BCH 462-Biotechnology and Genetic Engineering

Lecture 5- Industrial Microbial Enzymes and their applications

Lecture outline, aims, and objectives

- Introduction of industrial microbial enzymes biotechnology.
- Brief history and an example of the importance of industrial microbial enzymes biotechnology.
- The process of microbial enzyme development for their use in industry.
- Detailed examples of some industrial enzymes

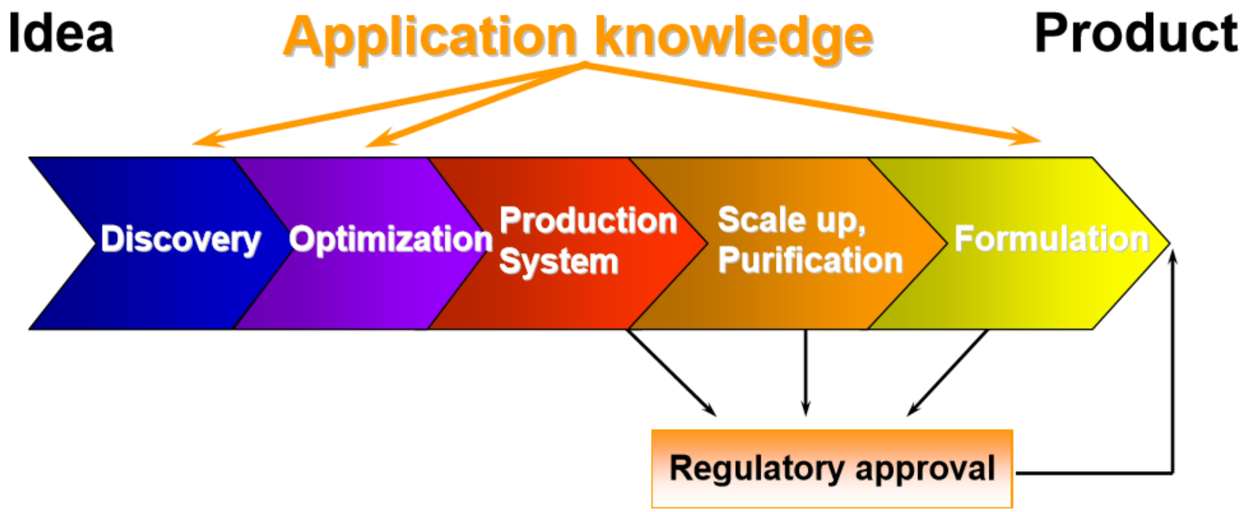
Development of an industrial enzyme

Industrial biotechnology companies use many specialized techniques to find and improve nature's enzymes.

Information from genomic studies on microorganisms is helping researchers capitalize on the wealth of genetic diversity in microbial populations.

Researchers first search for enzyme-producing microorganisms in the natural environment. Once isolated, such enzymes can be identified and characterized for their ability to function in specific industrial processes.

If necessary, they can be improved with biotechnology techniques.

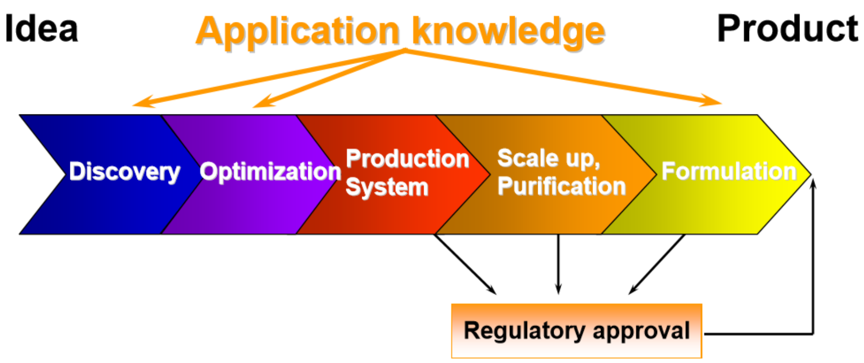


Definitions

Industrial enzymes biotechnology: The practical application and industrial use of enzymes to accomplish certain reactions outside the cell.

