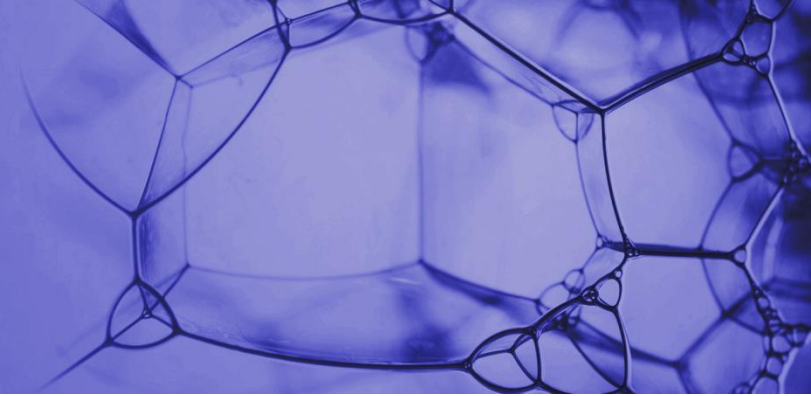


**LOSCHMIDT
LABORATORIES**



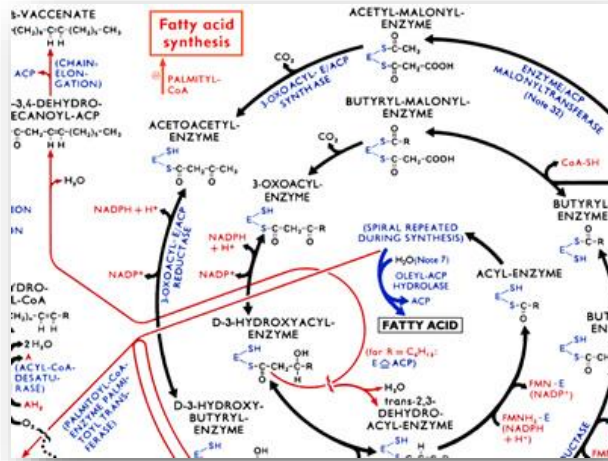
Molecular Biotechnology in Industry

Outline

- ❑ Enzymes and applications
- ❑ Definition of white biotechnology
- ❑ Enzyme sources
- ❑ Industrial production of proteins
- ❑ Enzyme and cells immobilization
- ❑ Examples of biocatalytic applications

Enzymes

- ❑ natural catalysts (biocatalyst)
- ❑ catalyze chemical reactions in living systems



- **oxidoreductases** - oxidation/reduction
- **transferases** - transfer of functional groups
- **hydrolases** – hydrolytic cleavage
- **lyases** - cleavage of C-C, C-N and C-O bonds
- **isomerases** - racemization, epimerization
- **ligases** - formation of C-C, C-N and C-O bonds

Enzyme applications

amylases
xylanases
proteases



proteases
peptidases
lipases
lactase



lipases
lactase



pectinases



cellulases
ligninases
xylanases
amylases



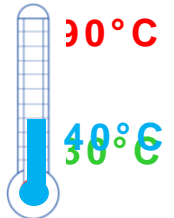
pectinases
glycosidases
esterases



amylases
glucanases
arabinoxylanases
proteases



amylases
proteases
cellulases
lipases



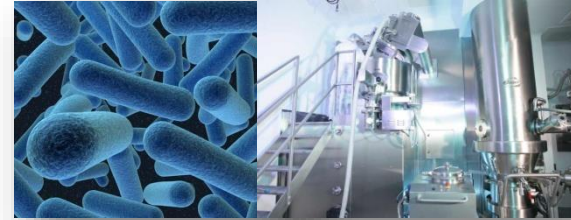
Enzyme applications

restrictases
DNA ligases
polymerases



phosphatases
peroxidases

amylases
proteases
cellulases
phytases
lipases



lipases
nitrilases
peptidases
amidases
aldolases

urokinases
proteases
alteplase



cellulases
ligninase
lipases

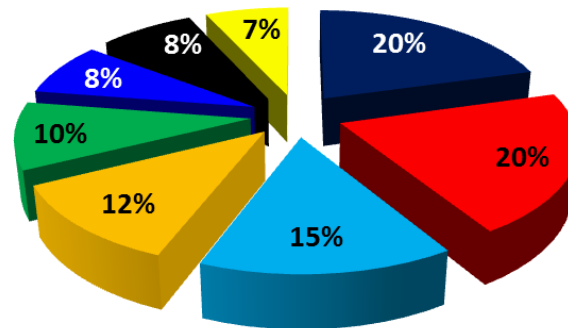
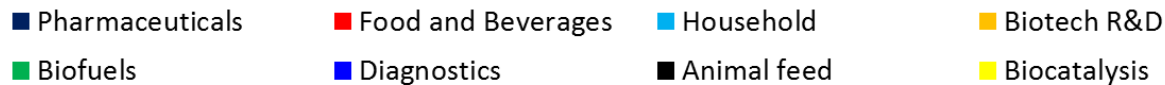
amylase
cellulases
catalase



dehalogenases
choline esterase
peroxidases

White (industrial) biotechnology

- ❑ biotechnology incorporated into production processes and products that **involve chemical reactions - biocatalysis**
- ❑ **sustainable** and **environmentally-friendly** industry
- ❑ provide **energy efficiency**, increased **productivity** and better **safety**
- ❑ uses **enzymes** and **micro-organisms** to make products and services in a wide range of industrial sectors



