Enzymes as Food Ingredient

According to the intended use food enzymes are categorized either as:

- **Food additives having a technological function**
- **Processing aids present only in residual amounts in food and not having a function anymore there**

Historically Speaking…..

- **Enzymes (biotech) have been impacting our food supply for *1000's of years!!!***
- **Use of enzymes became an offshoot of a biological or microbiological discovery (brewing and alcohol production, vinegar, baking) – involved fermented foods**
- **Modern enzyme applications included rennin for cheese, glucose oxidase for desugaring eggs and alpha amylase for starch processing**


PREMIUM
SOY SAUCE


Welch's
100%
Grape Juice
Welch's
100%
Grape Juice

- **But, typically those products were developed first as result of a biological processes.**

- **Foods, as the consumer recognizes them, made directly by addition of enzymes are limited……**

Foods Made with Enzymes

Extending shelf life with enzymes

- **Food Industry is looking for new, creative products with certain attributes:**
  - **Safe**
  - **Healthy**
  - **”Green” or sustainable**
  - **Cost effective**
  - **More natural**
- **However, responsibility being pushed back to the ingredient level**

Ingredient Development Driving Issues

- Reduce waste
- Improve functionality
- Be unique
- Enhance nutrition

Ingredient Development Driving Issues cont..

- Reduce chemical footprint from field to package
- Employ safe processing methods
- Improve safety throughout distribution cycle- both as an ingredient and in finished product

Enzymes are a Good Fit for Ingredient Development

- **Specificity**
- **Tools of biotechnology have greatly improved many areas:**
- **-cost**
- **-purity**
- **-uniqueness**
- **-stability**

Enzymes are a Good Fit for Ingredient Development cont…

- **Specialization**
- **Continuous discovery**
- **Greater libraries of products to choose from**

Today's Enzyme Capabilities- Ingredients

- **Modify cellulose for enhanced fiber properties**
- **-viscosity, mouthfeel**
- **Selective Hydrolysis of proteins for flavor, nutrition, and reduce waste**
- **Extraction of high value oils, flavors, extracts**

Today's Enzyme Capabilities- Ingredients cont…

- **Modify physical properties of starches**
- **Replace/remove chemicals in many ingredient processes**
- **Alter lipid profiles and functionality**

- **ENZYMES GET THE JOB DONE….they can be used to prolong the freshness of bread, to enhance the browning of the crust, to ensure a sufficient supply of fermentable sugars in frozen dough, to break down the pentosans in rye and wheat flour which hinder the development of gluten, etc…**

Industrial Application of Enzymes

Industry	Application	Enzymes
Fruit juice/Wine	**Juice extraction** **Clarification** **Starch hydrolys**	**Pectinase** **Cellulase** **Hemicellulase** **Starch hydrolysis**
Baking	**Dough conditioner** **Bread volume** **Crumb structure** **Crust colour** **Antistaling**	**α-amylase** **Amyloglucosidase** **protease**
Others	**Fat modification** **Oxygen removal** **Confectionery** **Softening**	**Lipase** **Glucose oxidase** **Invertase**

Industry	Application	Enzymes
Starch	**Glucose syrup**	**α-amylase** **β-amylase** **Amyloglucosidase** **Pullulanase** **Glucosisomerase**
Dairy	**Cheese** **Cheese flavour** **Lactose hydrolys**	**Rennin** **Lipase** **Protease** **Lactase**

Function of Enzymes in Baked Goods

Property	Target improvement	Enzymes used
Processability	Shorter mixing & proofing time, better dough stability	Proteases, hemicellulases, oxidases, lipases
Volume	Larger volume, esp. for high fibre products	α-amylase, hemicellulases, cellulases, lipases, protease
Stability	Antistalling effect, extended shelf life, improved freshness	α-amylase, hemicellulase

Property	Target improvement	Enzymes used
Texture	Softer crumb, fine & regular pore structure, better crispness, less hygroscopicity	α-amylase Proteases, hemicellulases
Colour	Browning effect, improved crust colour, bleaching effect	α-amylase, glucoamylases (hemicellulases), lipoxygenases
Flavour	Production of fermentation substrates & aroma precursors	α-amylase, protease, lipoxygenases, lipases, glucose oxidase

Property	Target improvement	Enzymes used
Nutritional properties	Increased amount of total & soluble dietary fibre, reduced fat baking	Hemicellulases, cellulases
Replacement of chemicals	Replace of bromate, sodium metabisulphate, vital gluten	α-amylase, glucose oxidase, hemicellulases, lipoxygenases, cellulases, lipases, proteases

Main Enzymes Types for Baking

Enzyme	Major substrate in bread flour
Amylolytic enzymes	**Starch**
α-amylase	Amylose & amylopectin
β-amylase	Amylose & amylopectin
Glucoamylase	Amylose & amylopectin
Pullulanase	Amylopectin
Cellulase & hemicellulase	**Cell-wall components: cellulose, β-glucan, pentosans**
Cellulase	Cellulose & β-glucan
Laminarinase	β-glucan
Licheninase	β-glucan
Xylanase	Arabinoxylan
α-arabinosidase	Arabinoxylan

