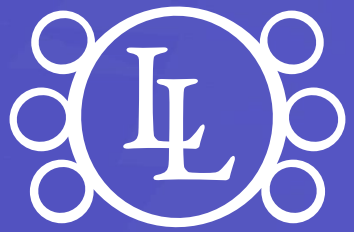


LOSCHMIDT
LABORATORIES

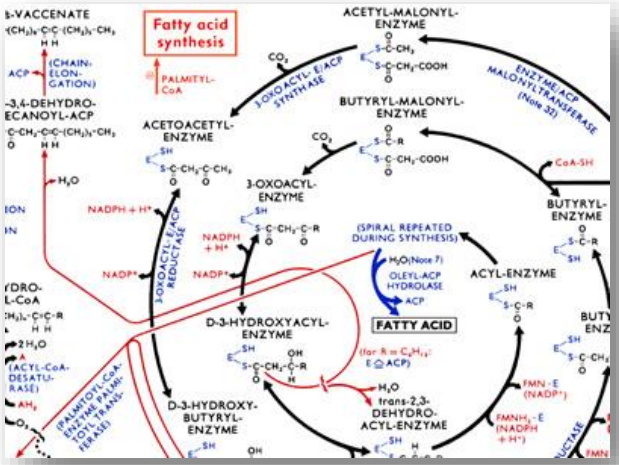
7. Molecular Biotechnology in Industry

Outline

- Enzymes and applications
- Definition of white biotechnology
- Sustainable development
- 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-Nand C-O bonds

Enzyme applications

restrictases
DNA ligases
polymerases

phosphatases
peroxidases

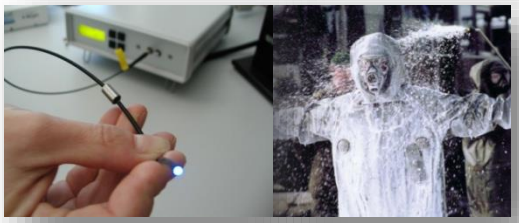
amylases
proteases
cellulases
phytases
lipases

lipases
nitrilases
peptidases
amidases
aldolases

asparaginase
DNase
urokinases
proteases

cellulases
ligninase
lipases

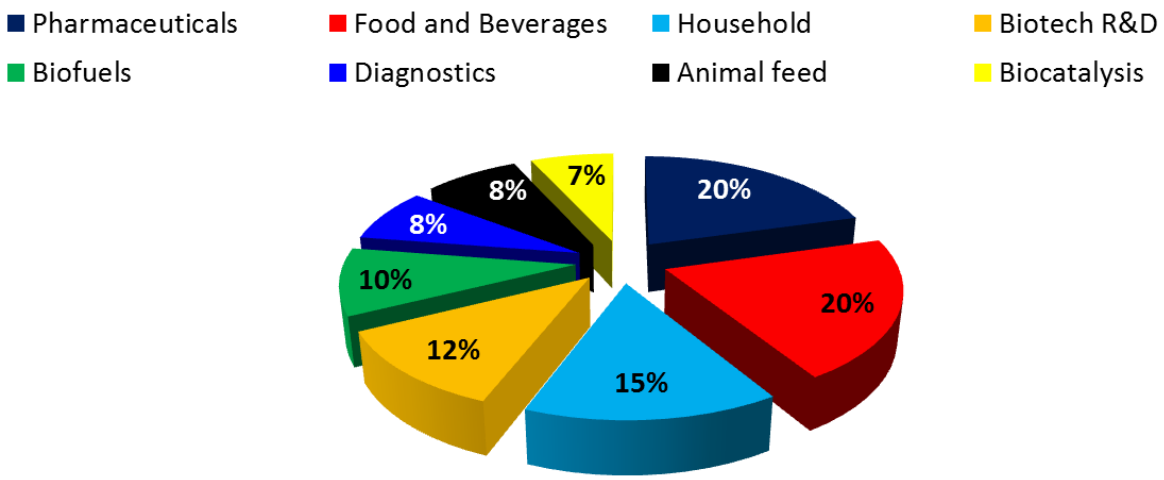
amylase
cellulases
catalase



dehalogenases
choline esterase
peroxidases

White (industrial) biotechnology

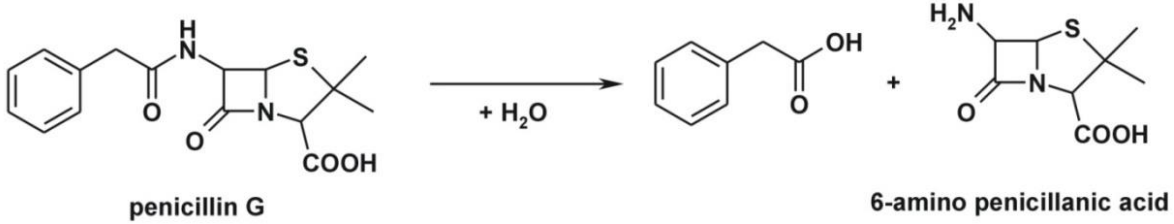
- biotechnology incorporated into production processes and products that **involve chemical reactions - biocatalysis**
- **sustainable** and **environmentally-friendly** industry
- provide **energy efficiency**, increased **productivity** and better **safety**
- uses **enzymes** and **micro-organisms**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



