ENZYMES and Their Application

DDG 17-4-2021

DDG 4-3-2022

What are Enzymes?

Introduction

- Enzymes are macromolecular biological catalysts.
- Enzymes accelerate, or catalyze, chemical reactions.
- The molecules at the beginning of the process are called substrates and the enzyme converts these into different molecules, called products.
- Microbial enzymes are the biological catalysts for the biochemical reactions leading to microbial growth and respiration, as well as to the formation of fermentation products.

Enzymes

- A class of proteins that serve
Catalysts are chemicals that
  - Increase the rate of a reaction
  - Are not changed by the reactio
used again)
  - Do not change the nature of th
reaction could have occurred
enzyme, just much slower

Mechanism of enzyme action

- Michaelis and Menton proposed a hypothesis for enzyme action.
- According to their hypothesis, the following steps occurs:

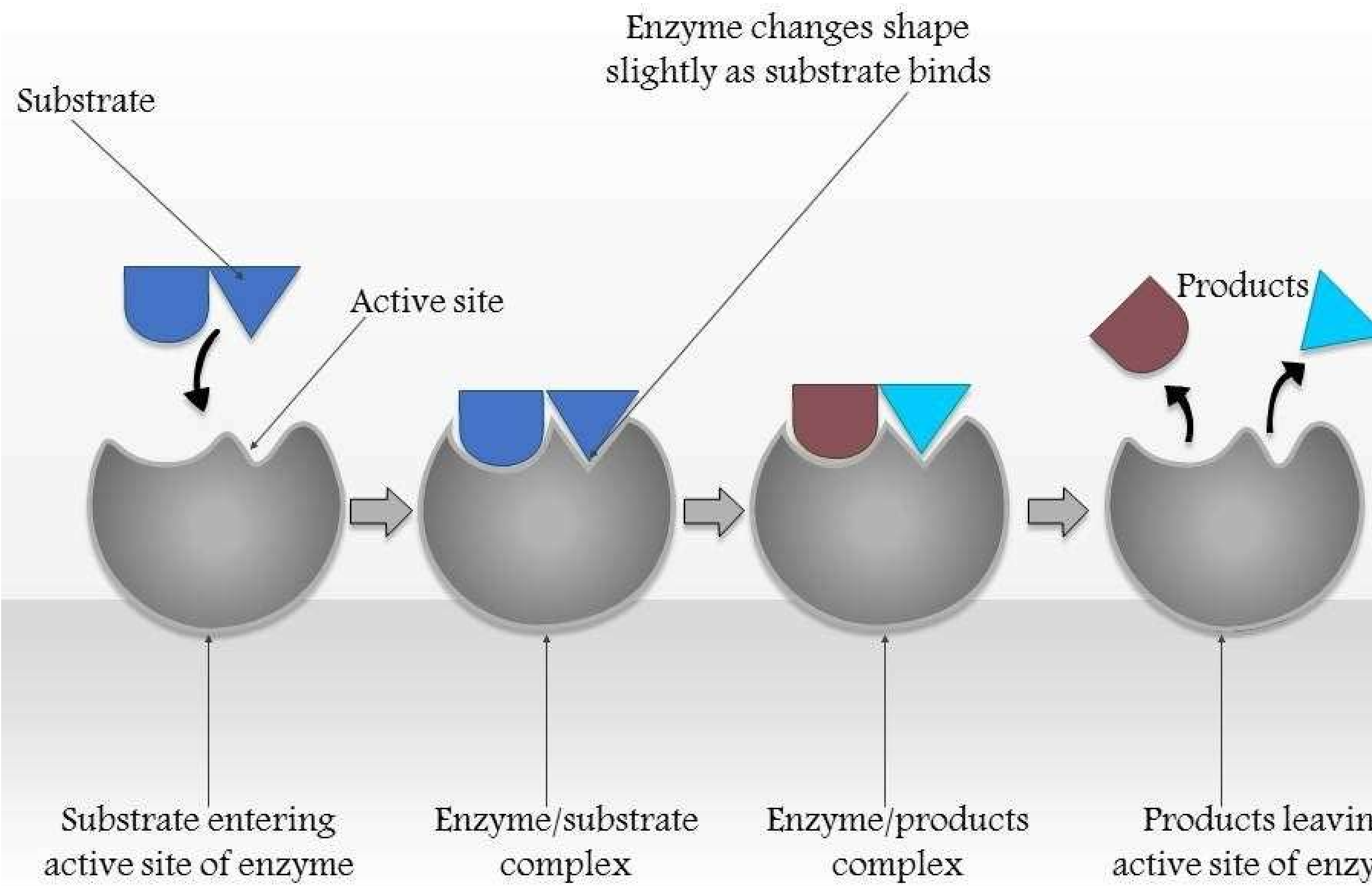
1. combination of enzyme with substrate

-substrate attaches on the active site of enzyme to form enzyme-substrate complex.

2. Breakdown of substrate

-active site loosens the chemical bonds in the substrate breaking down the substrate into products.