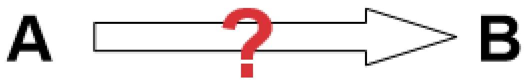
Enzymes: biocatalysts produced by living cells to bring about specific biochemical reactions. They are protein in nature, and may or may not have a non-protein prosthetic group. They are specific in their action on substrates and only accelerate the rate of particular reaction by lowering the activation energy without undergoing any permanent change in them, and therefore, are vital biomolecules that support life.

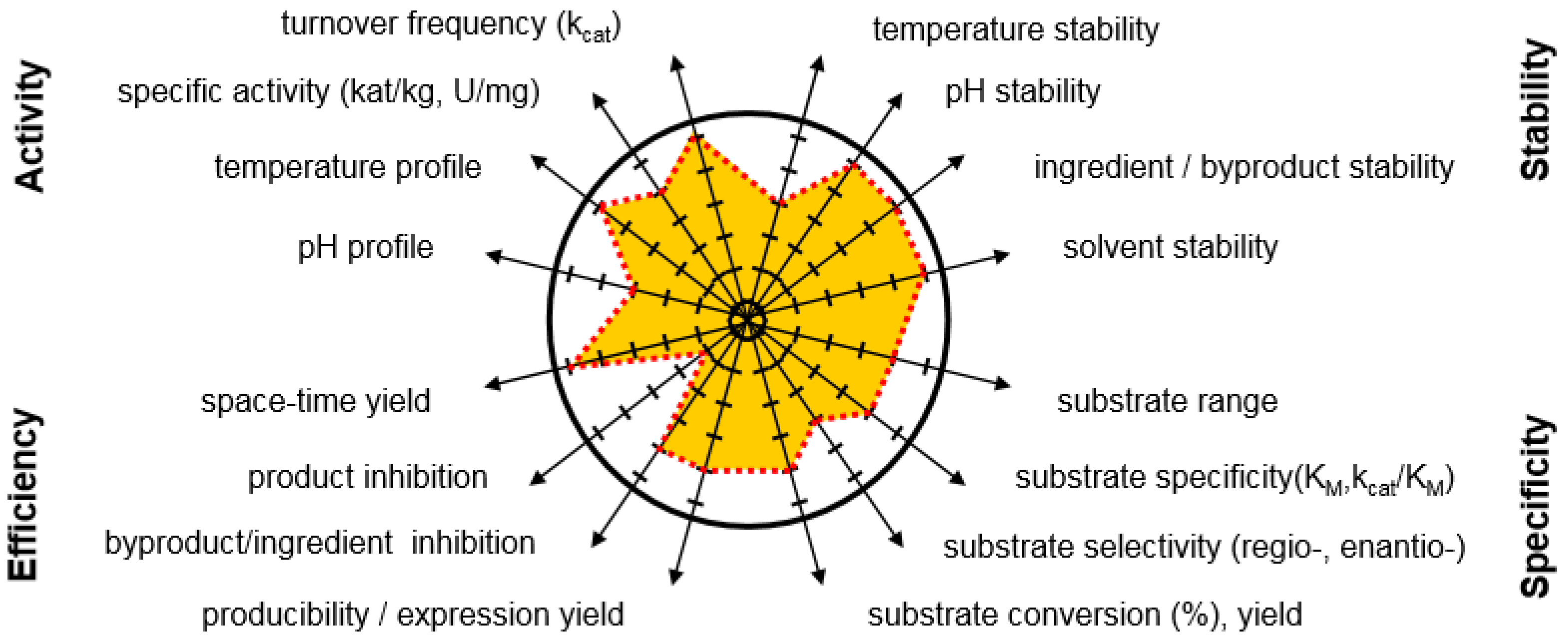
Searching for the ideal enzyme



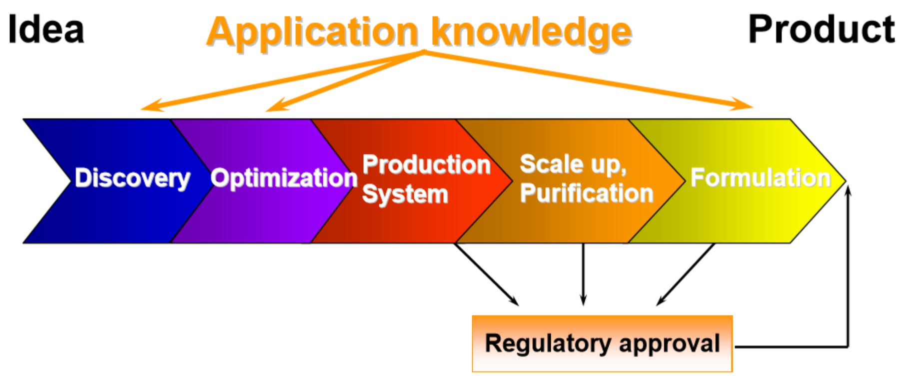
Desired Reaction

-> reaction constraints



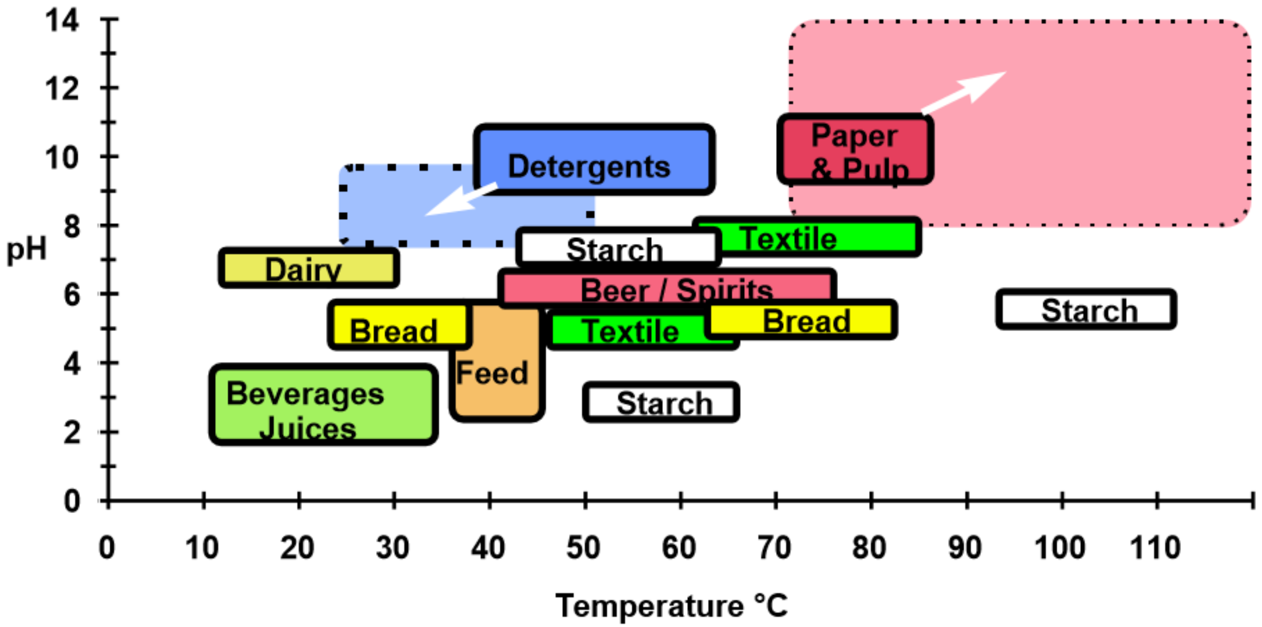


Optimization of pH and Temp requirements of industrial enzymes



Fine tune the enzyme properties for application conditions:

- Stability improvement
- Improve catalytic efficiency
- Improve compatibility with the chemistry of the application environment