Sustainable solutions

- innovative and competitive products and processes meeting **criteria of sustainability**
- transfer of biological solutions to **modern technologies** create the **future in balance** between economy, cleaner environment and better lives
- "… development that meets the **needs of the present without compromising** the ability of **future** generations to meet their own needs" (WCED, 1987)

Example of sustainable technology

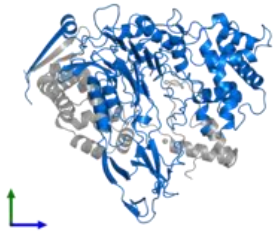


Chemical process (-40°C)

1000 t	penicillin G
160 t	ammonia
300 t	dimethylchlorosilane
800 t	*N,N*-dimethylaniline
600 t	phosphopentachloride
4,200 m³	dichloromethane
4,200 m³	*n*-butanol

Biocatalysis (+30°C)

1000 t	penicillin G
45 t	ammonia
10,000 m³	water
1 t	ENZYME
	(1 $/kg 6-APA)



Enzyme-based technologies

ADVANTAGES

- high catalytic efficiency
- broad substrate specifity
- 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

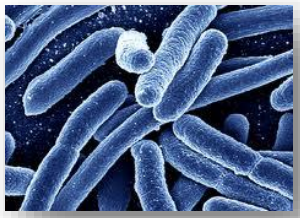
Enzyme sources

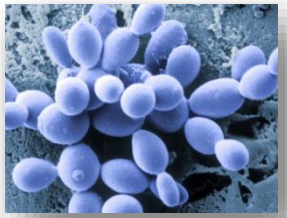
- **animal and plant tissues**
  - thousands years old developed empirically
  - pancreas (treatment of hides), calf stomach (cheese-making)
  - papaya, pineapple (meat tenderization)
  - **content up to 1%** enzyme of tissue weight
  - **less competitive** compared to fermentation of microorganism
  - **risk of contamination** with prions and viruses harmful to humans

Source	*Enzyme*	*Application*
Animal tissues		
Bovine and porcine pancreas	proteases (e.g., trypsine, chymotrypsine), amylases, lipases	digestive enzymes, anti-inflammatory agents, health food additives
Porcine stomach	pepsine	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

Enzyme sources

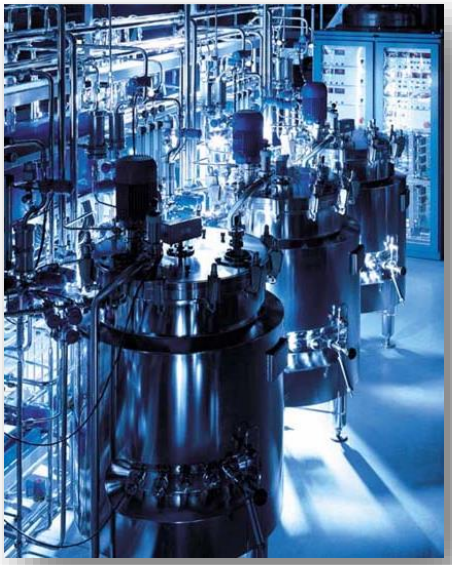
- **wild-type microorganisms**
  - enzymes from microorganisms long been safely used in food industry
  - food processing regulations - strict for non-recombinant enzymes
  - microorganisms used for screening for „new“ catalytic enzymes
  - screen for enzymes active at desired process conditions (e.g.,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