Enzyme-based technologies

ADVANTAGES

- ❑ high catalytic efficiency
- ❑ broad substrate specificity
- ❑ high selectivity
- ❑ compatibility of each other
- ❑ reusability
- ❑ sustainability
 - produced from biomass
 - non-toxic and biodegradable
 - operate at mild conditions
 - less byproducts and wastes

LIMITATIONS

- ❑ cofactor requirement
- ❑ prone to inhibitions
- ❑ highest activity in water
- ❑ less stable
- ❑ low selectivity
- ❑ expensive

Enzyme sources

❑ animal and plant tissues

- thousands years old developed empirically
- pancreas (treatment of hides), calf stomach (cheese-making)
- papaya, pineapple (meat tenderization)

<i>Source</i>	<i>Enzyme</i>	<i>Application</i>
Animal tissues		
Bovine and porcine pancreas	proteases (e.g., trypsin, chymotrypsin), amylases, lipases	digestive enzymes, anti-inflammatory agents, health food additives
Porcine stomach	pepsin	body fortifying agents
Liver and muscle	aldolases	fructose digestion
Porcine kidney	D-aminoacid oxidase	
Plant tissues		
Pineapple stem	bromelain (mixture of proteases)	anti-inflammatory agents, meat tenderizer
Papaya latex	papain (protease)	anti-inflammatory agents
Aspegillus	proteases, lipases, amylases, cellulases	natural food supplements, digestive enzymes

- **content up to 1%** enzyme of tissue weight
- **less competitive** compared to fermentation of microorganism
- **risk of contamination** with prions and viruses harmful to humans

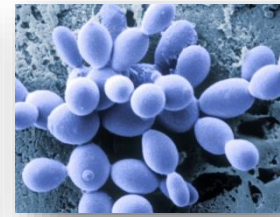
Enzyme sources

❑ wild-type microorganisms

- enzymes from microorganisms long been safely used in food industry
- food processing regulations - strict for non-recombinant enzymes
- microorganisms used for screening for „new“ catalytic enzymes
- screen for enzymes active at desired process conditions
(e.g., pH, temperature)

❑ recombinant microorganisms

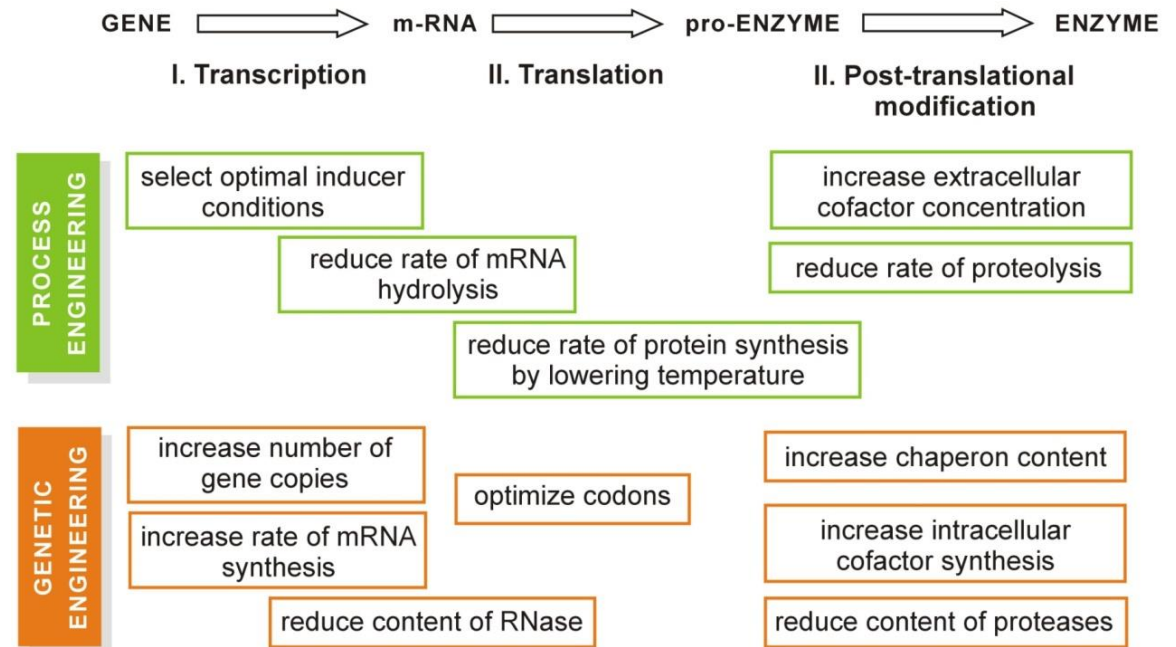
- most technical enzymes produced using **recombinant technology**
- when yield in wild type organism is low or desired enzyme is not in class I organism
- bacteria, fungi and yeasts
(e.g., *E.coli*, *Bacillus*, *Aspergillus*, *Saccharomyces*)



