LOSCHMIDT LABORATORIES

6. Industrial Applications of Molecular Biotechnology

Outline

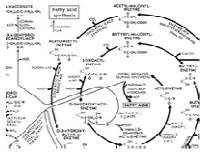
- Definition of white biotechnology
- Enzymes and applications
- Sustainable development
- Enzyme sources
- Industrial production of proteins
- Enzyme and cells immobilisation
- Examples of biocatalytic applications

White (industrial) biotechnology

- biotechnology incorporated into production processes and products that **involve chemical reactions - biocatalysis**
- uses **enzymes** and **micro-organisms** to make products and services in a wide range of industrial sectors
  - fine chemicals and pharmaceuticals
  - materials and polymers
  - paper, pulp and textiles
  - food and feed
  - detergents
- **sustainable** and **environmentally-friendly** industry
- **using biomass** rather than traditional petrochemicals
- provide **energy efficiency**, increased **productivity** and **better safety** and **environmental** characteristics

Enzymes

- **natural catalysts** (biocatalyst)
- catalyse **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

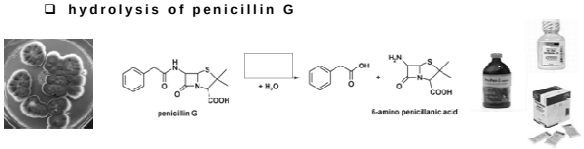
dehalogenases
OPH, BChE
peroxidases

Sustainable solution

- inovative 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)
  - reduce environmental impact
  - reduce consumption of resources (raw materials, energy, air, water)
  - use of renewable raw materials
  - reduce waste production
  - maximize waste recycling

Example of sustainable technology

- **hydrolysis of penicillin G**



Chemical process (-40°C)		**Biocatalysis** (+30°C)	
1000 t	**penicillin G**	**1000 t**	**penicillin G**
160 t	**ammonia**	**45 t**	**ammonia**
300 t	**dimethylchlorosilane**	**10,000 m³**	**water**
800 t	***N,N*-dimethylaniline**	**1 t**	**ENZYME**
600 t	**phosphopentachloride**		**(1 $/kg 6-APA)**
4,200 m³	**dichloromethane**		
4,200 m³	***n*-butanol**		

Enzyme-based technology

ADVANTAGES

- high catalytic **efficiency**
- broad substrate **specifity**
- high degree of **selectivity**
- **compatibility** of each other
- **reusability**
- **sustainablity**
  - produced from biomass
  - easily biodegradable
  - non-toxic, non-flamable
  - no reqirement of large quantity of toxic metals and solvents
  - less byproducts and wastes
  - oparate at mild conditions

LIMITATIONS

- **cofactor** requirement
- prone to **inhibitions**
- highest **activity in water**
- generaly **less stable**
- insufficient **selectivity**
- often **expensive**
- **allergies**

Enzyme sources

ANIMAL AND PLANT TISSUES

- **content** often **up to 1%** enzyme of tissue weight
- thousands years old developed empirically
- pancreas (earlier treatment of hides), calf stomach (cheese-making)
- papaya, pineapple (tenderization of meat), sodom apple leaves (milk clotting)
- **risk of contamination** with prions and viruses harmfull to humans
- **less competetive** compared to fermentation of microorganism

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
Aspergillus	proteases, lipases, amylases, cellulases	natural food supplements, digestive enzymes

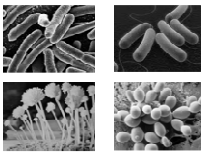
Enzyme sources

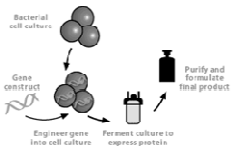
WILD-TYPE MICROORGANISMS

- enzymes from microorganisms long been safely used in **food industry**
- **food processing enzymes** - regulation strict for using 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

- 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)
- most technical enzymes produced using **recombinant technology**

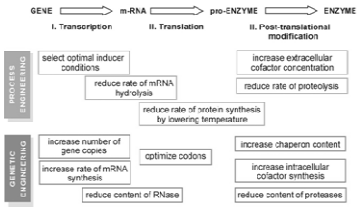




Industrial production of enzymes

FERMENTATION

- **non-recombinant** and **recombinant** organisms
- **steady and safe** (class I or GRAS) organisms
- **up-scale** and **optimisation**
  - **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)



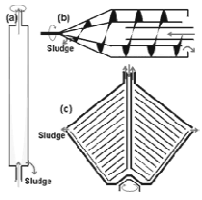
Downstream process

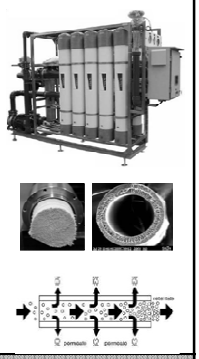
SEPARATION AND HOMOGENISATION

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

Intracellular | Periplasmatic | Extracellular

separation of cells from medim by centrifugation or microfiltration





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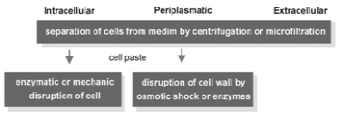
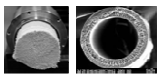
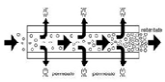