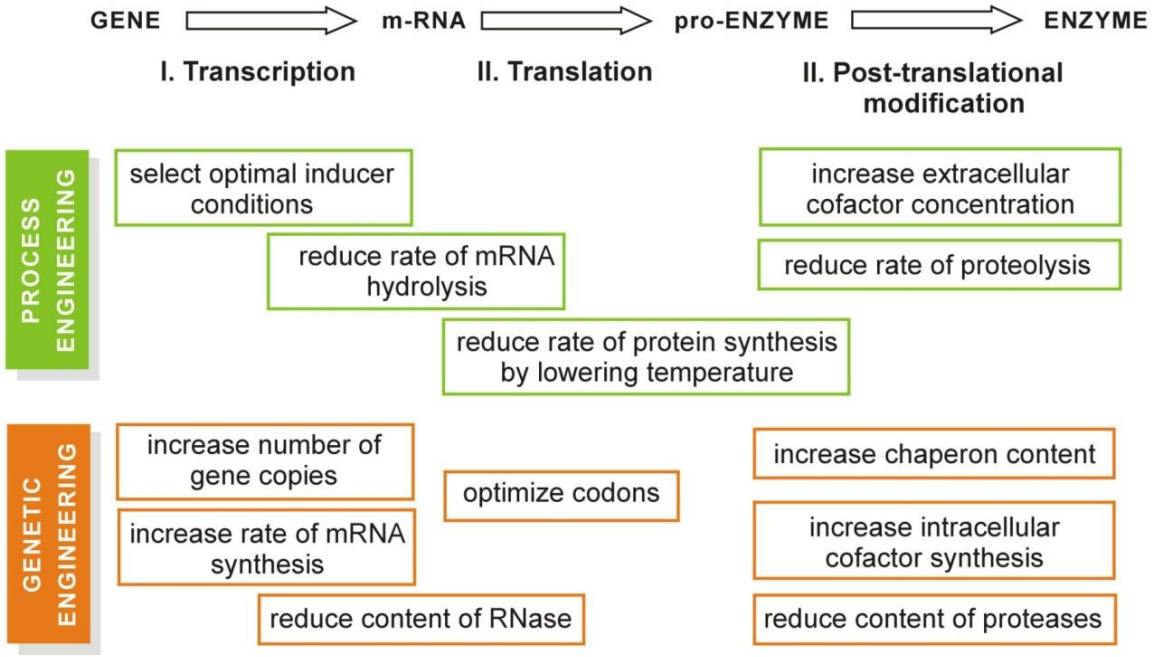


Industrial production of proteins

- **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)



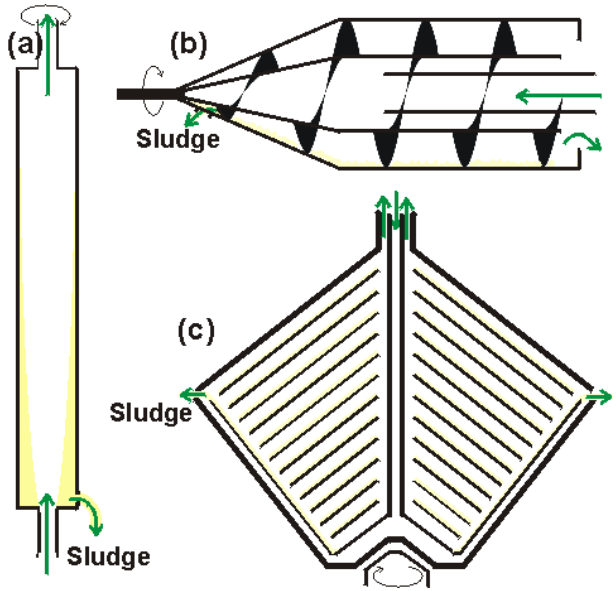


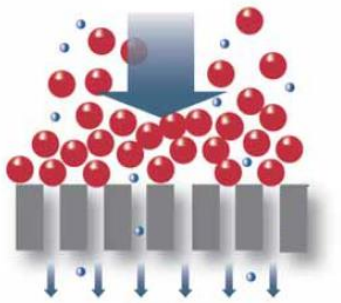
Downstream process

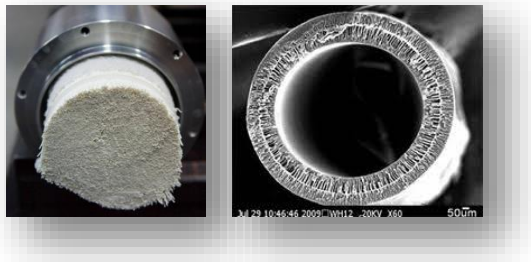
- **separation** and **homogenization**
  - dependent on application and required purity
  - **technical** enzymes - low to moderate purity
  - proteins for **therapy** and **diagnostics** - high purity

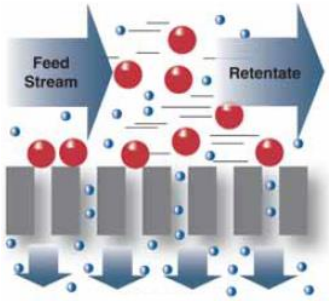
Intracellular | Periplasmatic | Extracellular

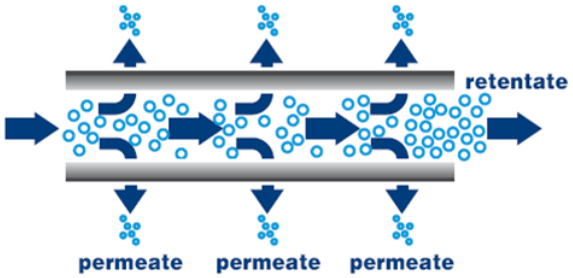
separation of cells from medim by centrifugation or microfiltration