The Mechanism of enzyme action



Define enzymes
(Enzymes as Biological Catalysts)

- **Enzymes** are proteins that increase the rate of reaction by lowering the energy of activation
- They catalyze nearly all the chemical reactions taking place in the cells of the body.
- Not altered or consumed during reaction.
- Reusable

14.2 What Role Does Transition-State Stabilization Play in Enzyme Catalysis?

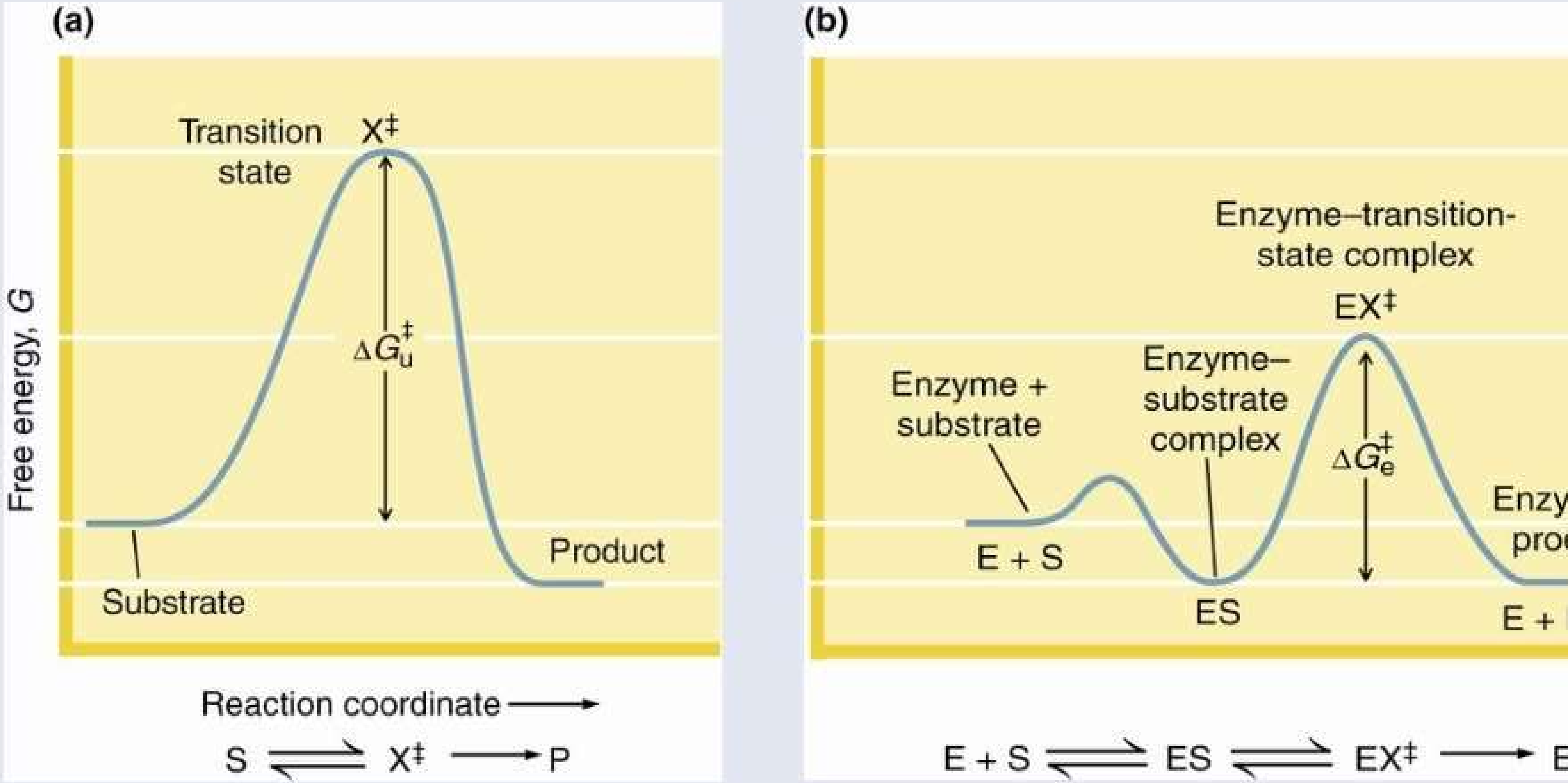
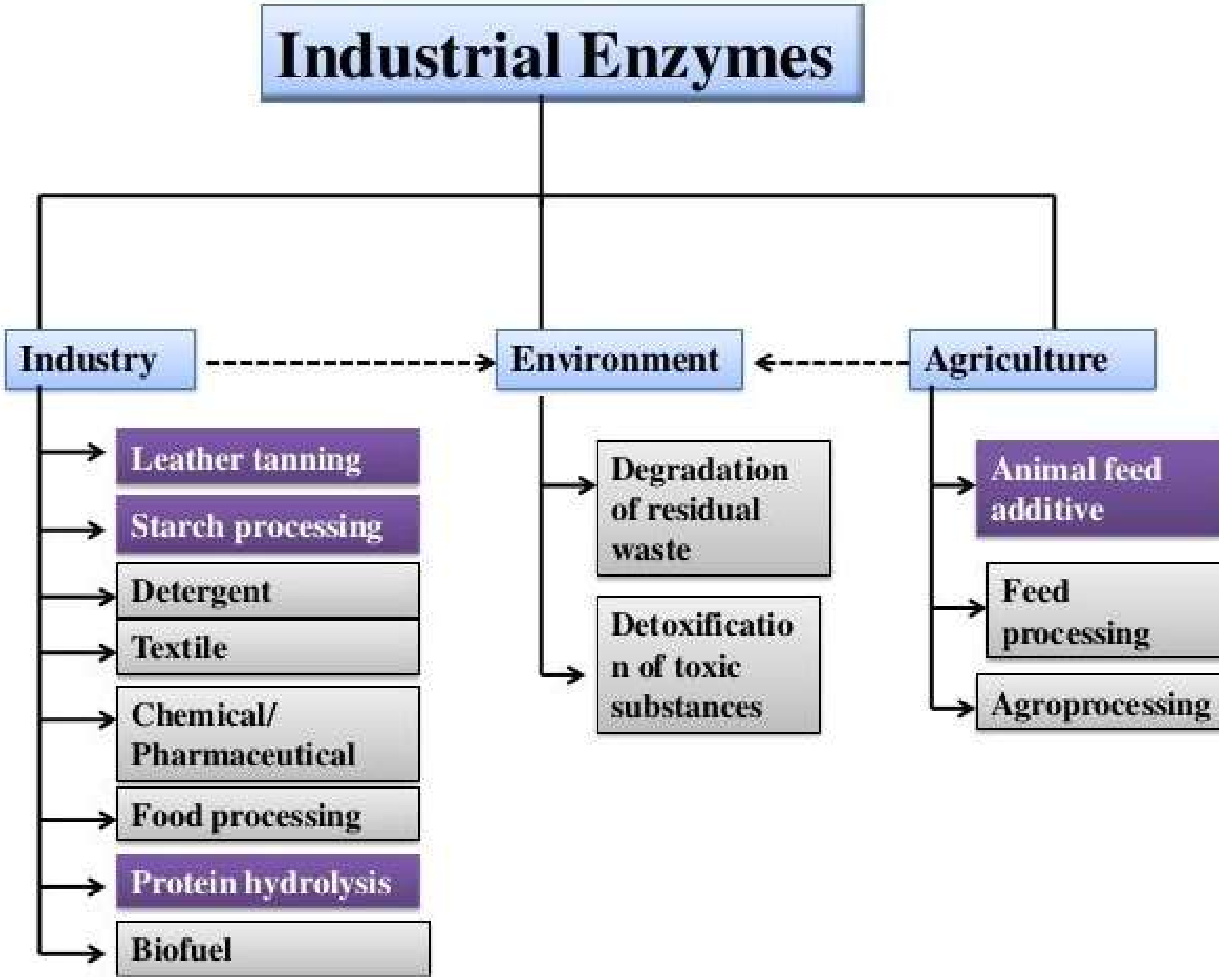