Industrial production of proteins

□ fermentation

- non-recombinant and recombinant organisms
- steady and safe (class I or GRAS) organisms
- up-scale and optimization
- **high cell density** fermentation (50 g cell dry weight per liter)
- upper limit of **protein concentration** (10 g.L^{-1} ; 40% of total cell protein)



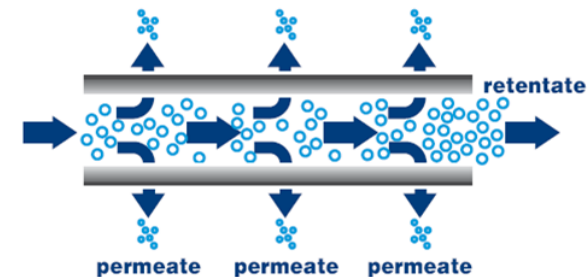
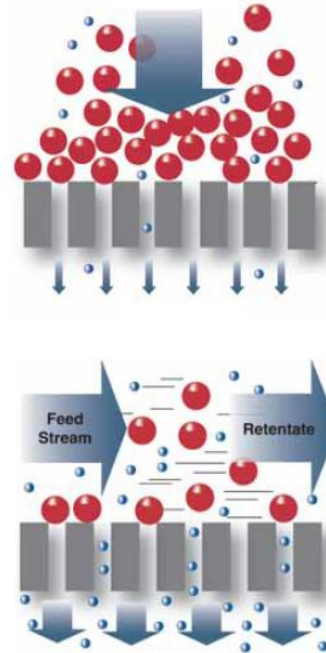
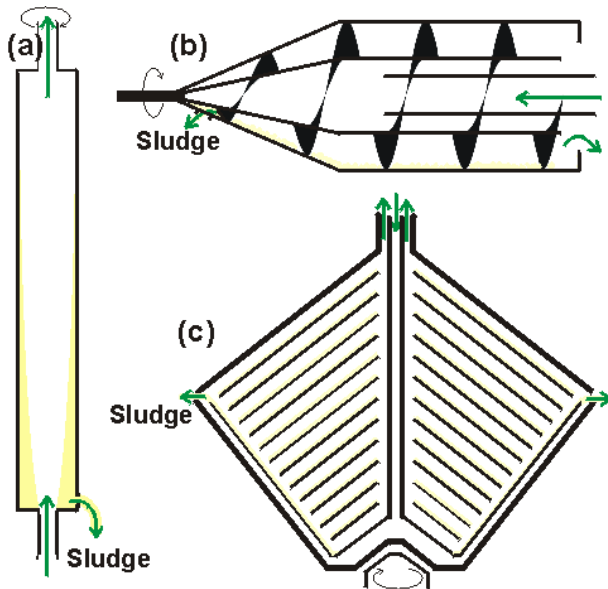
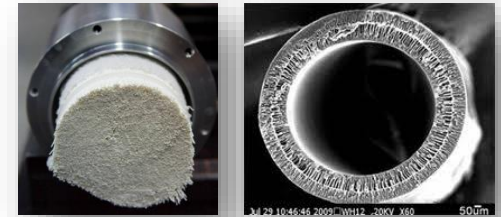
Downstream process