Protein engineering

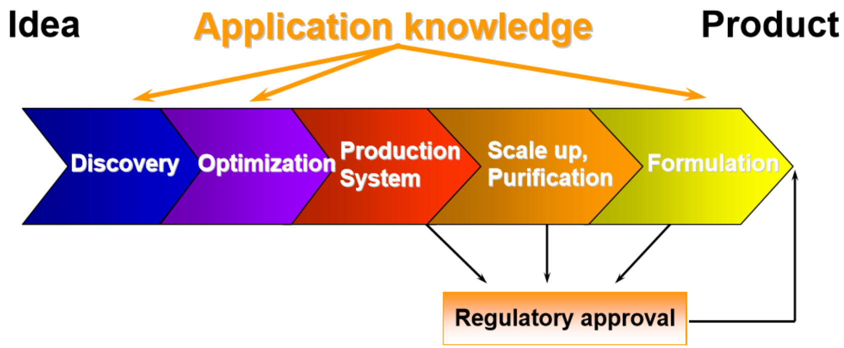
- **Often enzymes do not have the desired properties for an industrial application. E.g. extreme thermo stability or overproduction of the product.**

- **Protein engineering is used to improve commercially available enzyme to be a better industrial catalyst.**

- **Several enzymes have already been engineered to function better in industrial processes. These include proteinases, lipases, cellulases and few amylases**

- **Xylanases is a good example of engineered enzyme from *Trichoderma.* Its xylanase has been purified and crystallized. By designed mutagenesis its thermal stability has been increased about 2000 times at 70^{o}C and its pH-optimum shifted towards alkaline region by one pH-unit.**
- **It is used in pulp and paper industry and needs to be stable in high temperature.**

Production of microbial enzymes



Enzymes occur in every living cell, hence in all microorganisms.

Each single strain of organism produces a large number of enzymes, hydrolyzing, oxidizing or reducing, in nature.

The amounts of the various individual enzymes produced vary between species and even between strains of the same species.

It is important to select strains for the commercial production of specific enzymes which have the capacity for producing highest amounts of the particular enzymes desired.

Enzyme Production by Microbial Fermentation

- **Extracellular enzymes are secreted outside the cell which makes the recovery and purification process much simpler compared to production of intracellular enzymes .**

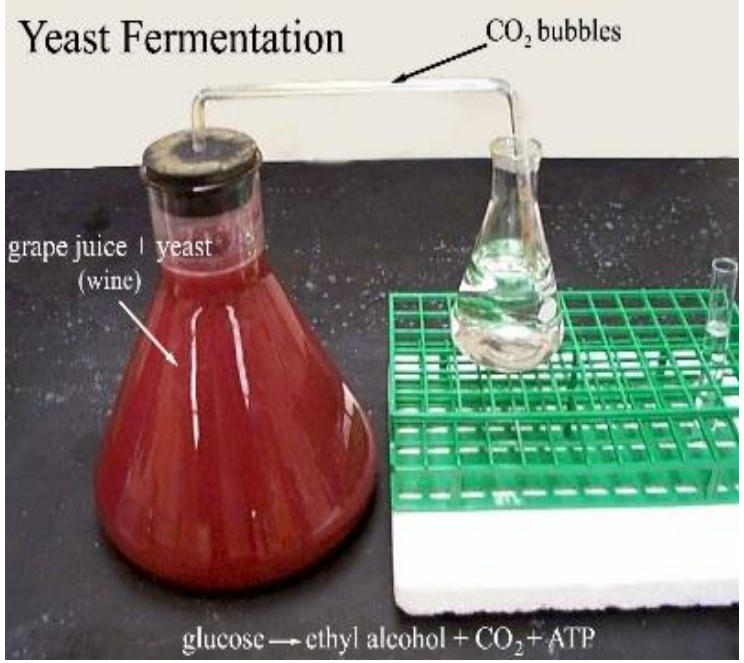
- Intracellular enzymes must be purified from thousands of different cell proteins and other components.

- The organism producing the enzymes should have a GRAS-status, which means that it is Generally Regarded as Safe. This is especially important when the enzyme produced by the organism is used in food processes .

