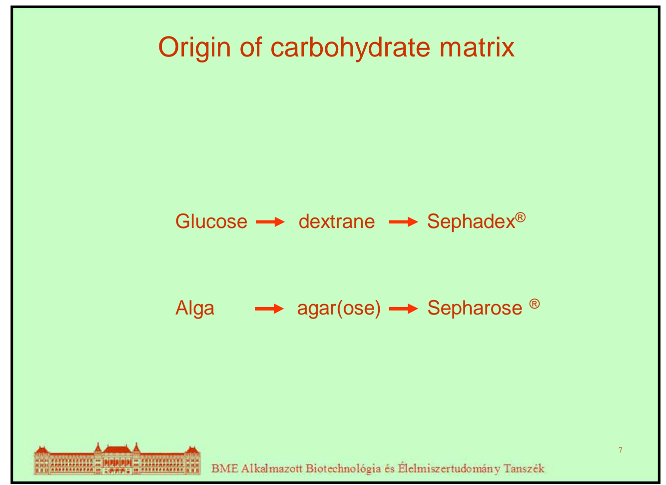

With what?
PHYSICAL METHODS
CHEMICAL METHODS
WHERE TO?
CROSSLINK TO ITSELF
TO CARRIER
ENTRAPMENT
CHEMICAL BOND
INTO GEL
IONIC BOND
MICROCAPSULE
BEHIND MEMBRANE
PHYSICAL ADSORPTION


CHEMICAL METHODS
Building of covalent bond:
free α-, β- or γ-COOH , α-, β –NH2 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
BME Alkalmazott Biotechnológia és Élelmiszertudomány Tanszék


CHEMICAL METHODS
LINK TO CARRIER WITH COVALENT BOND
M = MATRIX
CROSSLINK
CROSSLINKING WITH MUNTIFUNCTIONAL REAGENT


MATRIX: vicinal –OH goups like:
cellulose, Sepharose, Sephadex
CNBr
substituted iso-carbamide
N-substituted imido-carbamate
N-substituted carbamate
imidocarbamate
BME Alkalmazott Biotechnológia és Élelmiszertudomány Tanszék