Enzyme	Major substrate in bread flour
Proteolytic enzymes	Starch
Proteases	Proteins
Peptidases	Peptides

Action of Enzymes on Starch

- **Source of enzymes:**
  - **Wheat & barley malt**
  - **Fungal & bacterial α-amylase**

Damaged starch granules are attacked by

(a) α-amylase – dextrins &

(b) β-amylase - maltose

} **Provide fermentable sugars – yeast – CO_2 – higher bread loaf**

Action of Enzymes on Starch

- ***Starch hydrolysis by α-amylase also result in***

  - **Weakening of starch gel in the baked bread – improved crumb softness**
  - **Stabilization of gas cells – important in frozen dough products**

- ***Anti-staling effect of α-amylase***

  - **Interference of dextrins in amylopectin recrystallization**
  - **Dextrin promote the formation of amylopectin-lipid complex**

Action of Enzymes on Pentosans

- **Pentosan (mainly arabinoxylans) contribute 2-3% of wheat flour, up to 5% in wholemeal flour and 8% in rye flour**
- **Insoluble pentosans hinder the development of gluten**
- **Pentosans bind ~ x10 their own weight of water – 1/3 of water binding capacity of flour**

Action of Enzymes on Pentosans

- **Degradation of pentosan (e.g. b xylanases) causes water redistribution from pentosans to starch and gluten phase – dough become softer & easier to process**
- **Addition of xylanase alleviate problems caused by addition of dietary fibre**

- **So…**
- **Enzyme usage in food today more focused on value creation at ingredient level**
- **Breath of possibilities expanding due to tools of biotech**
- **Advancement of enzyme development has enhanced sustainability of many processes**

- **Microbial enzymes offer many unique possibilities based on the substrate**
- **Have become very cost effective as processing aids**

IMMOBILIZATION

Definition: „Immobilization means that the biocatalysts are limited in moving due to chemically or physically treatment“

- transformation of enzyme to insoluble form or inclusion to definite space
- method for **reuse and stabilisation** of enzyme
- **one-step reactions** - domain of immobilized enzymes

The attractions of immobilized enzymes from an analytical standpoint are primarily their reuseability,and hence cost saving, and the greater efficiency and control of their catalytic activity (e.g., potentially longer half-lives, predictable decay rates and more efficient multi-step reactions).

Immobilized enzyme

- An **immobilized enzyme** is enzyme an that is attached to an inert, insoluble material such as calcium alginate (produced by reacting a mixture of sodium alginate solution and enzyme solution with calcium chloride).

- This can provide increased resistance to changes in conditions such as pH or temperature.

- It also allows enzymes to be held in place throughout the reaction, following which they are easily separated from the products and may be used again - a far more efficient process and so is widely used in industry for reactions.

- An alternative to enzyme immobilization is whole cell immobilization

http://en.wikipedia.org/wiki/Immobilized_enzyme

ADVANTAGES OF IMMOBILIZED ENZYME

- Development of continuous processes allowing more economic organization of the operations, automation, decrease of labour, and investment/capacity ratio.
- Availability of the product in greater purity. Purity of the product is very crucial in food processing and pharmaceutical industry since contamination could cause serious toxicological, sensory, or immunological problems.
- Greater control over enzymatic reaction as well as high volumetric productivity with lower residence time, which are of great significance in the food industry, specially in the treatment of perishable commodities as well as in other applications involving labile substrates, intermediates or products

Commercial use

Immobilized enzymes are very important for commercial uses as they possess many benefits to the expenses and processes of the reaction of which include :

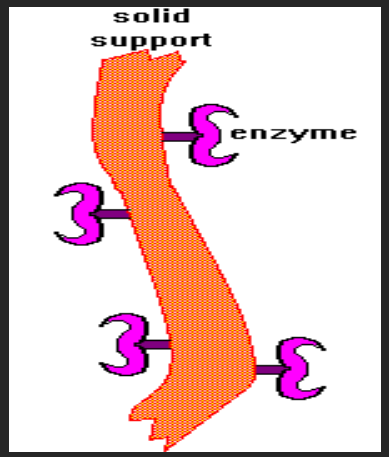
Convenience : Minuscule amounts of protein dissolve in the reaction, so workup can be much easier. Upon completion, reaction mixtures typically contain only solvent and reaction products.

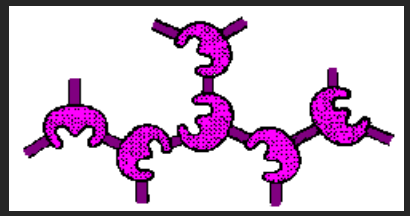
Economical : The immobilized enzyme is easily removed from the reaction making it easy to recycle the biocatalyst.