Figure 14.1 Enzymes catalyze reactions by lowering the activation energy. Here the free energy of activation for (a) the uncatalyzed reaction is larger than that of the enzyme-catalyzed reaction.



Industrial Enzymes
Industry
Environment
Agriculture
Leather tanning
Starch processing
Detergent
Textile
Chemical/ Pharmaceutical
Food processing
Protein hydrolysis
Biofuel
Degradation of residual waste
Detoxificatio n of toxic substances
Animal feed additive
Feed processing
Agroprocessing

Application of commercial enzyme

Industry	Analytical	Medicine
amylases	Glucose oxidase	asparaginase
proteases	Galactose oxidase	proteases
catalases	Alcohol dehydrogenase	lipases
isomerases	hexokinase	
Penicillin acylases	muramidase	
	Cholesterol oxidase	

Use of enzymes in washing pow

- Enzymes are added to washi clean clothes:
- Lipase is used to clear oily st breaks oils into simple Fatty and these are easily washed
- Blood stains and egg stains(p are washed using proteases proteins to Amino acids (col then washed easily by deterg

Applications

- Pectinase enzymes are commonly used in processes involving the degradation of plant materials, such as speeding up the extraction of fruit juice from fruit, including apples.
- Pectinases have also been used in wine production since the 1960s
- Helps to clarify fruit juices and grape must, for the maceration of vegetables and fruits and for the extraction of olive oil.
- By treatment with pectinase, the yield of fruit juice during pressing is considerably increased.

AMYLASE :

- enzymes that break down starch or glycogen
- produced by a variety of living organisms: bacteria, plants and humans.
- major advantage of using micro organisms for the production of amylases is in economical bulk production capacity and microbes are also easy to manipulate to obtain enzymes of desired characteristics
- α-amylase (alpha-amylase) - Reduces the viscosity of starch by breaking down the bonds at random, therefore producing varied sized chains of glucose

3. Production of α-Am

3.1. Sources

- α-Amylase can be isolated fro **animals or microorganisms.**
- The enzyme has been isolated barley and rice plants.
- It has been found that cassava ma water is a source of α-Amylas
- In the recent past, there has been research on microbial production Amylase.

1) What are amylases

- Amylases are important hydrolase enzymes which have been widely used since many decades.
- These enzymes randomly cleave in glycosidic linkages in starch mole
- To hydrolyze them and yield:
  (dextrins
  (oligosacc

2) Types of amylases

2.1. α-Amylase:

- α-Amylase is a hydrolase enzyme that catalyses the hydrolysis of **internal α-1, 4-glycosidiclinkages** in starch
- to yield products like glucose and maltose.
- It is a calcium **metalloenzyme** i.e. it depends on the presence of a metal co factor for its activity.
- The optimum pH for activity is found to be 7.0

TYPES OF AMYLASE

Various types of amylase associated with degradation of starch a
related polysaccharides structures have been detected and studie