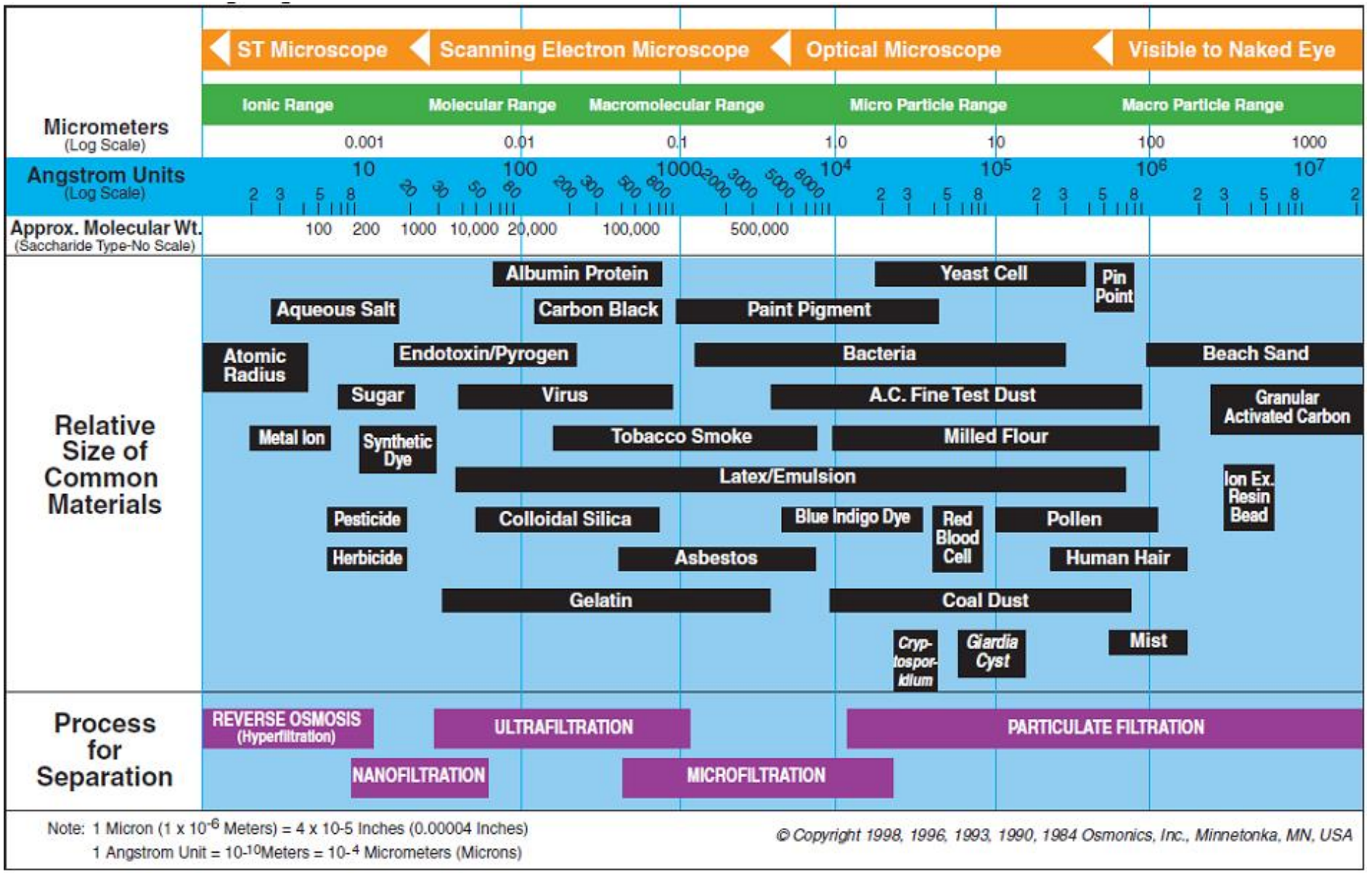








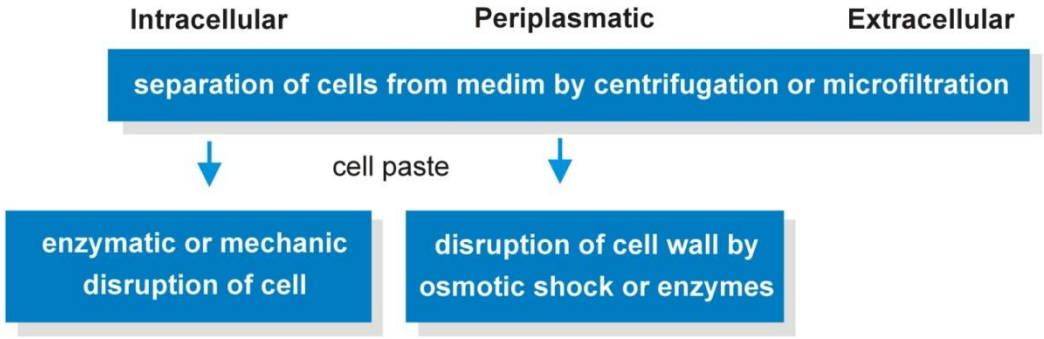
Downstream process



Note: 1 Micron (1 x 10^{-6} Meters) = 4 x 10-5 Inches (0.00004 Inches)
1 Angstrom Unit = 10^{-10}Meters = 10^{-4} Micrometers (Microns)

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

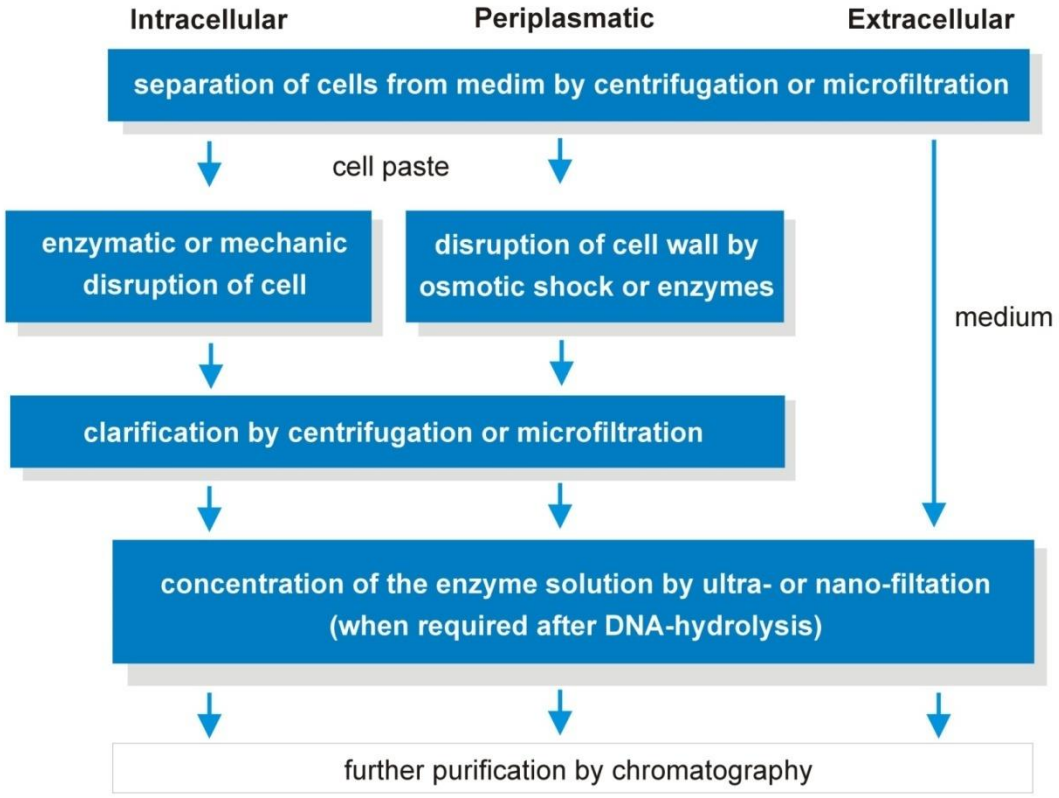
- **ultrasonic disruption** - cell lysis with high frequency sound
- **homogenizers** - high pressure (1500 bar) and expansion
- **freeze fracturing** - water crystals as abrasive
- **ball mills** and **blenders**

NON-MECHANICAL

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

Downstream process

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

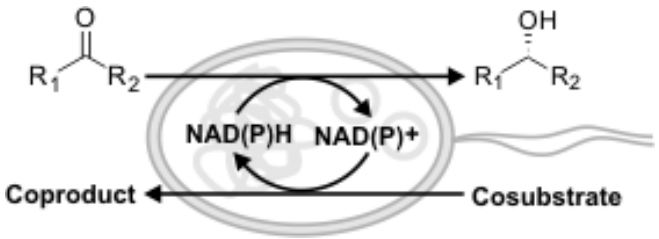


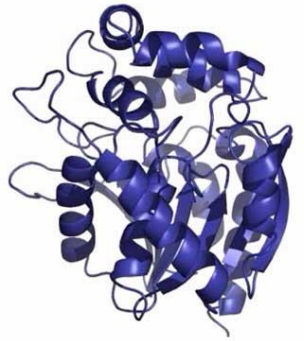
Downstream process

- **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)
    - isoelectric point (pI)
    - 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)