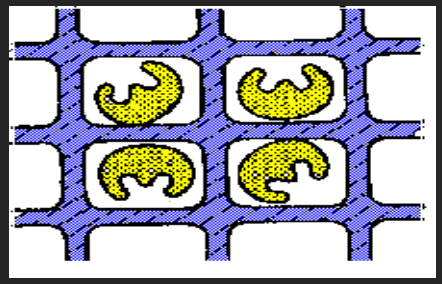
Stability : Immobilized enzymes typically have greater thermal and operational stability than the soluble form of the enzyme.

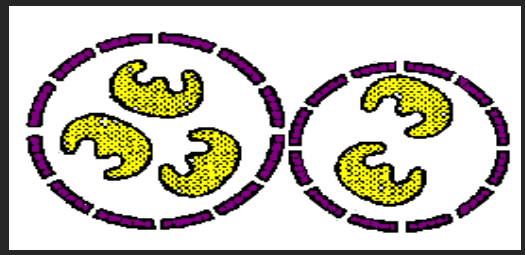
ENZYME IMMOBILIZATION METHOD

- "Carrier –binding"
  - Adsorpsi Fisik
  - Ikatan Ionik
  - Ikatan Kovalen
- "Cross-linking"
- "Entrapment"
  - Jenis Kisi
  - Jenis Mikrokapsul









Aspects of the immobilization procedure :

1. The properties of the free enzyme.
2. The type of support used.
3. The methods of support activation and enzyme attachment.

1. Properties of the Free Enzyme

- Source of the enzyme
- Purity (and method of purification)
- Catalytic activity and details of other constituents - - etc.

The above information permits direct comparison of enzymes from different sources.

2. Enzyme Support

The support material can have a critical effect on the stability of the enzyme and the efficiency of enzyme immobilization.

The most important requirements for a support material are that it must be insoluble in water, have a high capacity to bind enzyme, be chemically inert and be mechanically stable.

- The enzyme binding capacity is determined by the available surface area, both internal (pore size) and external (bead size or tube diameter), the ease with which the support can be activated and the resultant density of enzyme binding sites.

- The surface charge and hydrophilicity must be considered.

Parameters of Enzyme Immobilization

- Effective, easy, cheap, acceptable (non-toxic in food and medical applications)
- R**ate and yield** dependent on the parameters involved (e.g., type of carrier, concentrations, pH, temperature, method, reaction time)
- Empirical optimization
- External **protein surface** properties (e.g., hydrophobicity, ionic groups, functional groups for covalent binding)
- Protein **surface engineering**
- Introduction of functional groups increases binding interactions, stability (e.g., nanoparticles, protecting molecules) and activity (e.g., cofactors)

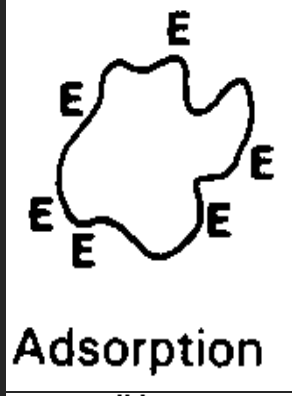
Method Immobilization Enzyme

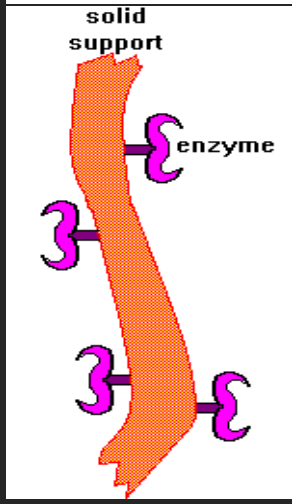
1. Adsorption on glass, alginate beads or matrix :