□ separation and homogenization

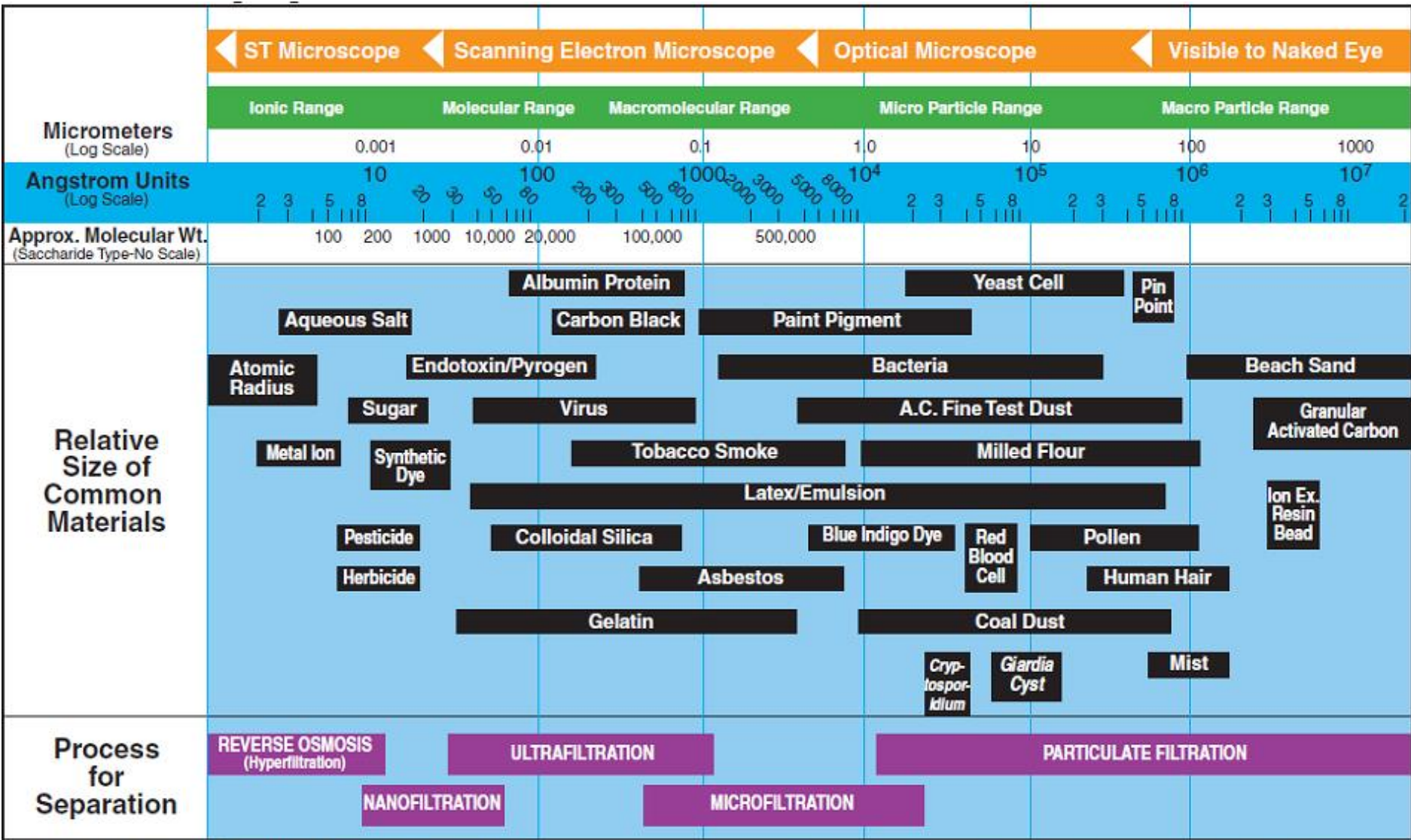
- dependent on application and required purity
- **technical** enzymes - low to moderate purity
- proteins for **therapy** and **diagnostics** - high purity

Intracellular Periplasmatic Extracellular

separation of cells from medim by centrifugation or microfiltration



Downstream process



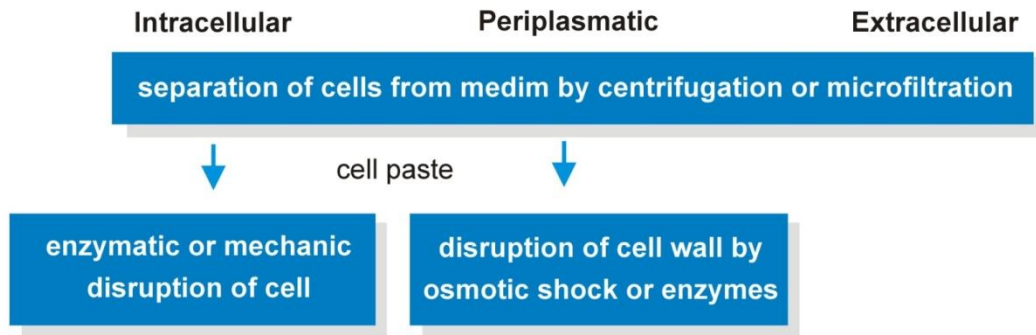
Note: 1 Micron (1 x 10⁻⁶ Meters) = 4 x 10⁻⁵ Inches (0.00004 Inches)
 1 Angstrom Unit = 10⁻¹⁰Meters = 10⁻⁴ Micrometers (Microns)

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Downstream process

□ separation and homogenization

- dependent on application and required purity
- **technical** enzymes - low to moderate purity
- proteins for **therapy** and **diagnostics** - high purity



MECHANICAL

- **ultrasonic disruption** - cell lysis with high frequency sound
- **homogenizers** - high pressure (1500 bar) and expansion
- **freeze fracturing** - water crystals as abrasive
- **ball mills** and **blenders**

