LOSCHMIDT LABORATORIES

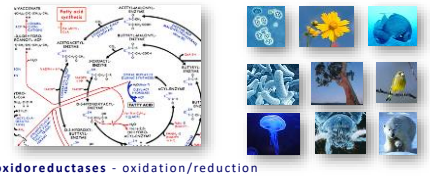
Molecular Biotechnology in Industry

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-Nand C-O bonds

Enzyme applications

Enzyme applications

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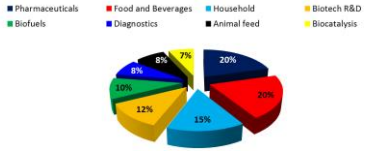
White (industrial) biotechnology

- 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



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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

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Enzyme sources

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

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

  - **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

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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*)

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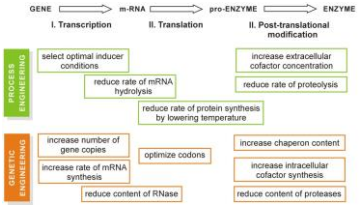
Industrial production of proteins

- **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)



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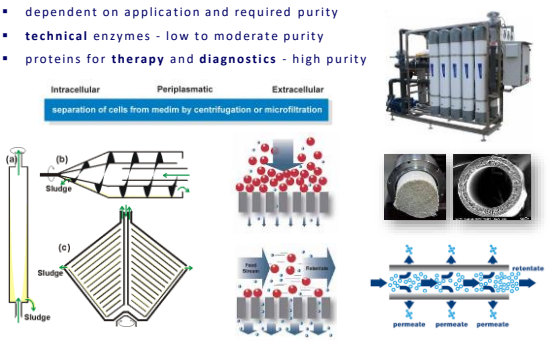
Downstream process

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



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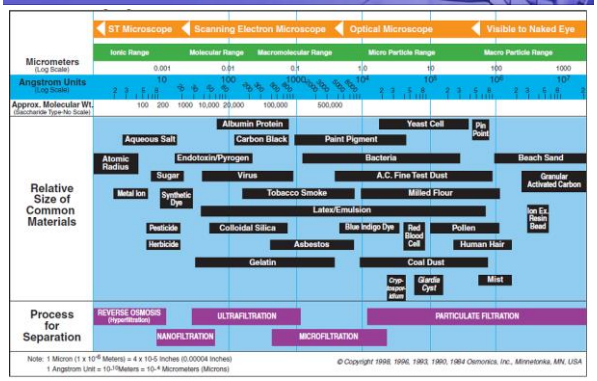
Downstream process



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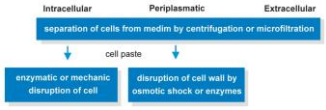

MECHANICAL

- **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)

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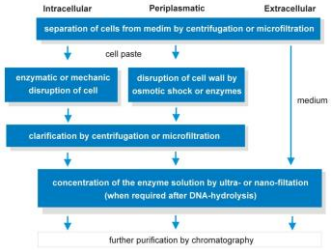

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Downstream process

- **enzyme purification**
  - **impurities** (e.g., proteins, DNA and others)
  - further purification when **safety** (e.g., recombinant DNA, viruses) or **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)

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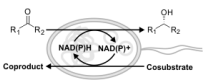
Whole cell vs. isolated enzyme

advantages

- allow more enzymes
- cofactor regeneration
- cheap

disadvantages

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

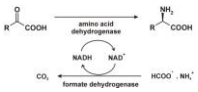


advantages

- smaller reactors
- less side reactions
- higher productivity

disadvantages

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



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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

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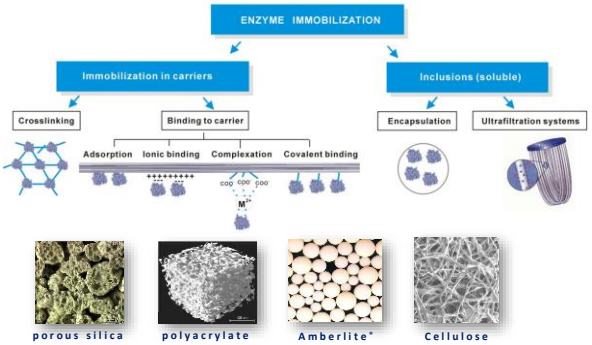
Immobilisation of enzyme



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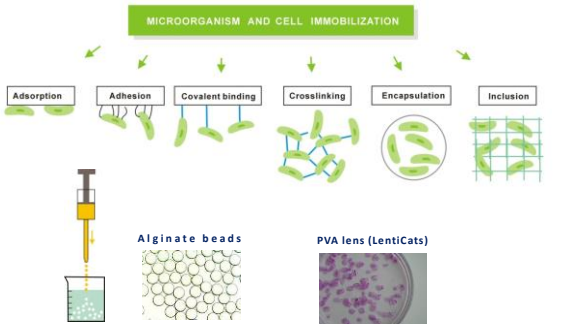
Immobilisation of cell



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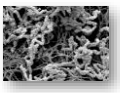
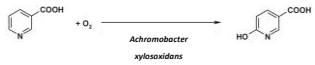
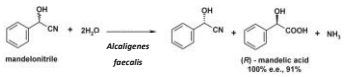
Examples of whole cell biocatalysis



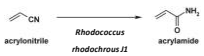
- synthesis of **agrochemical intermediates** by microbial hydroxylation of heteroatomics (Lonza)



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



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



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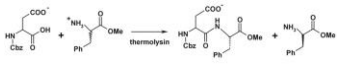
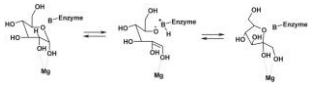
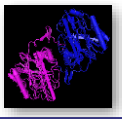
Examples of enzyme biocatalysis

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

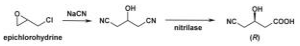


- synthesis of **high fructose syrup** from corn starch (10 million tons per year)





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



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Let´s make world better

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

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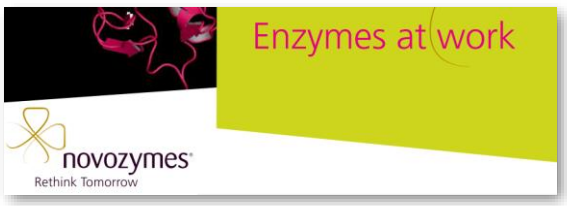
Reading

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



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