Fermentation process

- The function of the fermenter or bioreactor is to provide a **suitable environment** in which an organism can efficiently produce a target product—the target product might be
- · Cell biomass
- · Metabolite
- · Bioconversion Product
- The sizes of the bioreactor can vary over several orders of magnitudes.
- The microbial cell culture (few mm3), shake flask (100 - 1000 ml), laboratory fermenter (1 – 50 L), pilot scale (0.3 – 10 m^3) to plant scale (2 – 500 m^3) are all examples of bioreactors.

Cell culture fermenter



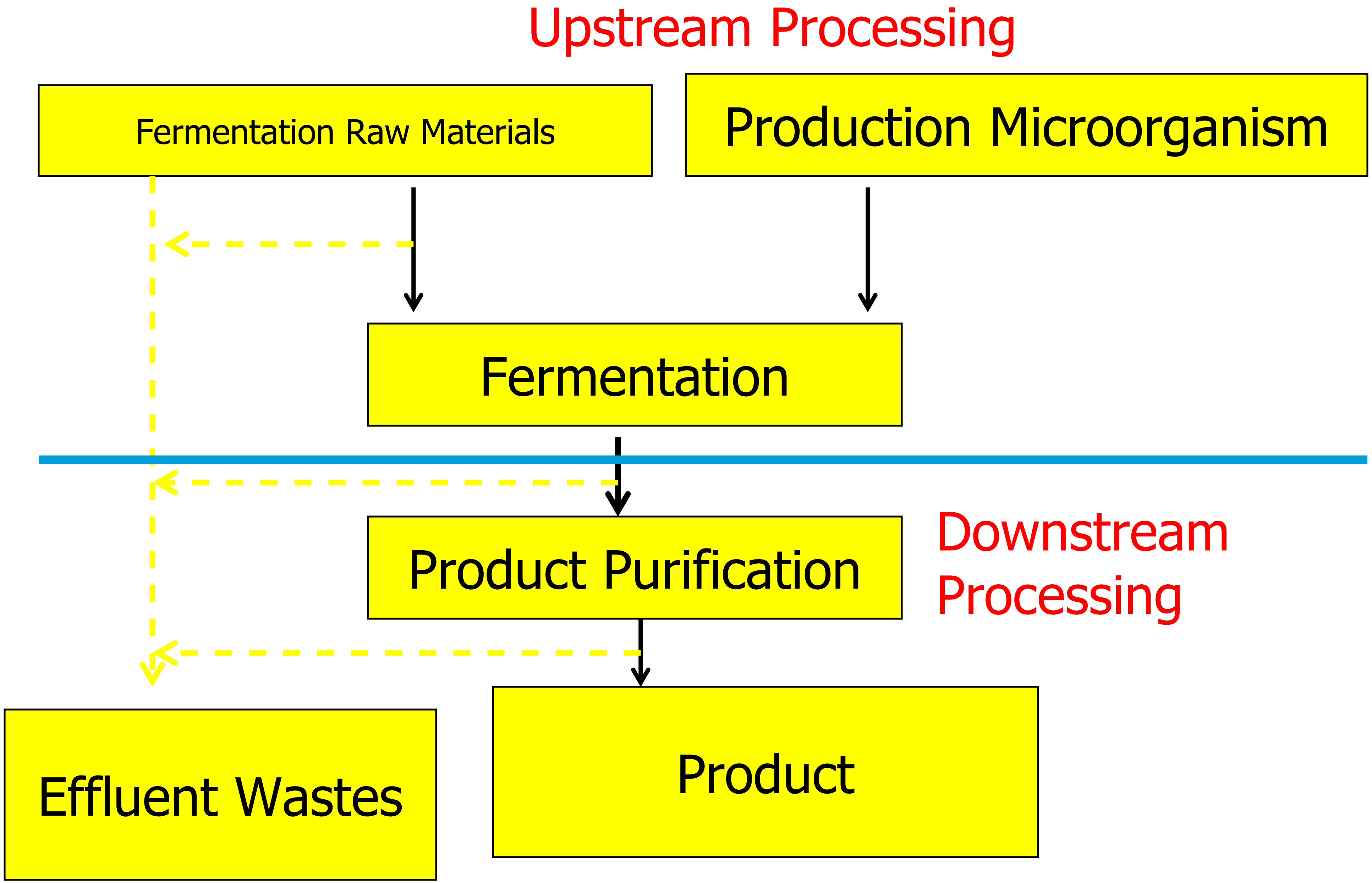
Shake flask fermenter

laboratory fermenter

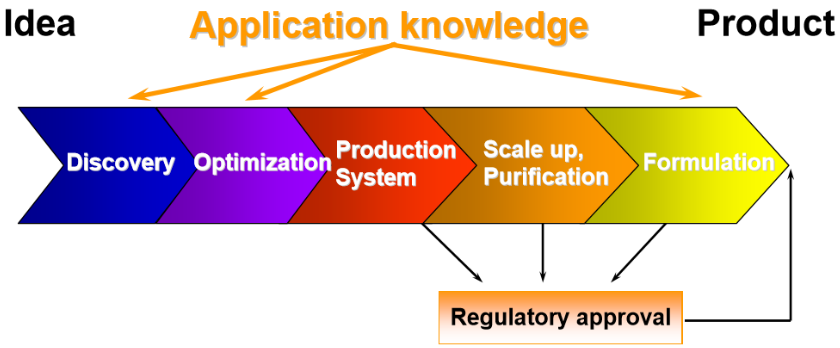
Pilot fermenter

Plant fermenter

Fermentation Process

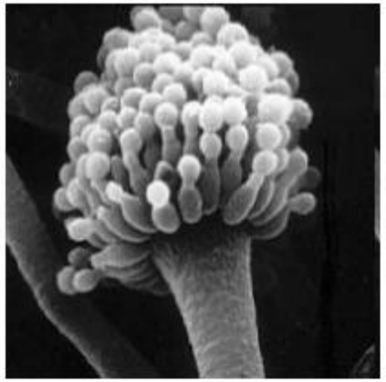