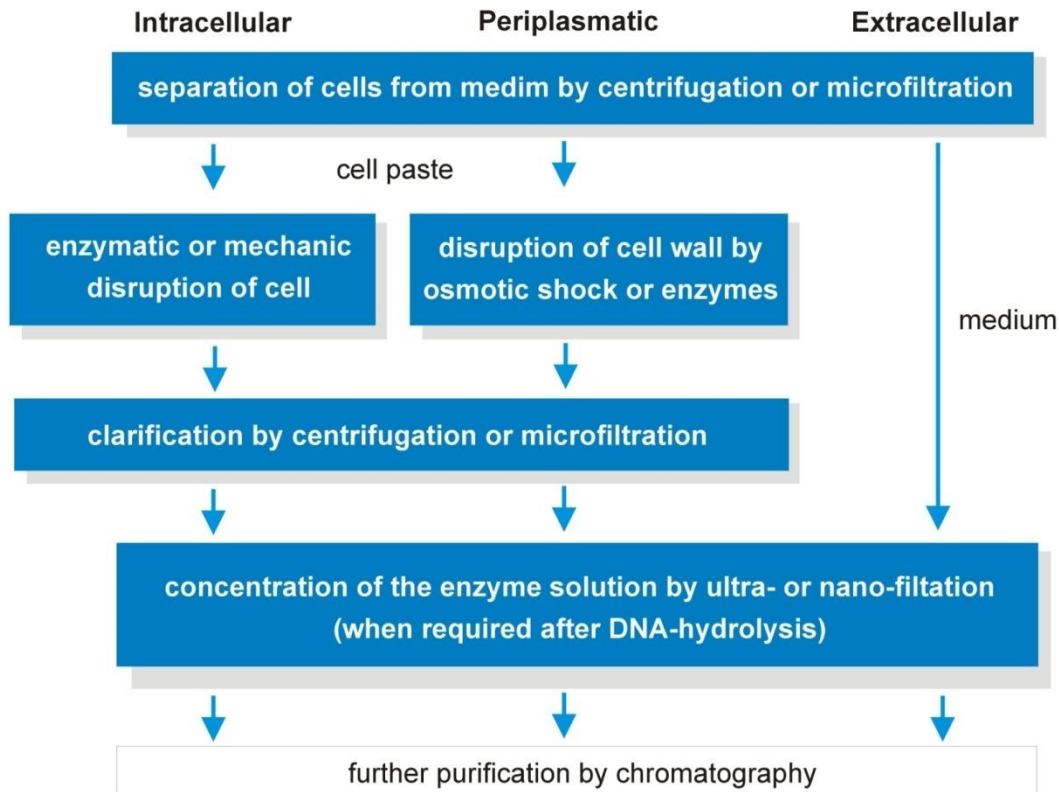
NON-MECHANICAL

- **osmotic shock** (e.g., high sucrose medium)
- **chemical permeabilization** (e.g., solvents, surfactants, antibiotics)
- **enzymatic permeabilization** (e.g., glycanases, proteases, mannanase)

Downstream process

□ separation and homogenization

- dependent on application and required purity
- **technical** enzymes - low to moderate purity
- proteins for **therapy** and **diagnostics** - high purity



Downstream process

❑ enzyme purification

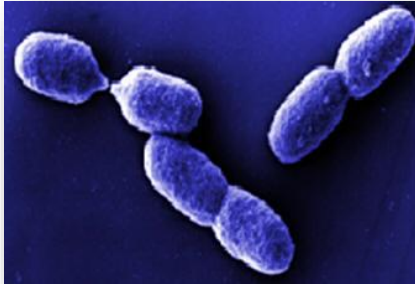
- **impurities** (e.g., proteins, DNA and others)
- further purification when **safety** (e.g., recombinant DNA, viruses) or **function** reasons (impurities disturbing catalytic function)
- basic knowledge of **protein properties** necessary

- molecular weight (MW)

WHAT ARE THE RELEVANT PROTEIN PROPERTIES?

- cofactors
 - pH range
 - temperature stability
- **methods** of protein purification
 - precipitation and differential solubilization (e.g., ammonium sulfate, pH, solvents)
 - membrane filtration
 - chromatographic methods (e.g., size exclusion, ion exchange, hydrophobic, metal affinity, biospecific)
 - **more steps -> higher purity** (each step loss >10% of enzyme)

Whole cell vs. isolated enzyme

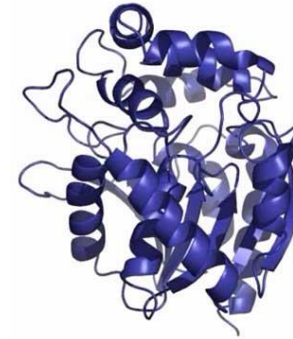
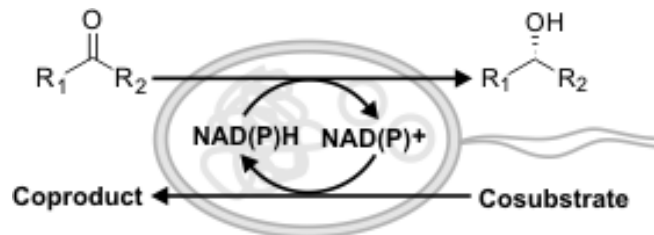