Origin of carbohydrate matrix
Glucose → dextrane → Sephadex®
Alga → agar(ose) → Sepharose ®
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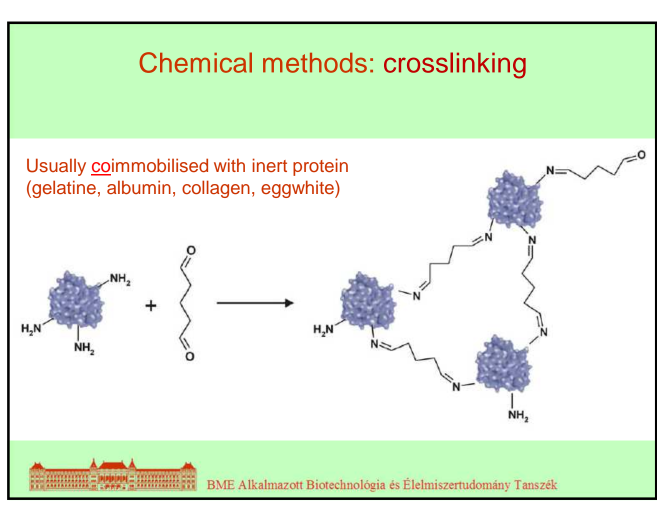
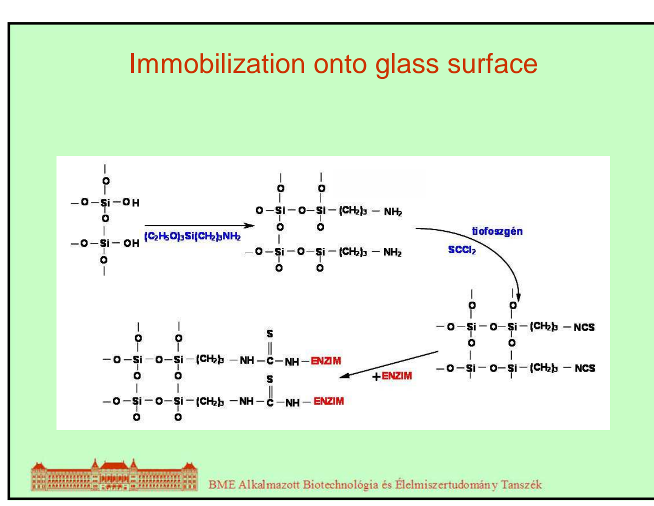
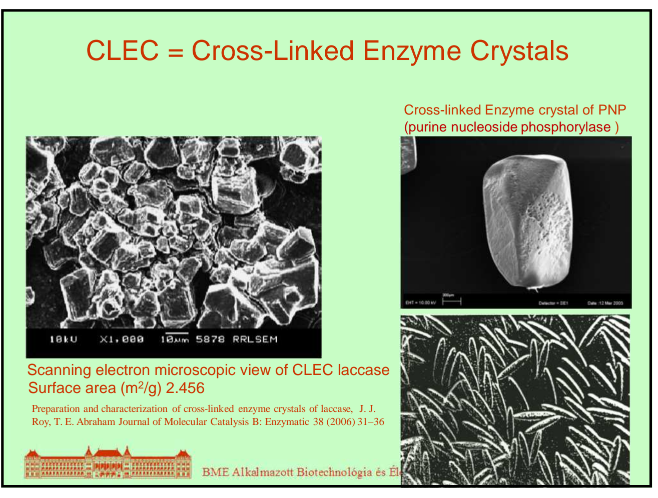
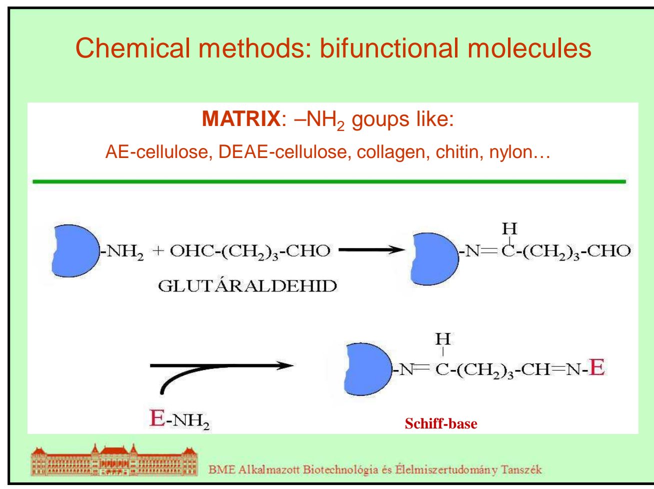

Chemical methods: crosslinking
Usually coimmobilised with inert protein
(gelatine, albumin, collagen, eggwhite)
BME Alkalmazott Biotechnológia és Élelmiszertudomány Tanszék


Immobilization onto glass surface
tiofoszgén
SCCl2
+ENZIM
BME Alkalmazott Biotechnológia és Élelmiszertudomány Tanszék


CLEC = Cross-Linked Enzyme Crystals
Cross-linked Enzyme crystal of PNP
(purine nucleoside phosphorylase)
Scanning electron microscopic view of CLEC laccase
Surface area (m2/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