1. Enzymes that hydrolyze α-1,4 bonds e.g. **α -amylase** (endoacti
amylases).

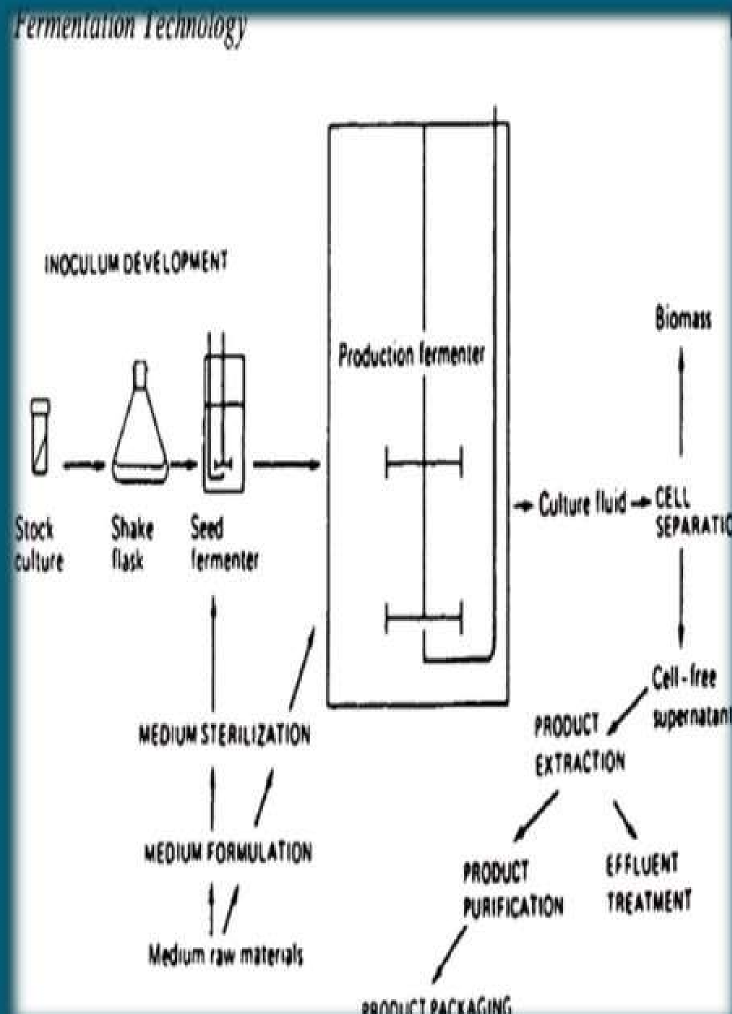
2. Enzymes that hydrolyze α -1,4 e.g. -**β amylase** (exoacting amyl
producing maltose as a major end product).

3. Enzymes that hydrolyze terminal 1,4 linked α D-glucose residue
glucoamylase.

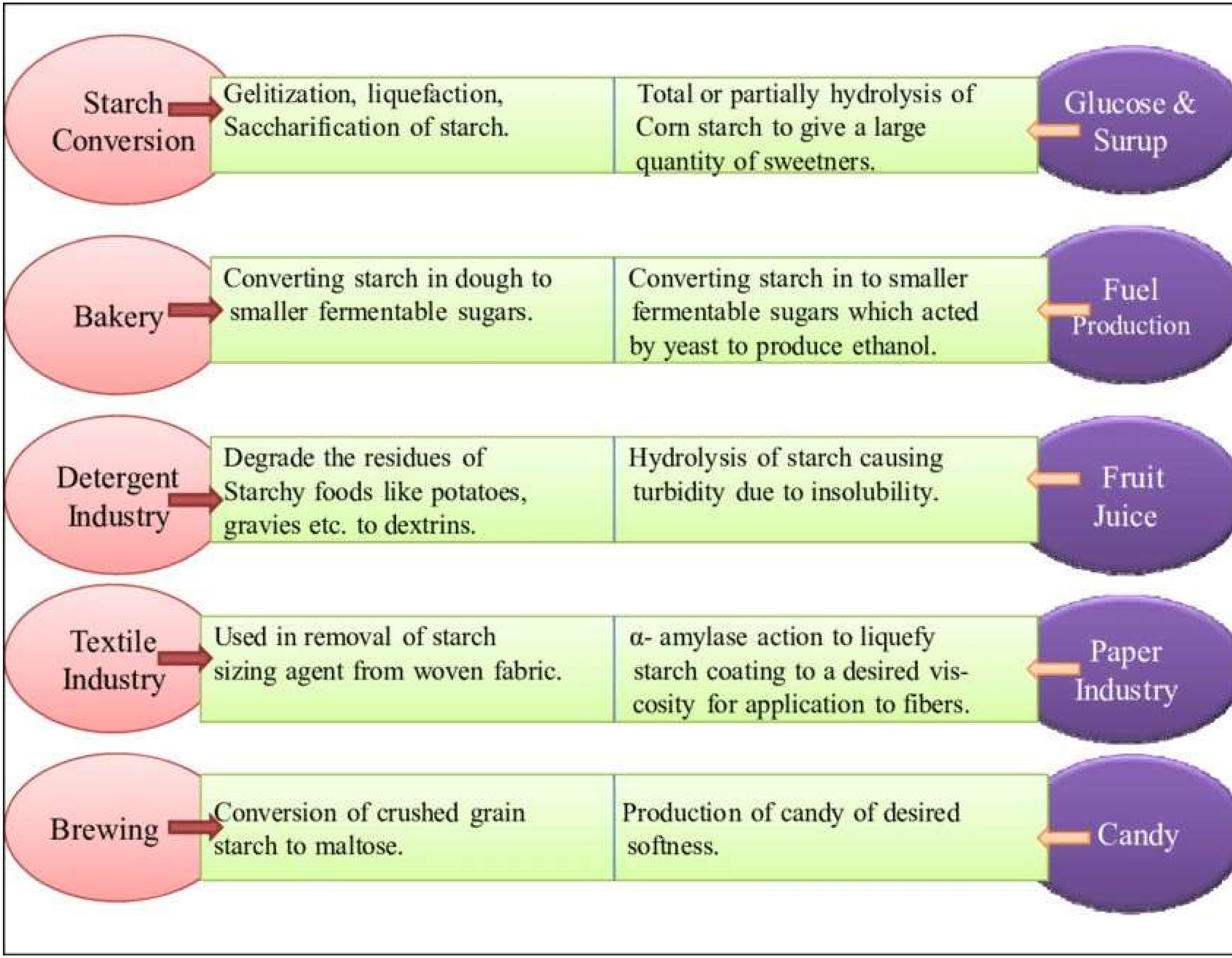
4. Enzymes that hydrolyze only α -1,6 linkages e.g. **pullulanase** .