□ advantages

- allow more enzymes
- cofactor regeneration
- cheap

□ disadvantages

- side-reactions
- low tolerance to solvents
- low productivity

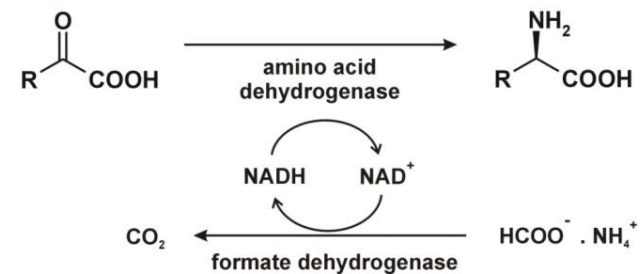


□ advantages

- smaller reactors
- less side reactions
- higher productivity

□ disadvantages

- more expensive
- addition of cofactors
- less stable outside cell



Immobilisation methods

❑ biocatalysts (enzyme or cell) **limited in moving** due to **chemical** or **physical treatment**

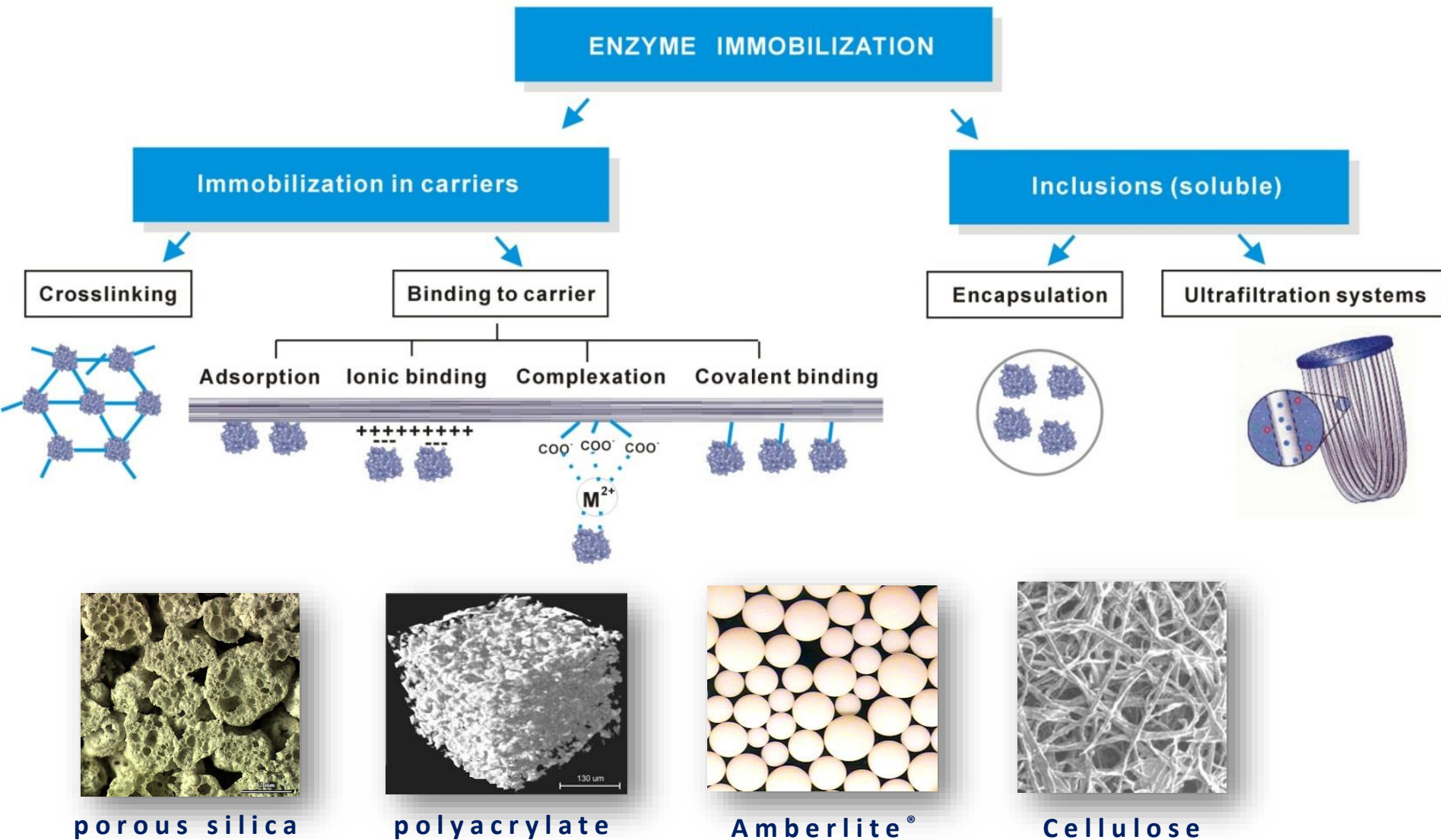
❑ **benefits**

- **stabilization** by immobilization
- **easy separation** of product
- **repeated use** of biocatalyst
- **continuous** bioprocessing