Chemical methods: bifunctional molecules
MATRIX: –NH2 goups like:
AE-cellulose, DEAE-cellulose, collagen, chitin, nylon…
-NH2 + OHC-(CH2)3-CHO
GLUTÁRALDEHID
-N=C-(CH2)3-CHO
E-NH2
-N=C-(CH2)3-CH=N-E
Schiff-base
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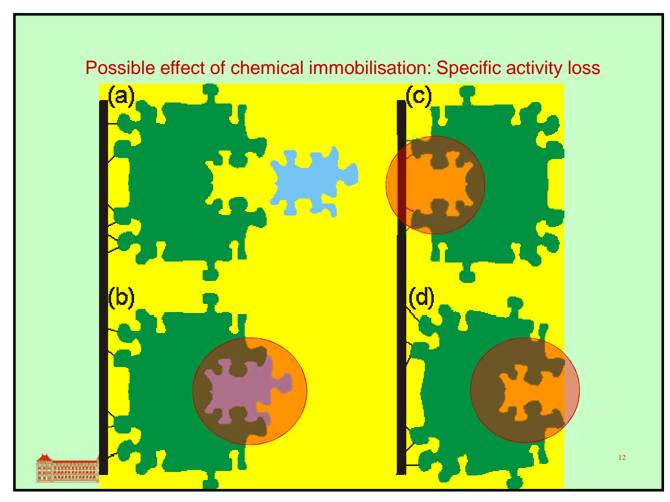
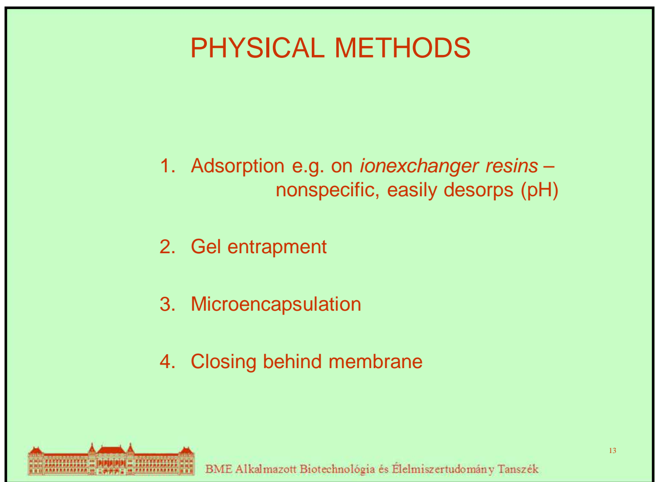
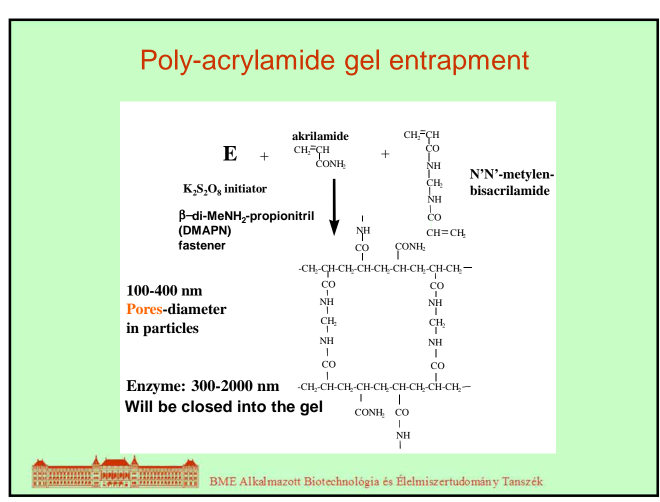
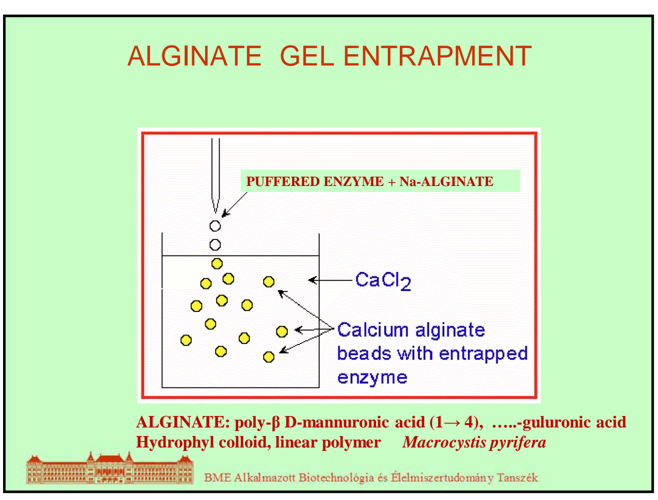
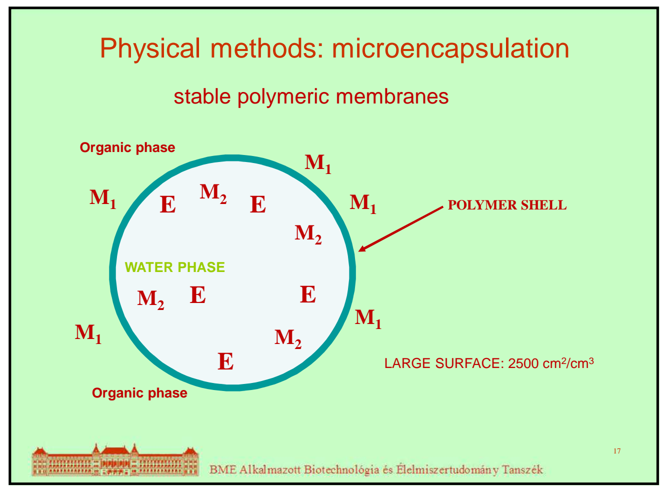
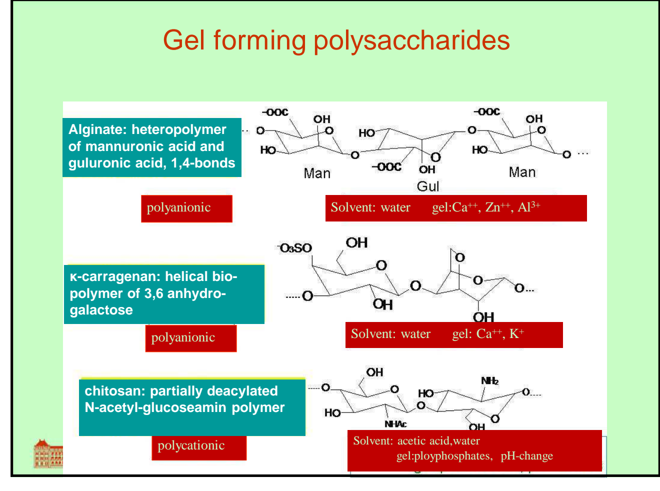