5. Enzymes that hydrolyze preferentially α -1,4 linkages in short c

MICROBIAL PRODUCTION OF AMYLASE



- MICROORGANISM: **Bacillus spp. Why bacteria? Why not fungus?**
- INOCULUM AND FERMENTATION MEDIUM: **addition of sterile distilled water into nutrient agar slants, and incubated.**
- FERMENTATION MEDIUM:
  -Bacteriological peptone
  -Magnesium sulfate
  -Potassium chlorate
  -Starch
- EXTRACTION OF AMYLASE FROM FERMENTATION MEDIUM: **fermentat medium is harvested by centrifugation at 5000 rpm for 20 min at 4°C. The supernatant is collected to estimate t amylase activity**
- SUBMERGED FERMENTATION: **why advantageous?**
- OPTIMUM TEMPERATURE: **35°C**
- OPTIMUM pH: **7**
- OPTIMUM TIME: **10hours**



Starch Conversion
Gelitization, liquefaction, Saccharification of starch.
Total or partially hydrolysis of Corn starch to give a large quantity of sweetners.
Glucose & Surup
Bakery
Converting starch in dough to smaller fermentable sugars.
Converting starch in to smaller fermentable sugars which acted by yeast to produce ethanol.
Fuel Production
Detergent Industry
Degrade the residues of Starchy foods like potatoes, gravies etc. to dextrins.
Hydrolysis of starch causing turbidity due to insolubility.
Fruit Juice
Textile Industry
Used in removal of starch sizing agent from woven fabric.
α- amylase action to liquefy starch coating to a desired viscosity for application to fibers.
Paper Industry
Brewing
Conversion of crushed grain starch to maltose.
Production of candy of desired softness.
Candy

Applications Of Industrial Amylases:

ENZYMES	SOURCE	APPLICATIONS
Alpha- amylase	Bacterial α amylase (e.g., Bacillus subtilis), Fungal α amylase (e.g., Aspergillus niger)	Textiles, starch syr laundry and dishwashing detergents, paper desizing , fermenta
β- amylase	From a strain of Bacillus	Brewing, maltose syrup
γ-Amylase	Aspergillus niger	Manufacture of dextrose syrup anc high fructose syrup production.

Sources of Enzymes

Biologically active enzymes may be extracted from any living organism:

Of the hundred enzymes being used industrially,

- over a half are from fungi

- over a third are from bacteria with the remainder divided between animal (8%) and plant (4%) sources .

Advantages of using i
enzymes?

Direct

- Foreign currency
  - Export of enzymes
  - Value addition to raw materials
- Job creation
  - Enzyme production
  - Value addition

- Reduct
pollutio
  -
- Help to
curren
  -

PRODUCTION OF MICROBIAL ENZYMES AND ITS APPLICATIONS

- Enzymes are the catalysts of biological systems.
- They classifies into
- Oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases.
- Sources of enzymes:
- Animal, plant and microbial.
- Amylase takadiastase, first fungal enzyme used for digestive disorders.
- proteases – detergent industry.
- Amylases and amyloglucosidases – glucose from starch.
- Glucose isomerase – production of fructose.
- Microbial renin – cheese production.