❑ **limitations**

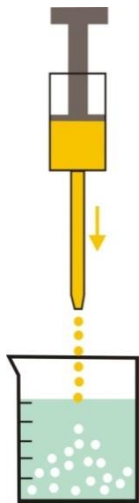
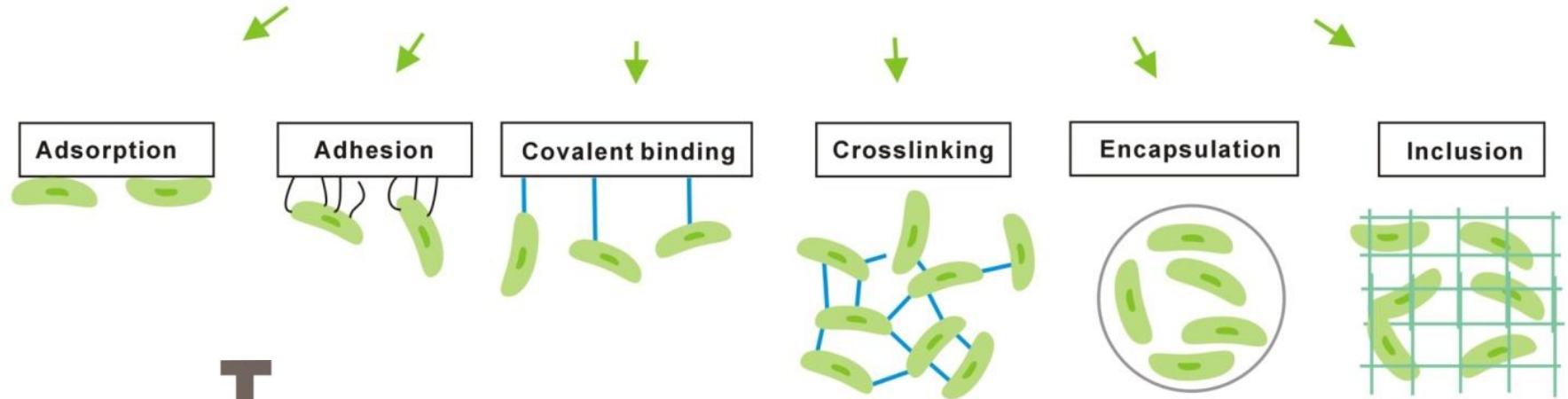
- **expenses** of carriers and immobilization
- **activity loss** during immobilization
- **changes in properties** of biocatalyst
- **mass transfer** limitations

Immobilisation of enzyme

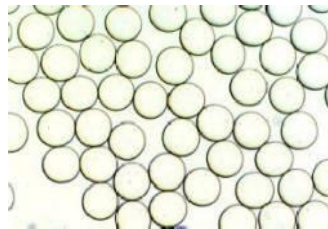


Immobilisation of cell

MICROORGANISM AND CELL IMMOBILIZATION



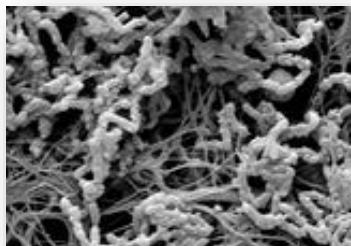
Alginate beads



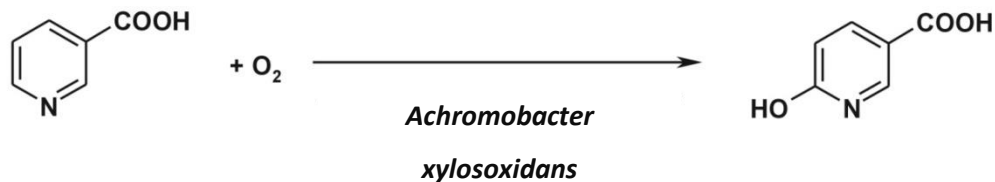
PVA lens (LentiCats)



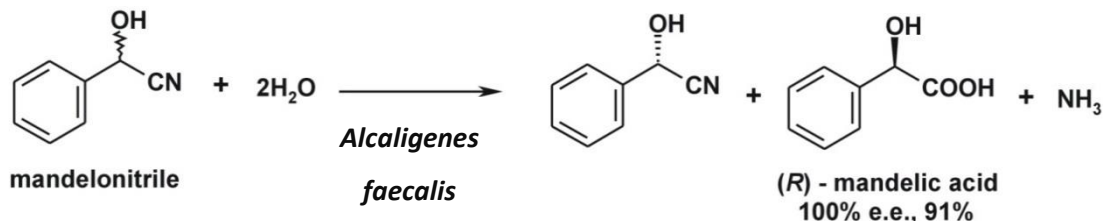
Examples of whole cell biocatalysis



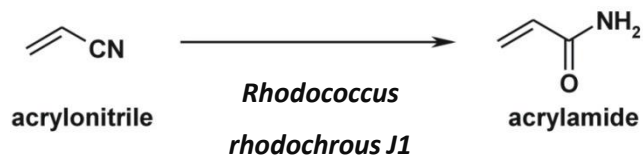
- synthesis of **agrochemical intermediates** by microbial hydroxylation of heteroatomics (Lonza)



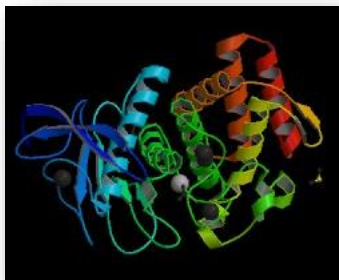
- mandelic acid - **urinary antiseptic, skin care cosmetics** (du Pont, Nitto Chemicals, etc.)