Possible effect of chemical immobilisation: Specific activity loss
(a)
(b)
(c)
(d)
12

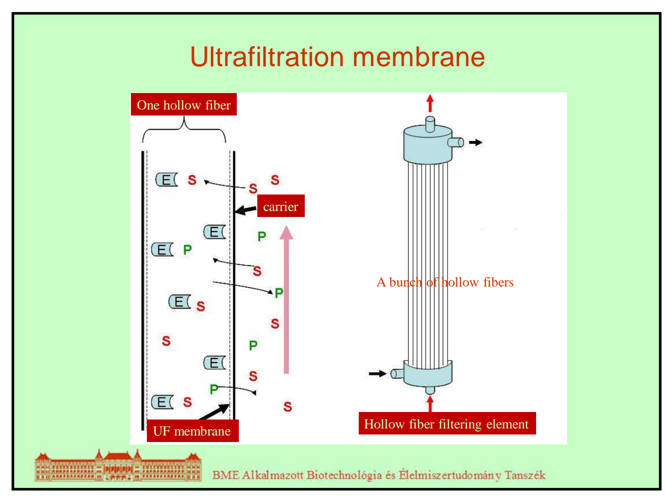

PHYSICAL METHODS
1. Adsorption e.g. on *ionexchanger resins* – nonspecific, easily desorps (pH)
2. Gel entrapment
3. Microencapsulation
4. Closing behind membrane
BME Alkalmazott Biotechnológia és Élelmiszertudomány Tanszék