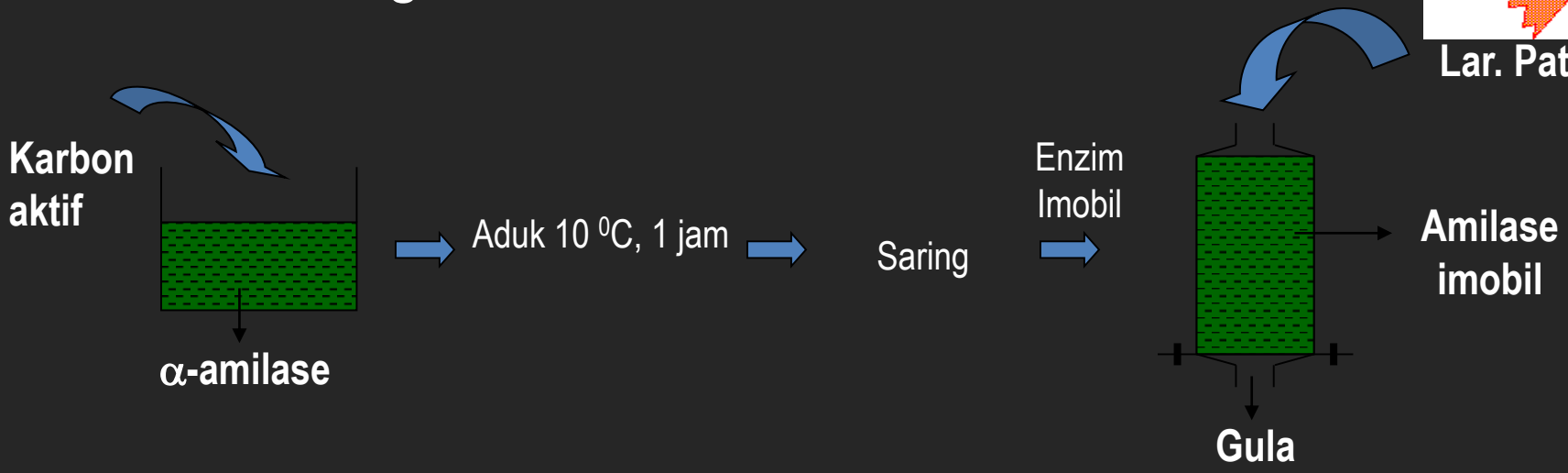
Enzyme is attached to the outside of an inert material. In general, this method is the slowest among those listed here. As adsorption is not a chemical reaction, the active site of the immobilized enzyme may be blocked by the matrix or bead, greatly reducing the activity of the enzyme.

Adsorption

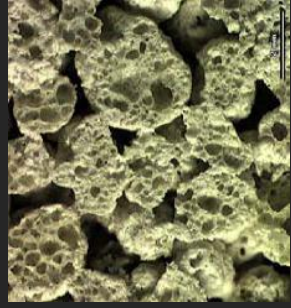
- Binding onto silica, clay or ion-exchange materials by weak interactions (e.g., ionic, electrostatic, hydrophobic)
- Dependent on process conditions (e.g., pH, temperature, ionic strength, hydrophobicity)
- Simple and cost-effective, reversible (stabilized by cross linking), but may cause enzyme unfolding



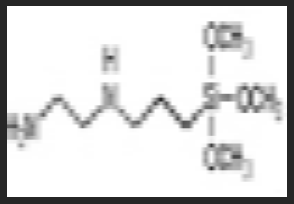




Inorganic Carriers



- High **pressure stability**
- May undergo **abrasion** in stirred vessels
- **SiO_2** based carriers functionalized by introduction of **amino groups** (e.g., treating with aminopropyl triethoxysilane)



- **Porous glass** (Corning, Waters, Schuller)
- **Silica** (Grace, Solvay, Degussa)
- **Mineral** materials (clays)
- **Celite** - adsorption and stabilisation of enzyme in organic media
- **Bentonite** - excellent adsorption capacity (up to 1.5 g protein per g bentonit) used for enzyme isolation by adsorption/desorption

➔ Crosslinking with glutaraldehyde prevents desorption

Organic Carriers from Natural Sources

- Favorable **compatibility** with proteins
- High range of **polysacharides** and derivatives used for immobilization
- Wide network structure
- Hydrophilic properties - weak interactions with proteins
- **Cellulose derivatives**
  - DEAE-cellulose (diethylaminoethyl-cellulose)
  - CM-cellulose (carboxymethyl-cellulose)
- **Dextran**
  - widely used for enzyme immobilization
  - activated by cyanogen bromide
  - mechanical stability limited
- **Other polysacharides**
  - agarose, starch, pectine and chitosan
- **Proteins** (gelatine)

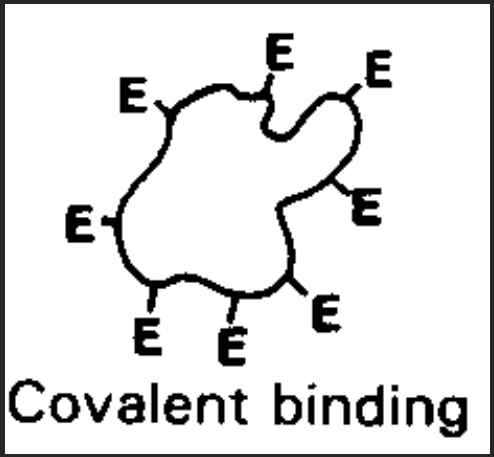
Table 11.14 Adsorption carriers for immobilization of enzymes

Type of carrier	Carrier	Enzyme used
Inorganic	Aluminium oxide	Glucoamylase
	Bentonite	Invertase
	Glass	Lipase
	Ca phosphate gel	Aspartase
Organic	Activated carbon	Glucose oxidase
		α-Amylase
		β-Amylase
		Glucoamylase
		Invertase
	Starch	α-Amylase
	Tannin aminohexyl cellulose	Glucose isomerase
		Aminoacylase

2. Covalent Binding

- Better stabilization of enzyme on carrier
- Introduction of functional group (e.g., amino, epoxy, thiol, cyanide)