Enzyme production system

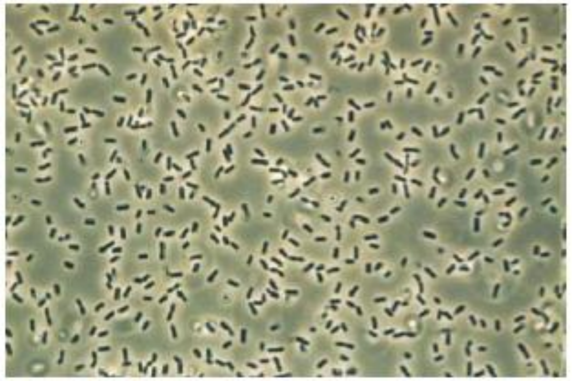


Commercial enzymes are produced from strains of molds, bacteria, and yeasts. A microbe can be manipulated and cultured in large quantities to meet increased demand and the enzyme would be produced in excess.

- High yield and purity
- Compatibility with existing fermentation equipment



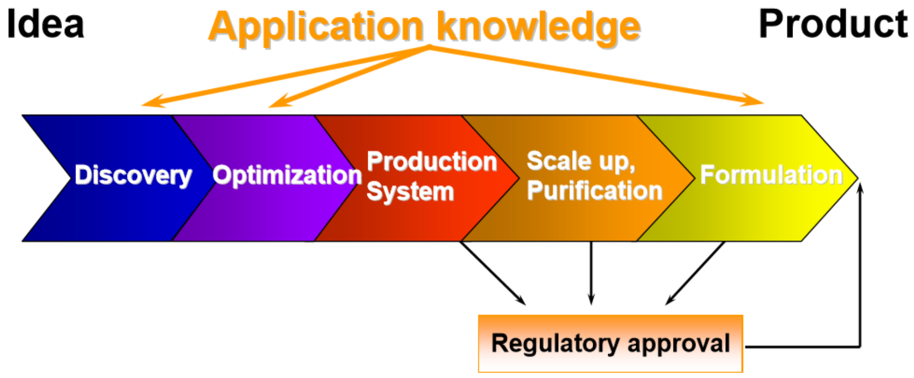
Aspergillus fumigatus (Courtesy of NIH)



Bacillus Subtilis (Courtesy of Wikipedia)

Saccharomyces cerevisiae

Escherichia coli (Courtesy of NIH)



Scale up/purification

- Transfer of production system into production plants
- Simple purification protocol with high purification yields

Production plant

Enzyme Technology

Enzyme technology involves how to use enzymes.