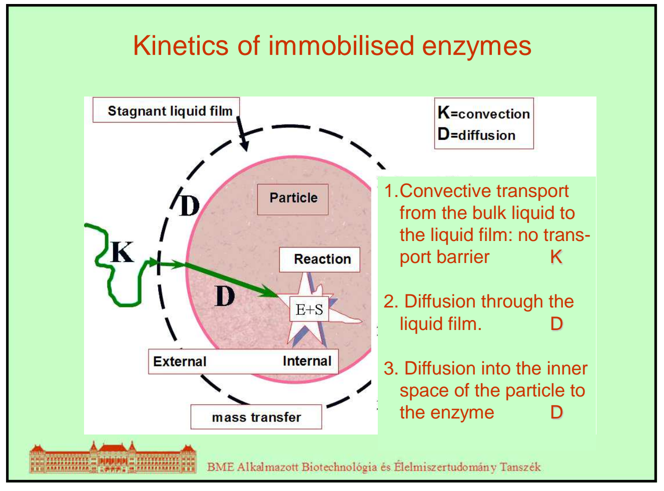

Poly-acrylamide gel entrapment
E
akrilamide
N'N'-metylen-bisacrilamide
K2S2O8 initiator
β–di-MeNH2-propionitril (DMAPN) fastener
100-400 nm Pores-diameter in particles
Enzyme: 300-2000 nm
Will be closed into the gel
BME Alkalmazott Biotechnológia és Élelmiszertudomány Tanszék

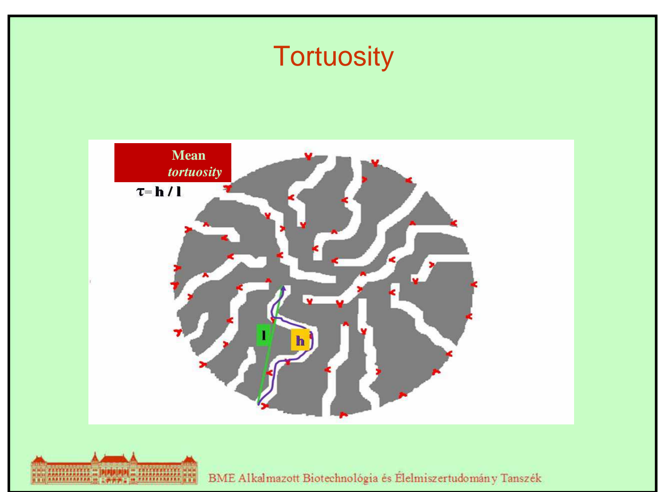

ALGINATE GEL ENTRAPMENT
PUFFERED ENZYME + Na-ALGINATE
CaCl2
Calcium alginate beads with entrapped enzyme
ALGINATE: poly-β D-mannuronic acid (1→ 4), …..-guluronic acid
Hydrophyl colloid, linear polymer Macrocystis pyrifera
BME Alkalmazott Biotechnológia és Élelmiszertudomány Tanszék

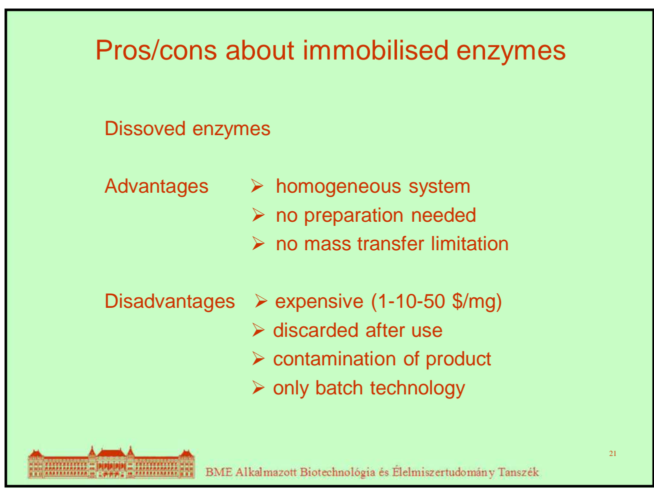

Physical methods: microencapsulation
stable polymeric membranes
Organic phase
M1
M2
E
WATER PHASE
POLYMER SHELL
LARGE SURFACE: 2500 cm2/cm3
Organic phase
BME Alkalmazott Biotechnológia és Élelmiszertudomány Tanszék