- Principle :
  1. activation
  2. derivatization
  3. binding of enzyme



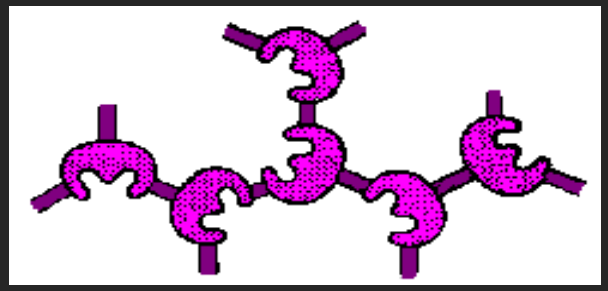
Covalent binding

3. **Crosslinked** :

- The enzyme is covalent bonded to a matrix through a chemical reaction.

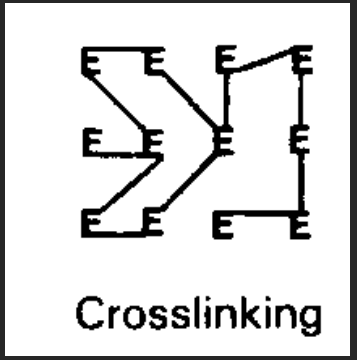
- This method is by far the most effective method

- As the chemical reaction ensures that the binding site does not cover the enzyme's active site, the activity of the enzyme is only affected by immobility.

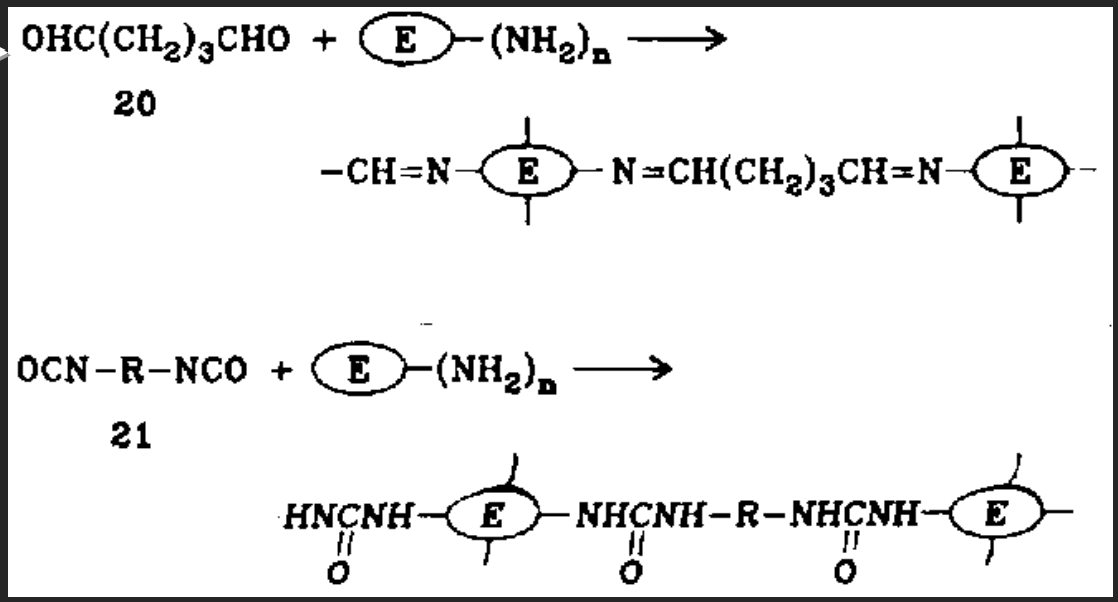


Crosslinked

Use reagent which usually has 2 identical functional groups ➔ reacted with amino acid residue of the enzyme



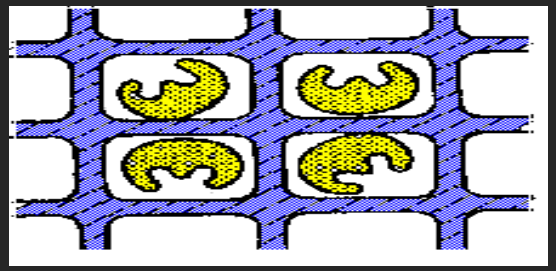
Glutaraldehyde ➔



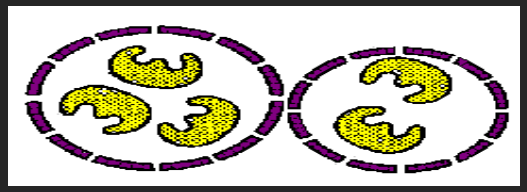
Diisocyanate ➔

4. Entrapment

The enzyme is trapped in insoluble beads or microspheres, such as calcium alginate beads. However, this insoluble substances hinders the arrival of the substrate, and the exit of products.