- **The simplest way to use enzymes is to add them into a process stream where they catalyse the desired reaction and are gradually inactivated during the process .**

- **Example liquefaction of starch with amylases, bleaching of cellulose pulp with xylanases or use of enzymes in animal feed.**

- **An alternative way to use enzymes is to immobilize them so that they can be reused.**

- **One method of immobilization is to use ultrafiltration membranes in the reactor system. The large enzyme molecules cannot pass the membrane but the small molecular reaction products can. Therefore enzymes are retained in a reaction system and the products leave the system continuously .**

Microbial enzymes and their use in industry

It was found that certain microorganisms produce enzymes similar in action to the amylases of malt and pancreas, or to the proteases of the pancreas and papaya fruit.

This led to the development of processes for producing such microbial enzymes on a commercial scale.

Some commercial enzymes and source microorganisms

Source	Enzyme	Microorganism
Fungal	Amylases Glucosidases Proteases	*Aspergillus oryzae* *Aspergillus flavus* *Aspergillus niger*
	Pectinases	*Aspergillus niger*
	Glucose oxidase Catalase	*Penicillium notatum* *Aspergillus niger*
Bacterial	Amylases Proteases Penicillinase	*Bacillus subtilis*
Yeast	Invertase	*Saccharomyces cerevisiae*
	Lactase	*Saccharomyces fragilis*

Carbohydrases

Enzymes which hydrolyze polysaccharides or oligosaccharides.

Of the carbohydrases, amylases have the greatest commercial application.

$$\text{Starch} \xrightarrow[\text{(liquefying amylase)}]{\alpha\text{-amylase}} \text{dextrins} + \text{maltose}$$

The liquefied starch is usually sold as 'maltodextrins' to the food industry mainly for use as bulking agents and in baby food.

Other industrial microbial enzymes

In Fruit Juice manufacturing, Pectinase is used to remove pectin. Addition of pectic enzymes to grapes or other fruits during crushing or grinding results in increased yields of juice on pressing.

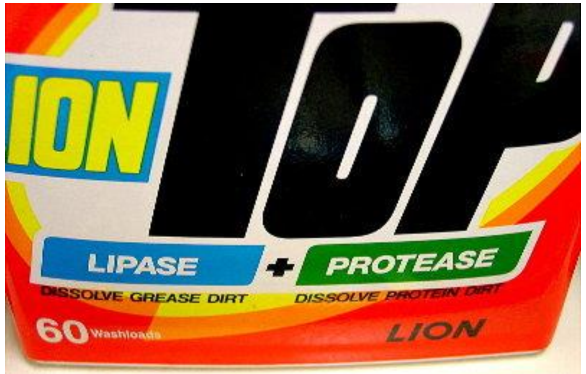
Most consumers prefer clear fruit juices. The cloud, such as in fresh cider, is usually material held in suspension by pectin. The safest way to accomplish pectin removal without affecting color or flavor is to treat the juice with a pectic enzyme.

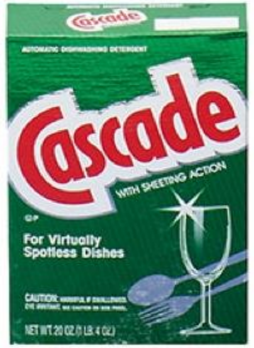
Rennet, a combination of chymosin and pepsin, is used for coagulation of milk into solid curds for cheese production.