- **mechanical**
  - homogenizers - high pressure (1500 bar) followed by expansion
  - ball mills - small abrasive particles
  - ultrasonic disruption - cell lysis with high frequency sound
  - blenders - blades rotate at speeds of 6,000 - 50,000 rpm
  - freeze fracturing - water crystals as abrasive
- **non-mechanical**
  - chemical permeabilization (e.g., solvents, surfactants, antibiotics)
  - enzymatic permeabilization (e.g., glycanases, proteases, mannase)
  - osmotic shock (e.g., high sucrose medium)



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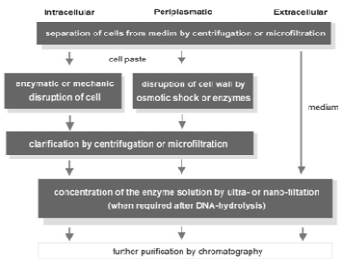
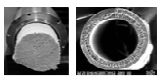
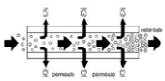






Downstream process

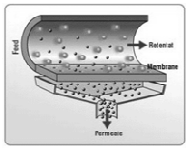
ENZYME PURIFICATION

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

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 (size exclusion, ion exchange, hydrophobic, metal affinity, biospecific)



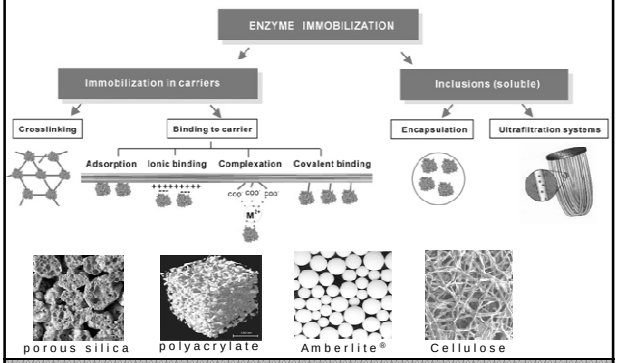
- **more steps -> higher purity** (multi-step manipulation, loss >10% of enzyme)

Immobilisation methods

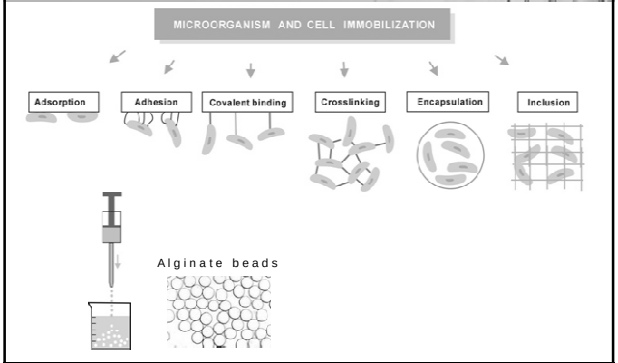
- biocatalysts (enzyme or cell) **limited in moving** due to **chemical or physical treatment**

REASONS	LIMITATIONS
▪ **reuse** of enzyme (reducing cost)	▪ **cost** of carriers and immobilisation
▪ easy product **separation**	▪ changes in **properties** (selectivity)
▪ **continous** processing	▪ mass transfer **limitations**
▪ **stabilization** by immobilisation	▪ **activity loss** during immobilisation

Immobilisation of enzyme



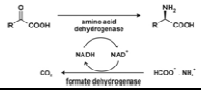
Immobilisation of cell



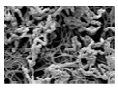
Whole cell vs. isolated enzyme

- **advantages**
  - allow more enzymes
  - cofactor regeneration
  - cheap
- **disadvantages**
  - side-reactions from other enzymes
  - low tolerance to organic solvents
  - low productivity

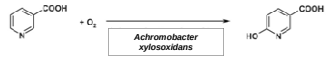
- **advantages**
  - smaller reactors
  - less side reactions
  - higher productivity
- **disadvantages**
  - more expensive
  - addition of cofactors
  - less stable outside cell

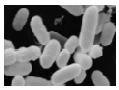


Examples of whole cell biocatalysis

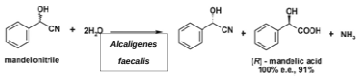


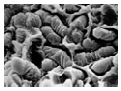
- synthesis of **agrochemical intermediates** by microbial hydroxylation of heteroatomics (**Lonza**)



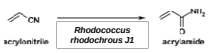


- **mandelic acid** - urinary antiseptic, skin care cosmetics (**du Pont, Nitto Chemicals,** etc.)



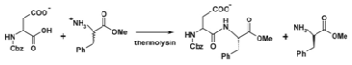


- large-scale production of commodity chemical - **acrylamide** (**Mitsubishi, Nitto Chemicals**)

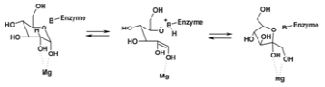


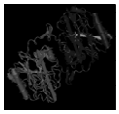
Examples of enzyme biocatalysis

- large scale production of **Aspartame**, low-calorie sweetner (**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$ 125 billion)