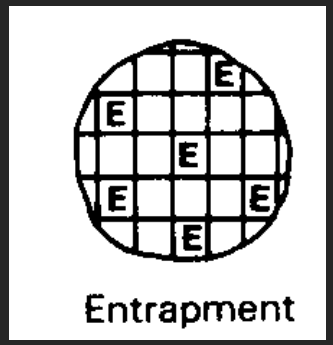
⇒ **lattice type** (alginat, k-caragenan, Poliacrylamide)



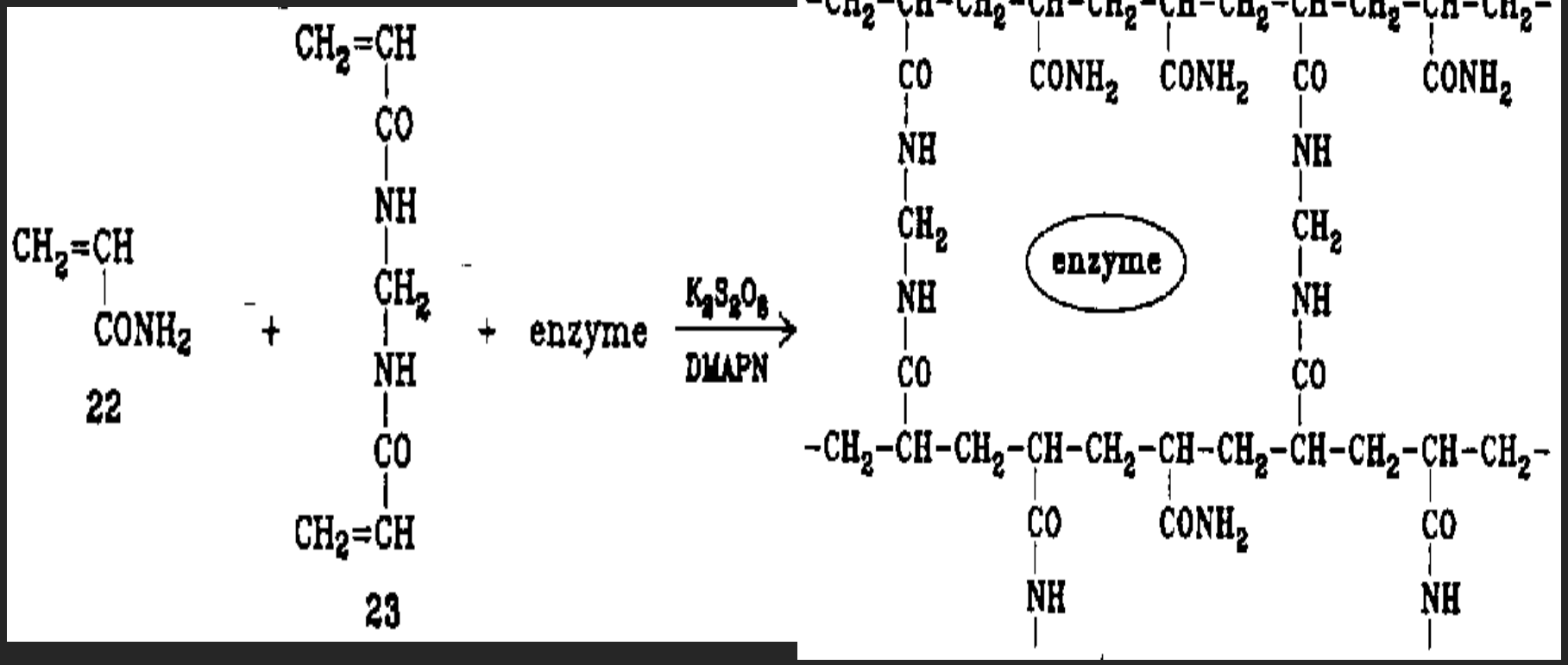
- ⇒ **microcapsule type**
  - 1 – 300 mμ
    - Permanently polymer Membran
    - Nopermanently

- Nilon
- Poliurea
- Etil selulosa
- Polistiren
- Kolodion
- Nitroselulosa
- Butil asetat selulosa

Immobilization by Entrapment



Poliacrylamide Gel



Perubahan Sifat Enzim Terimobilisasi

1. Aktivitas

V_1 (aktivitas relatif)
⇒ Perbandingan aktivitas enzim imobil vs enzim larut dalam jumlah sama

V_2 (aktivitas spesifik absolut)
⇒ Kecepatan reaksi per unit berat atau unit volume seluruh katalis

V_1 ⇒ tidak deaktivasi enzim akibat imobilisasi
V_2 ⇒ kemungkinan untuk mengimobilisasi enzim lebih banyak/sedikit per unit volume katalis

Penyebab penurunan aktivitas :

- Konfigurasi ⇒ menghalangi substrat
- Grup reaktif pada sisi aktif ikut terikat pada matriks
- Terbentuk konfigurasi tidak aktif
- Kondisi reaksi ⇒ denaturasi