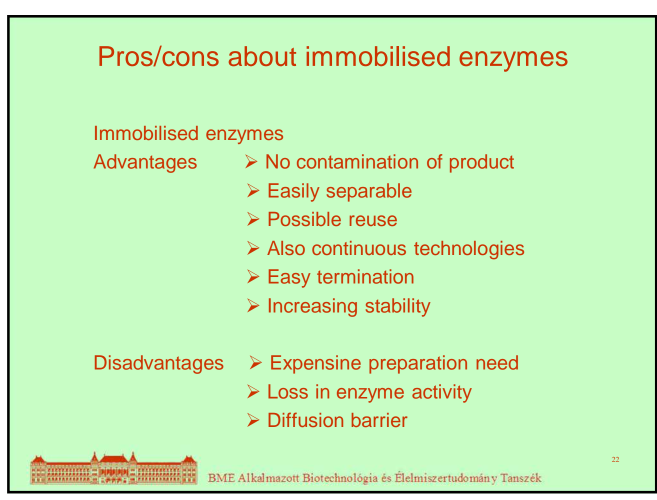

Gel forming polysaccharides
Alginate: heteropolymer of mannuronic acid and guluronic acid, 1,4-bonds
Man
Gul
Man
polyanionic
Solvent: water gel:Ca++, Zn++, Al3+
κ-carragenan: helical bio-polymer of 3,6 anhydro-galactose
polyanionic
Solvent: water gel: Ca++, K+
chitosan: partially deacylated N-acetyl-glucoseamin polymer
polycationic
Solvent: acetic acid,water gel:ployphosphates, pH-change

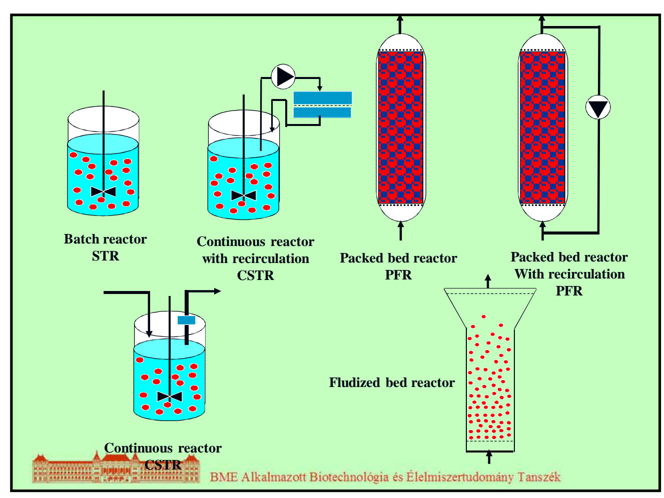

Ultrafiltration membrane
One hollow fiber
carrier
A bunch of hollow fibers
UF membrane
Hollow fiber filtering element
BME Alkalmazott Biotechnológia és Élelmiszertudomány Tanszék