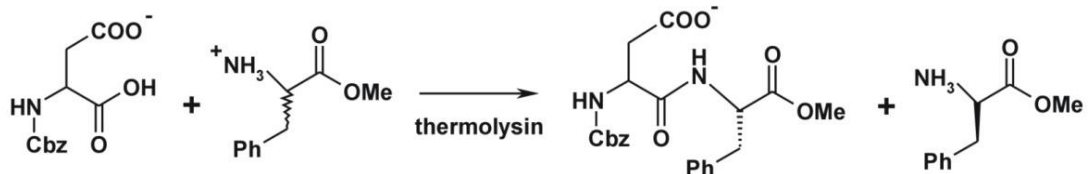
- large-scale production of **commodity chemical** - acrylamide (Mitsubishi, Nitto Chemicals)



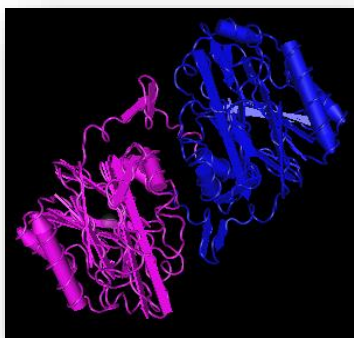
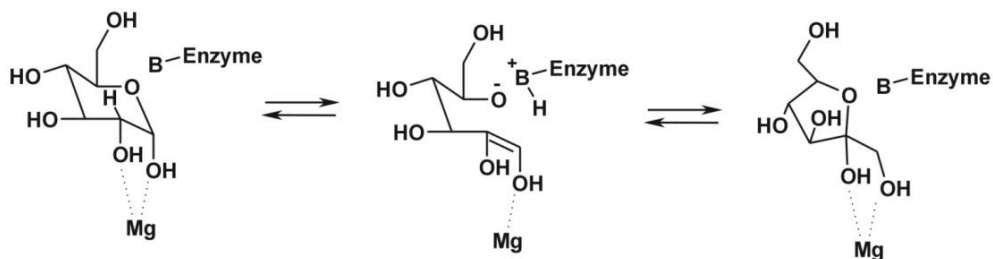
Examples of enzyme biocatalysis



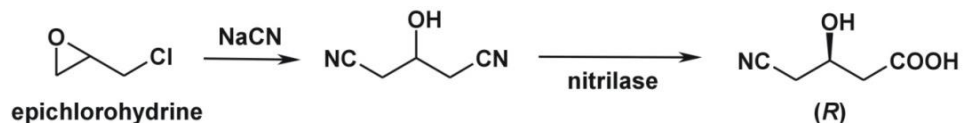
- large scale production of **Aspartame**, low-calorie sweetener (DSM, NutraSweet)



- synthesis of **high fructose syrup** from corn starch (10 million tons per year)

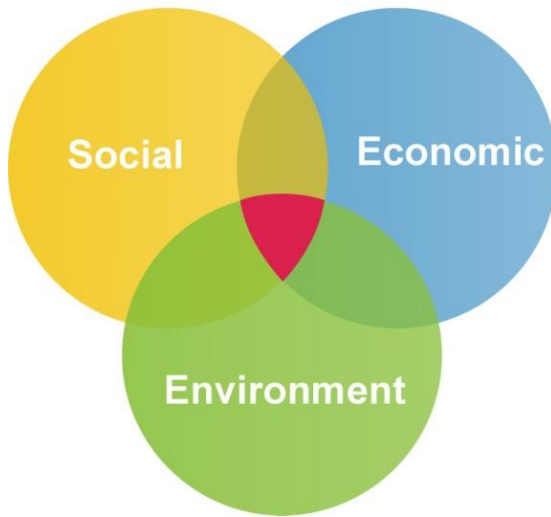


- synthesis of **atorvastatin, Lipitor[®]**, intermediate (Pfizer - sales since 1996 exceed US\$ 150 billion)



Let's make world better

- ❑ **sustainable** and **environmentally-friendly** industry
- ❑ **biomass** rather than traditional petrochemicals
- ❑ **energy efficient**, increased **productivity** and better **safety**



- ❑ **Enzymes at work** (Novozymes, Denmark)
 - 1. Why use enzymes for industrial processes?
 - 2. The nature of enzymes
 - 3. Industrial enzyme production