Enzyme production

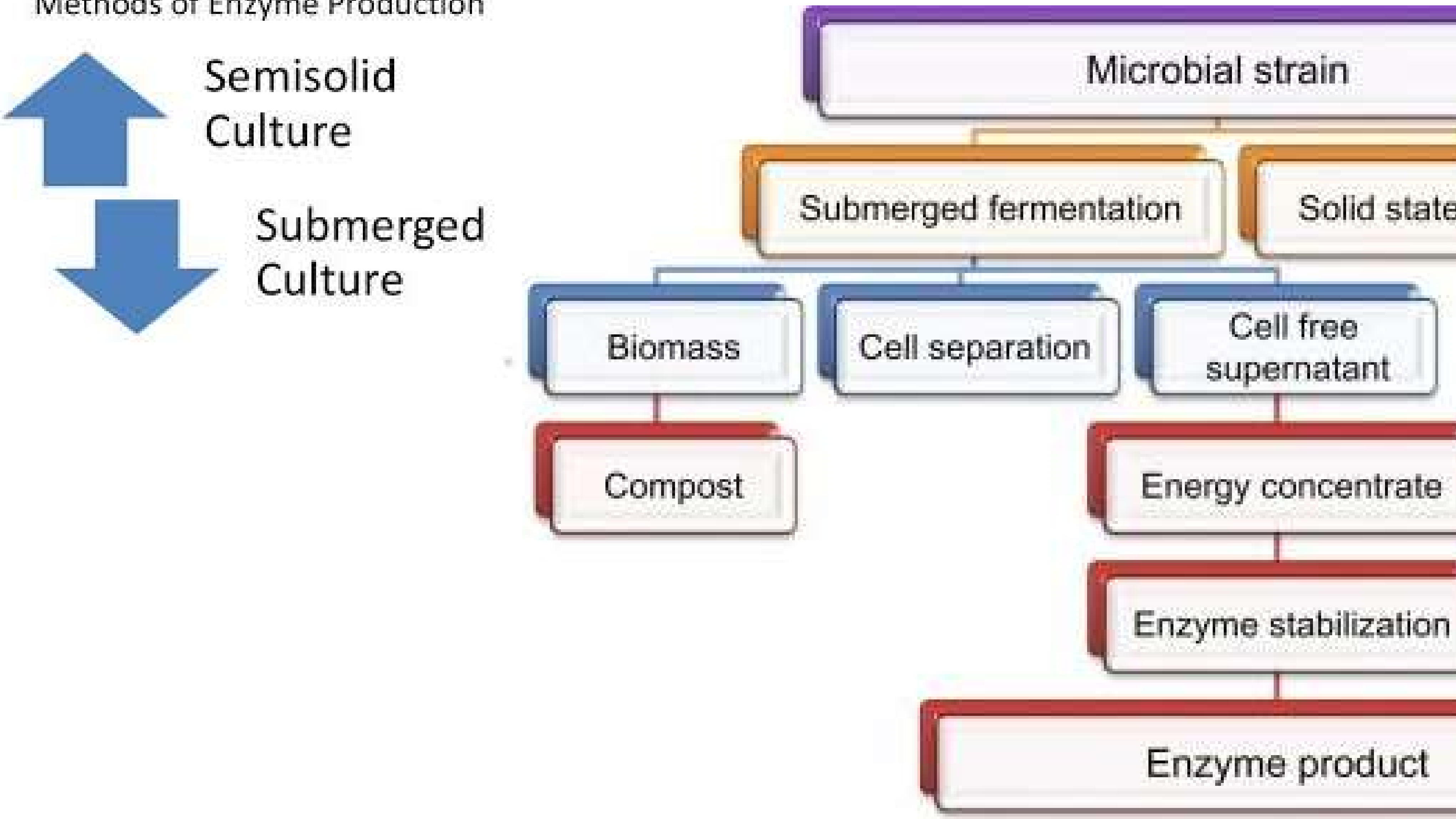
- The production of enzymes by fermentation was an established business before modern microbial biotechnology.
- However, recombinant DNA methodology was so perfectly suited to the improvement of enzyme production technology that it was almost immediately used by companies involved in manufacturing enzymes.
- Important enzymes are proteases, lipases, carbohydrases, recombinant chymosin for cheese manufacture and recombinant lipase for use in detergents.
- Recombinant therapeutic enzymes already have a market value of over US$2 billion, being used for thromboses, gastrointestinal and rheumatic disorders, metabolic diseases and cancer.
- They include tissue plasminogen activator, human DNAase and Cerozyme.