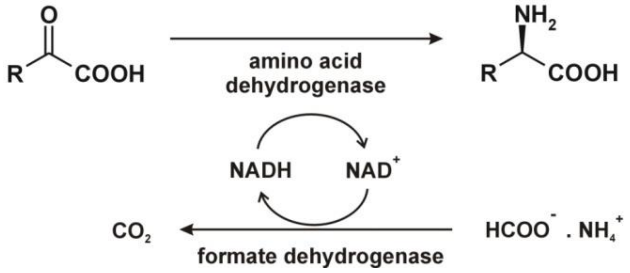
Whole cell vs. isolated enzyme

- **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



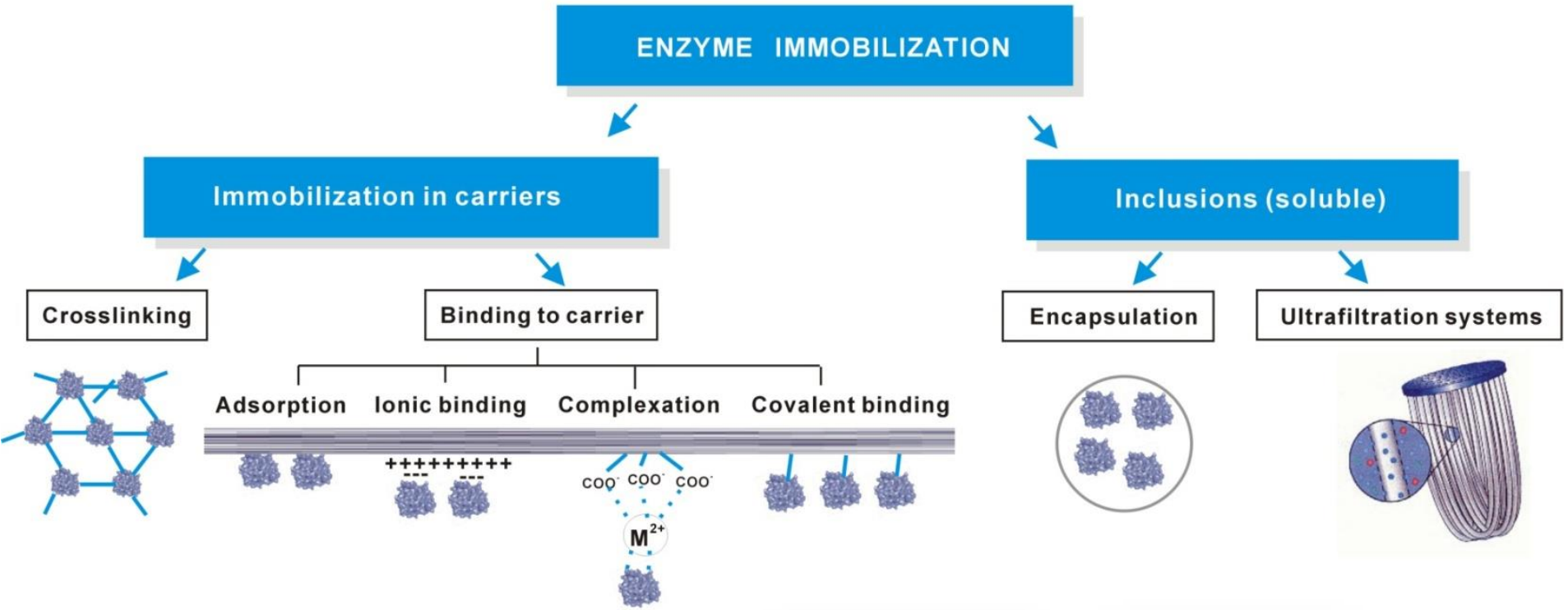
Immobilisation methods

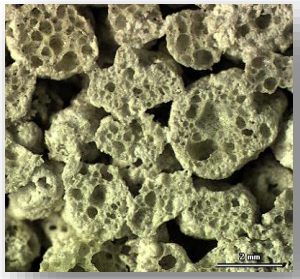
- biocatalysts (enzyme or cell) **limited in moving** due to **chemical** or **physical treatment**

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

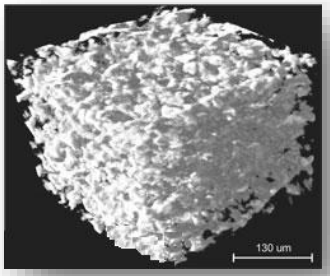
- **limitations**
  - **expenses** of carriers and immobilization
  - **activity loss** during immobilization
  - **changes in properties** of biocatalyst
  - **mass transfer** limitations