2. pH optimum enzim imobil

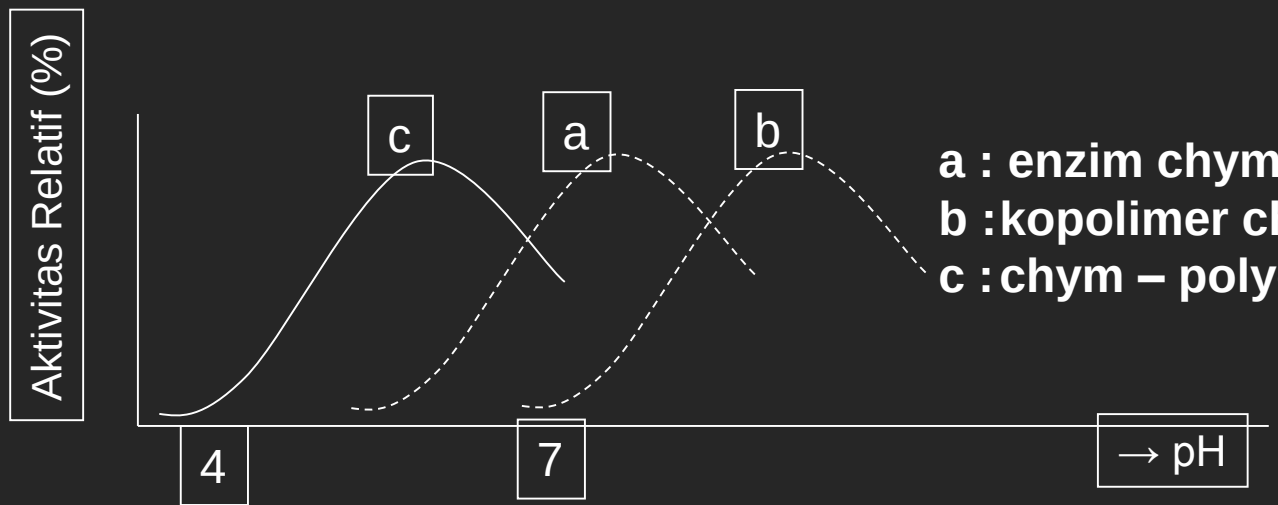
Penyebab perubahan pH : ⇒ distribusi yang tidak seragam dari ion H^+, ion OH^- dan substrat bermuatan

***Carrier* bermuatan negatif ⇒ pH optimum bersifat basa**

- CMC
- Maleac anhydride/etilen
- Asam galakturonat
- Asam poliaspartat

***Carrier* bermuatan positif ⇒ pH lebih asam**

- DEAE-selulosa
- Polimer polyornithyl



a : enzim chymotripsin larut
b : kopolimer chym – anhydride ethylene (-)
c : chym – polyornithyl (+)

3. Stabilitas

⇒ **Stabilitas operasi = $t_{1/2}$ (half-life)**
= waktu dimana terjadi kehilangan 50 % dari aktivitas enzim semula

$$t_{1/2} = \frac{0.693}{k} \qquad k = \frac{2.303}{t} \log \frac{E_0}{E}$$

k = konstanta kerusakan enzim
t = waktu operasi
E_0 = aktivitas enzim mula-mula
E = aktivitas enzim pada wktu t

Stabilitas operasi ditentukan oleh :

- **Jenis enzim**
- **Cara imobilisasi**
- **Jenis reaktor**

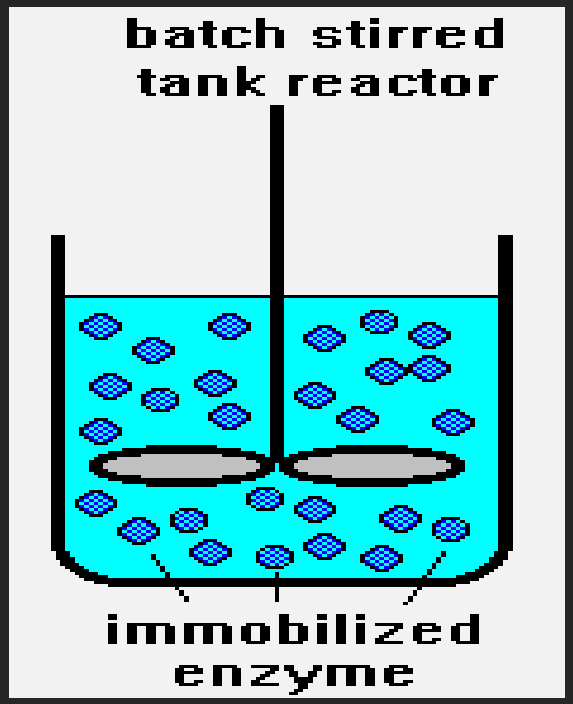
Enzim	NIlai $t_{1/2}$ pada gelas berpori*)		
	Substrat	Suhu (^{0}C)	$T_{1/2}$(hari)
L-asam amino aksidase	L-leusin	37	43
Alkalin fosfatase	P-nitrofenil fosfat	23	55
Papain	Kasein	45	35
Laktase	Laktosa	50	20
Glukoamilase	Pati	45	645