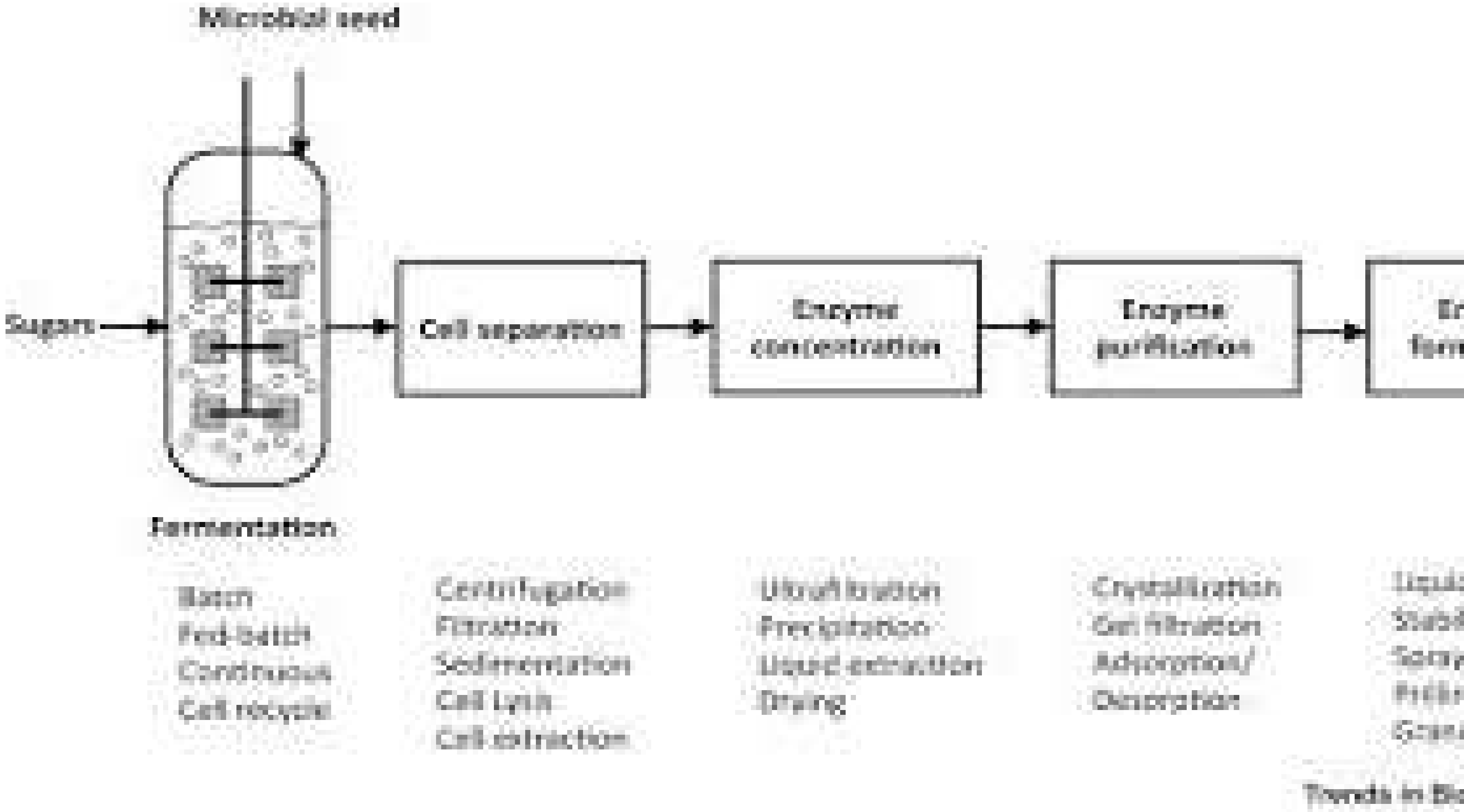
Fungal Enzymes

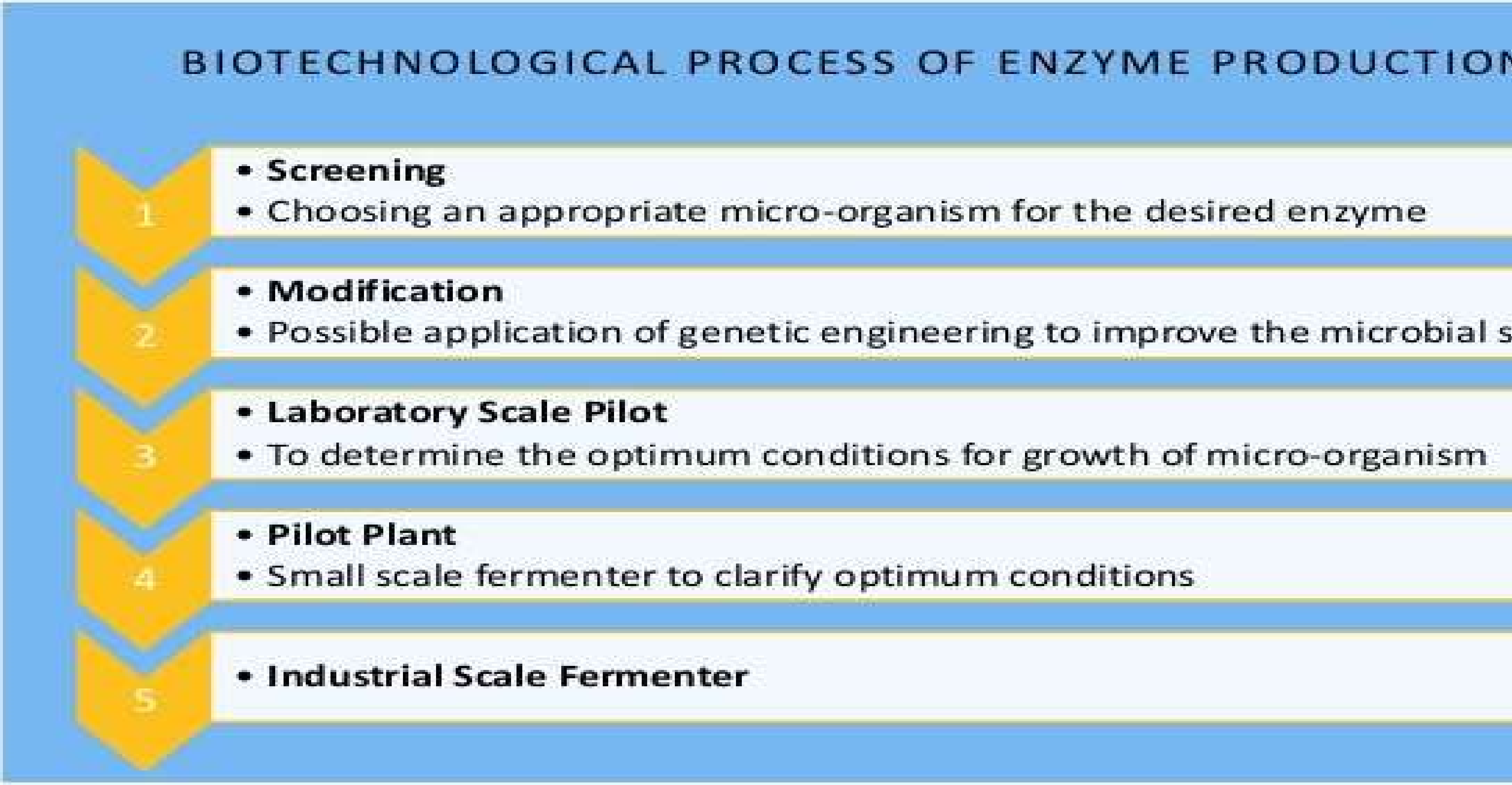
Enzyme	**EC**	**Sources**		Application
α-Amylase	3.2.1.1	*Aspergillus*	E	Baking
Catalase	1.11.1.6	*Aspergillus*	I	Food
Cellulase	3.2.1.4	*Trichoderma*	E	Waste
Dextranase	3.2.1.11	*Penicillium*	E	Food
Glucose oxidase	1.1.3.4	*Aspergillus*	I	Food
Lactase	3.2.1.23	*Aspergillus*	E	Dairy
Lipase	3.1.1.3	*Rhizopus*	E	Food
Rennet	3.4.23.6	*Mucor miehei*	E	Cheese
Pectinase	3.2.1.15	*Aspergillus*	E	Drinks
Protease	3.4.23.6	*Aspergillus*	E	Baking