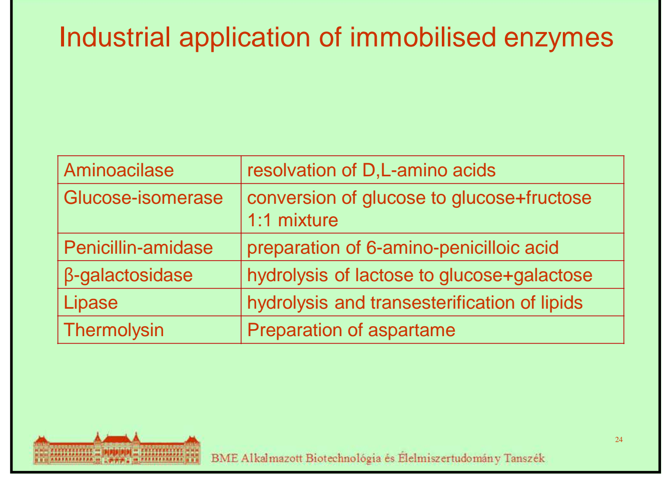




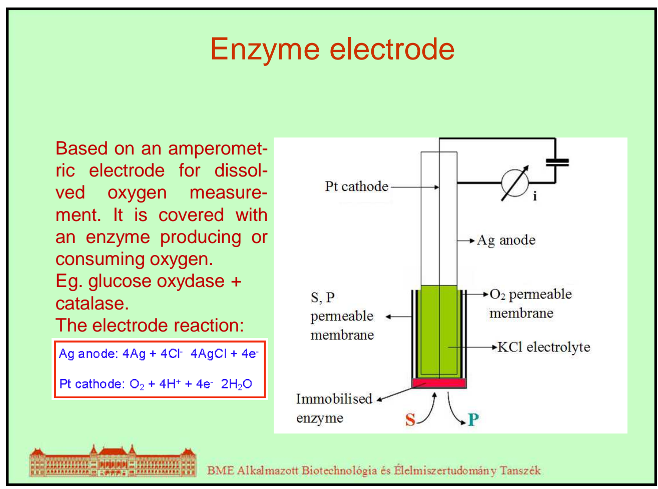








Aminoacilase	resolvation of D,L-amino acids
Glucose-isomerase	conversion of glucose to glucose+fructose 1:1 mixture
Penicillin-amidase	preparation of 6-amino-penicilloic acid
β-galactosidase	hydrolysis of lactose to glucose+galactose
Lipase	hydrolysis and transesterification of lipids
Thermolysin	Preparation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


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- 4AgCl + 4e-
Pt cathode: O2 + 4H+ + 4e- 2H2O
Pt cathode
Ag anode
S, P permeable membrane
O2 permeable membrane
KCl electrolyte
Immobilised enzyme
S
P
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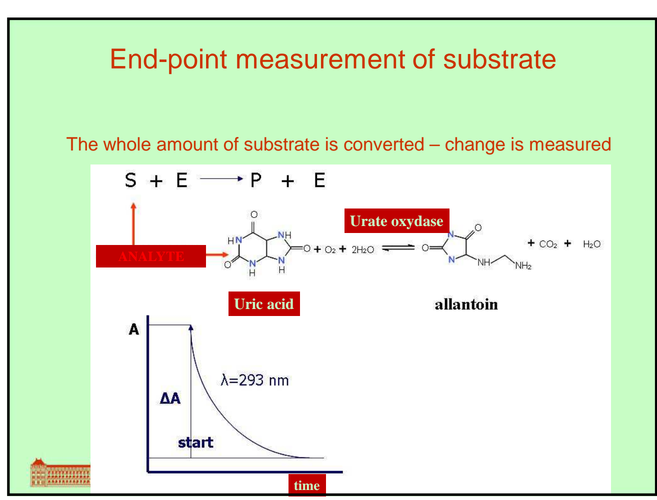
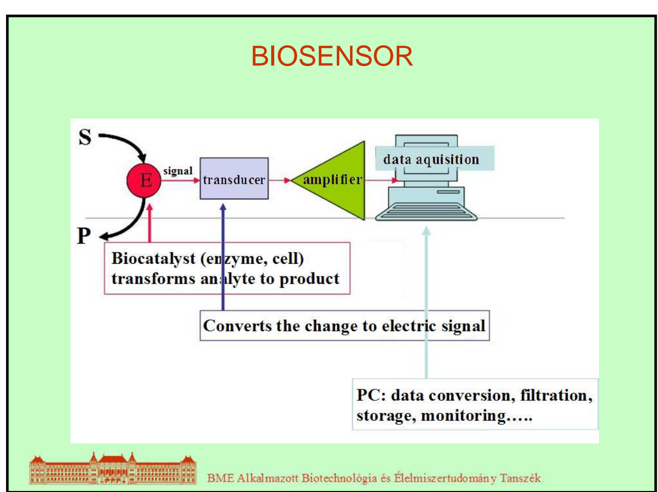

End-point measurement of substrate
The whole amount of substrate is converted – change is measured
S + E → P + E
Urate oxydase
ANALYTE
Uric acid
allantoin
A
ΔA
λ=293 nm
start
time