Immobilisation of enzyme

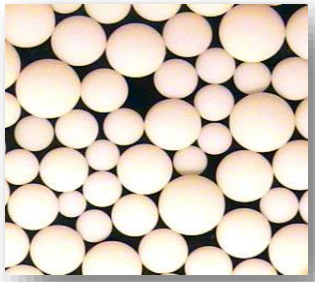




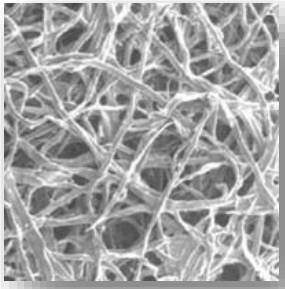
porous silica



polyacrylate

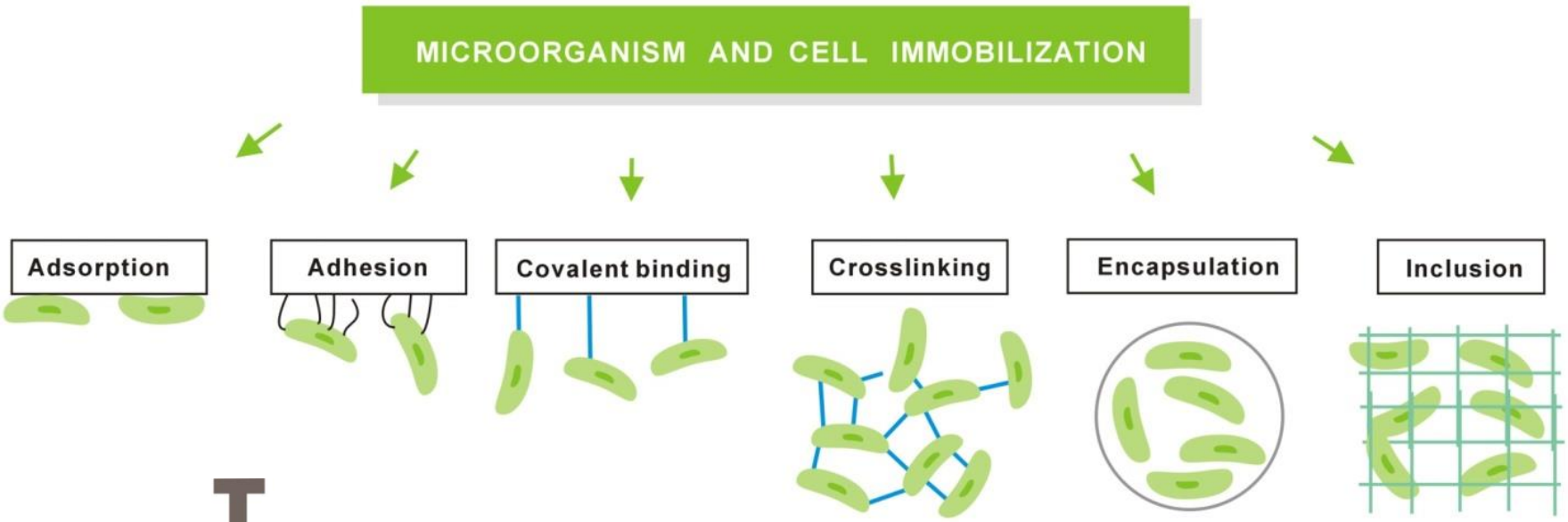


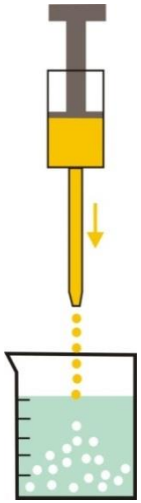
Amberlite®



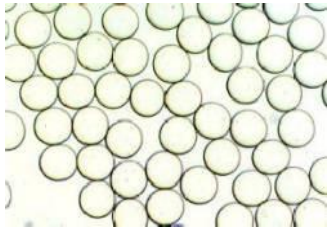
Cellulose

Immobilisation of cell



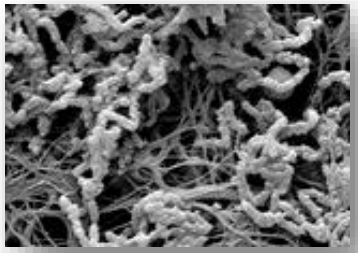


Alginate beads

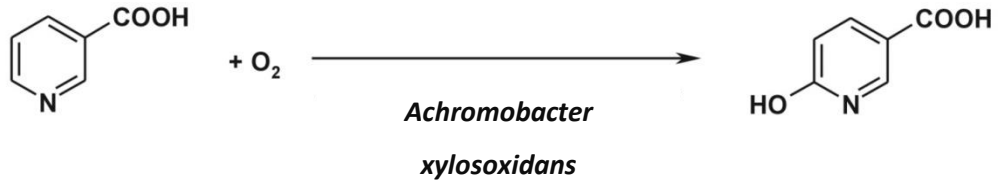


PVA lens (LentiCats)