Kinetika Enzim Imobil

Nilai Km (konstanta Michaelis-Menten)

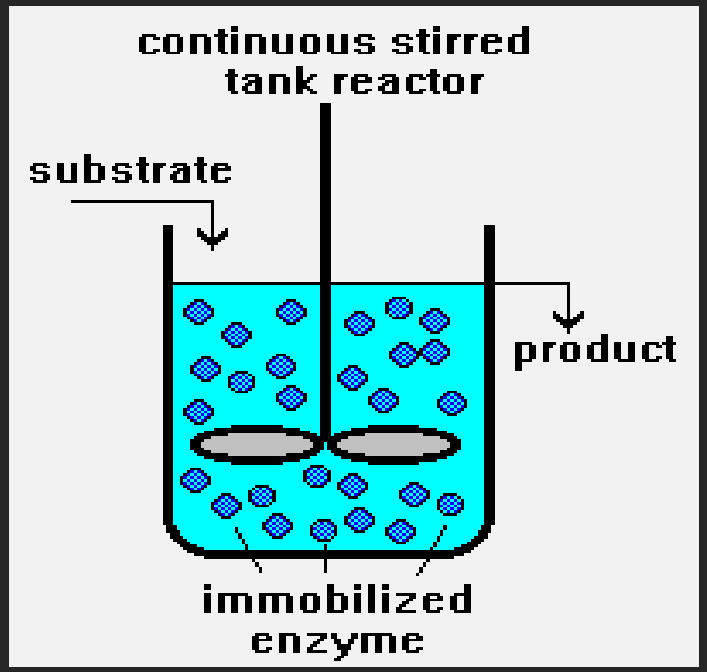
Enzim	Substrat	Km (milimolar)	
		Larut	Imobil
Invertase	Sukrosa	0.448	0.448
Arilsulfatase	P-nitrofenil-fosfat	1.85	1.57
Glukoamilase	Pati	1.22	0.30
Alkalin-fosfatase	P-nitrofenil-fosfat	0.10	2.90
Urease	Urea	10.0	7.60
Glu.oksidase	Glukosa	7.70	6.80
L-asam amino oksidase	L-leusin	1.00	4.00

Bioreaktor Enzim Imobil



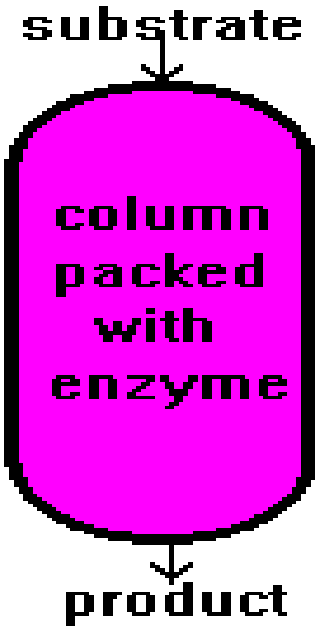
Reaktor Curah (Batch) :

- Sederhana
- Viskositas tinggi & aktivitas enzim rendah



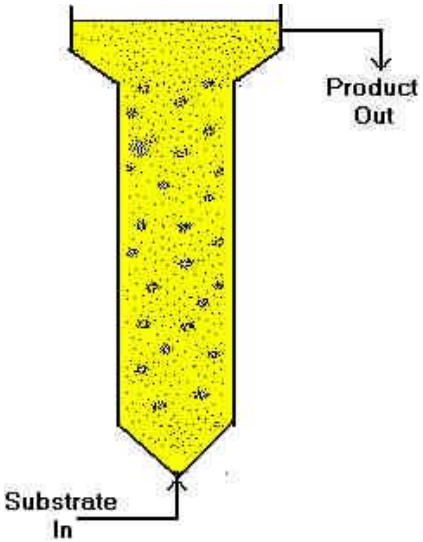
CSTR :

- Pengontrolan lbh mudah
- Cocok untuk kasus inhibisi (penghambatan) substrat
- Menghindari kontak enzim oleh substrat dan produk yang terlalu lama



Fixed-bed PFR (Unggun Diam/Terkemas) :

- Sinambung ➔ paling sering digunakan
- Aliran substrat dpt dari atas, bawah atau daur-ulang



Fluidized-bed (Unggun Terfluidisasi) :

- Untuk viskositas tinggi & terbentuk gas
- Laju fluidisasi perlu diatur agar enzim imobil tak rusak

Recycle Packed Column Reactor :

- allow the reactor to operate at high fluid velocities.
- a substrate that cannot be completely processed on a single pass