Large scale enzyme applications

Detergents

- **Detergents were the first large scale application for microbial enzymes.**
- **Bacterial proteinases are still the most important detergent enzymes. Some products have been genetically engineered to be more stable in the hostile environment of washing machines with several different chemicals present.**





Large scale enzyme applications

Textiles

- The use of enzymes in textile industry is one of the most rapidly growing fields in industrial enzymology.
- Starch has for a long time been used as a protective glue of fibers in weaving of fabrics. This is called sizing.

- Enzymes are used to remove the starch in a process called desizing. Amylases are used in this process since they do not harm the textile fibers.

- Laccase – a polyphenol oxidase from fungi is used to degrade lignin the aromatic polymer found in all plant materials . Faded look in Jeans is by Laccase.



Large scale enzyme applications

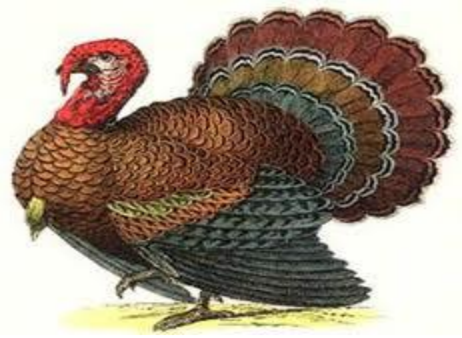
Animal Feed

- The net effect of enzyme usage in feed has been increased animal weight.

- The first commercial success was addition of beta-glucanase into barley based feed diets. Barley contains beta-glucan, which causes high viscosity in the chicken gut.

- **Xylanase, from *Trichoderma,* are added to wheat-based broiler feed and are nowadays routinely used in feed formulations and animals gain weight.**

- **Enzymes have become an important aspect of animal feed industry. In addition to poultry, enzymes are used in pig feeds and turkey feeds.**




Large scale enzyme applications

Leather

- Leather industry uses proteolytic and lipolytic enzymes in leather processing.

- Enzymes are used to remove animal skin, hair, and any unwanted parts.

Large scale enzyme applications

Leather

- The used enzymes are typically alkaline bacterial proteases.
- Lipases are used in this phase or in bating phase to specifically remove grease.

Large scale enzyme applications

Enzymes in Personal Care products

Personal care products are a relatively new area for enzymes and the amounts used are small but worth to mention as a future growth area.

Large scale enzyme applications

Enzymes in Personal Care products

- One application is contact lens cleaning. Proteinase and lipase containing enzyme solutions are used for this purpose. Hydrogen peroxide is used in disinfections of contact lenses. The residual hydrogen peroxide after disinfections can be removed by a heme containing catalase enzyme, which degrades hydrogen peroxide.

Large scale enzyme applications

Enzymes in Personal Care products

- Some toothpaste contains glucoamylase and glucose oxidase.
- Dentures can be cleaned with protein degrading enzyme solutions.
- Enzymes are also used for applications in skin and hair care products.



Large scale enzyme applications

Enzymes in DNA-technology

- DNA-technology has revolutionized both traditional biotechnology and opened totally new fields for scientific study.

- Recombinant DNA-technology allows one to produce new enzymes in traditional overproducing and safe organisms .

Large scale enzyme applications

Enzymes in DNA-technology

- **Protein engineering is used to modify and improve existing enzymes.**

- **DNA is basically a long chain of deoxyribose sugars linked together by phosphodiester bonds. Organic bases, adenine, thymine, guanine and cytosine are linked to the sugars and form the alphabet of genes. The specific order of the organic bases in the chain constitutes the genetic language.**

Large scale enzyme applications

Enzymes in DNA-technology

- **Genetic engineering means reading and modifying this language. Enzymes are crucial tools in this process. E.g.:**

Enzymes in DNA-technology

1. Restriction enzymes recognise specific DNA sequences and cut the chain at these recognition sites.
2. DNA modifying enzymes synthesize nucleic acids, degrade them, join pieces together and remove parts of the DNA.
3. DNA-polymerases synthesize new DNA-chains. Many of them need a model template, which they copy.
4. Ligases join adjacent nucleotides together.