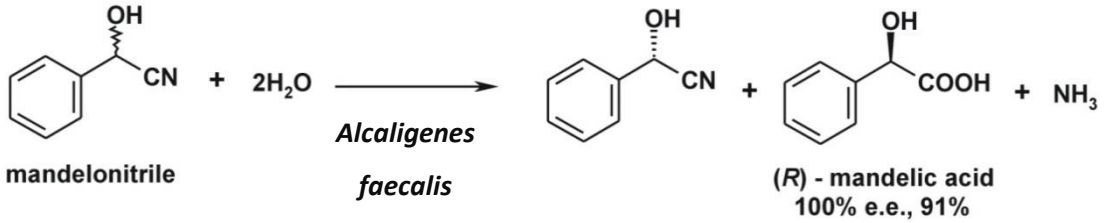
Examples of whole cell biocatalysis

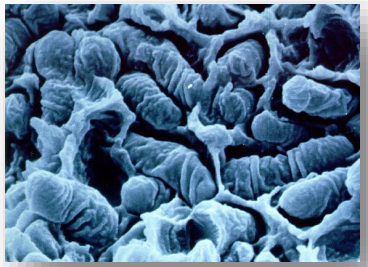


- synthesis of **agrochemical intermediates** by microbial hydroxylation of heteroatomics (Lonza)

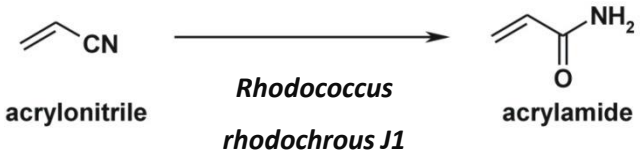


- mandelic acid - **urinary antiseptic, skin care cosmetics** (du Pont, Nitto Chemicals, etc.)



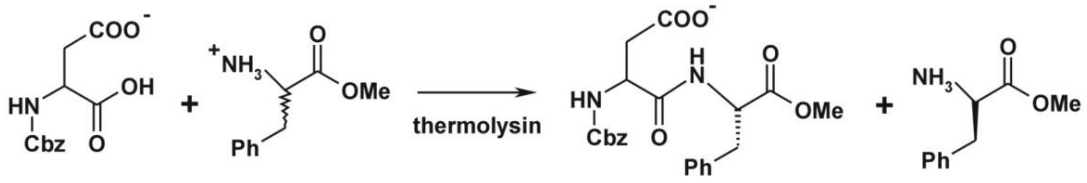


- large-scale production of **commodity chemical** - acrylamide (Mitsubishi, Nitto Chemicals)

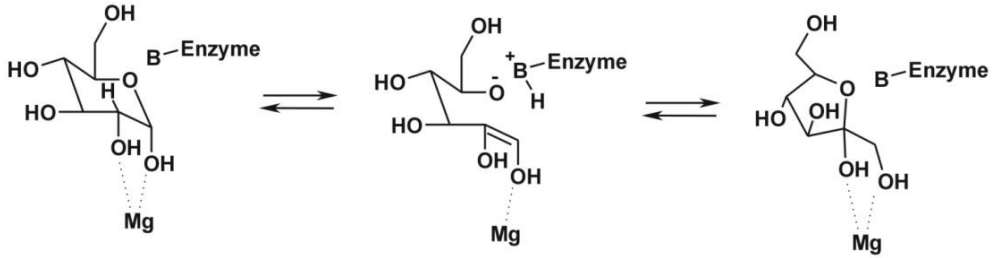


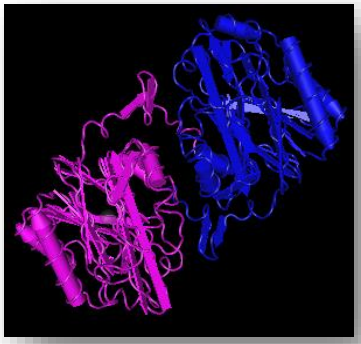
Examples of enzyme biocatalysis

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

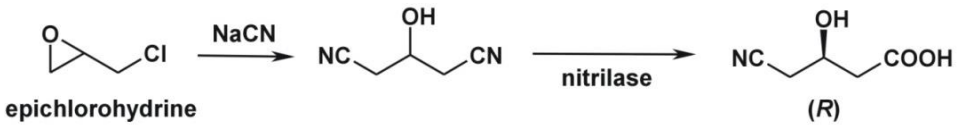


- synthesis of **high fructose syrup** from corn starch (10 million tons per year)





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



Reading

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