BIOSENSOR
S
P
E
signal
transducer
amplifier
data aquisition
Biocatalyst (enzyme, cell) transforms analyte to product
Converts the change to electric signal
PC: data conversion, filtration, storage, monitoring…..
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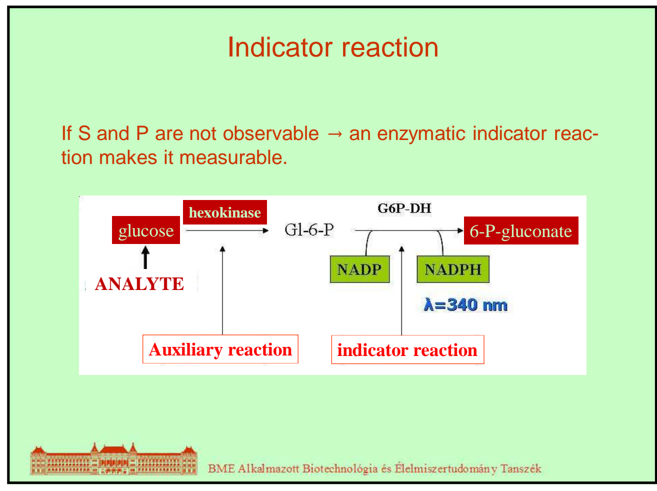
Indicator reaction
If S and P are not observable → an enzymatic indicator reaction makes it measurable.
glucose
hexokinase
Gl-6-P
G6P-DH
6-P-gluconate
NADP
NADPH
ANALYTE
λ=340 nm
Auxiliary reaction
indicator reaction
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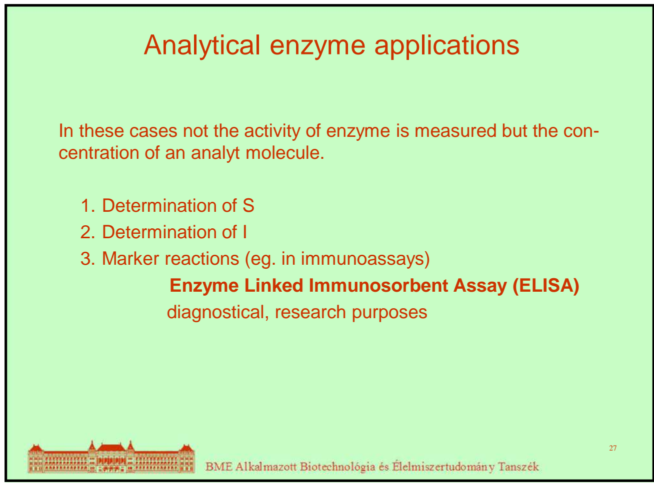
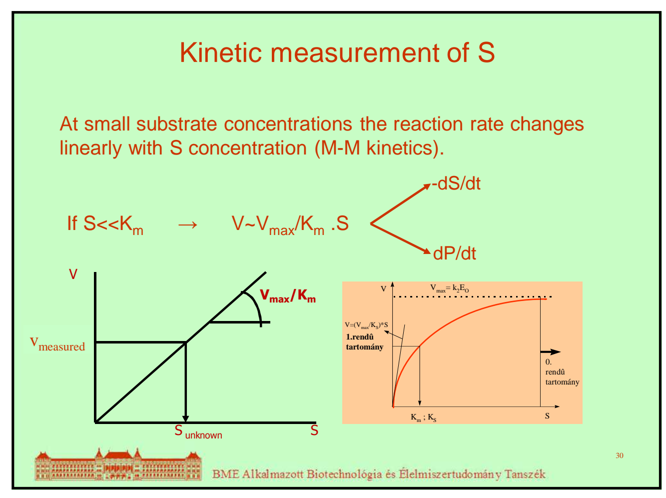

Analytical enzyme applications
In these cases not the activity of enzyme is measured but the concentration of an analyt molecule.
1. Determination of S
2. Determination of I
3. Marker reactions (eg. in immunoassays)
Enzyme Linked Immunosorbent Assay (ELISA)
diagnostical, research purposes
27
BME Alkalmazott Biotechnológia és Élelmiszertudomány Tanszék


Kinetic measurement of S
At small substrate concentrations the reaction rate changes linearly with S concentration (M-M kinetics).
If S<<Km → V~Vmax/Km .S
-dS/dt
dP/dt
V
Vmeasured
Vmax/Km
Sunknown
S
30
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