E: extracellular enzyme; I: intracellular enzyme

Methods of Enzyme Production




Microbial seed
Sugars
Fermentation
Batch
Fed-batch
Continuous
Cell recycle
Cell separation
Centrifugation
Filtration
Sedimentation
Cell Lysis
Cell extraction
Enzyme concentration
Ultrafiltration
Precipitation
Liquid extraction
Drying
Enzyme purification
Crystallization
Gel filtration
Adsorption/
Desorption


BIOTECHNOLOGICAL PROCESS OF ENZYME PRODUCTIO
1
• Screening
• Choosing an appropriate micro-organism for the desired enzyme
2
• Modification
• Possible application of genetic engineering to improve the microbial
3
• Laboratory Scale Pilot
• To determine the optimum conditions for growth of micro-organism
4
• Pilot Plant
• Small scale fermenter to clarify optimum conditions
5
• Industrial Scale Fermenter

Introduction

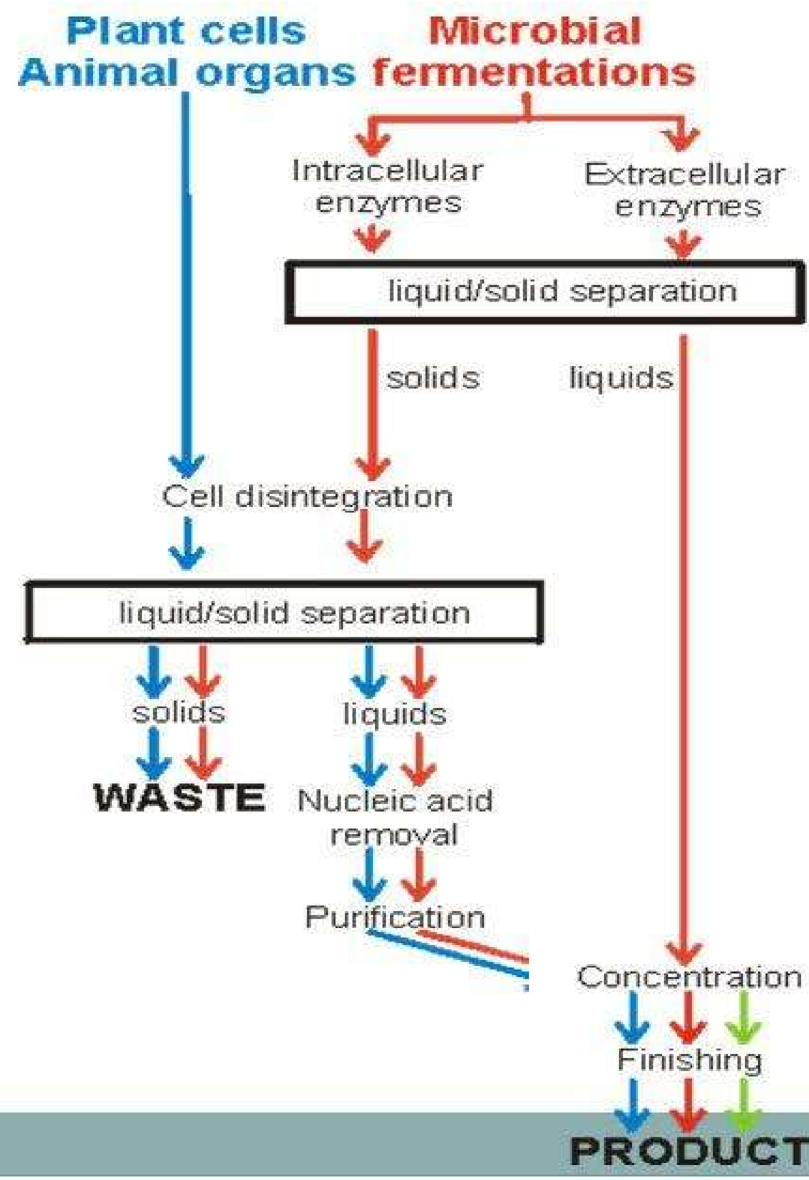
- Protease (Mixture of Peptidases and Proteinases) are enzymes that perform the hydrolysis of Peptide bonds.
- Peptide bonds links the amino acids to give the final structure of a protein.
- Proteinases are extracellular and Peptidases are endocellular.
- Second most important enzyme produced on a large scale after Amylase

INTRODUCTION

- A **protease** is proteolytic enzyme breaks
A *protease* is any enzyme that conducts pro
begins protein catabolism by hydrolysis
bonds that link amino acids
the polypeptide chain forming the protein.

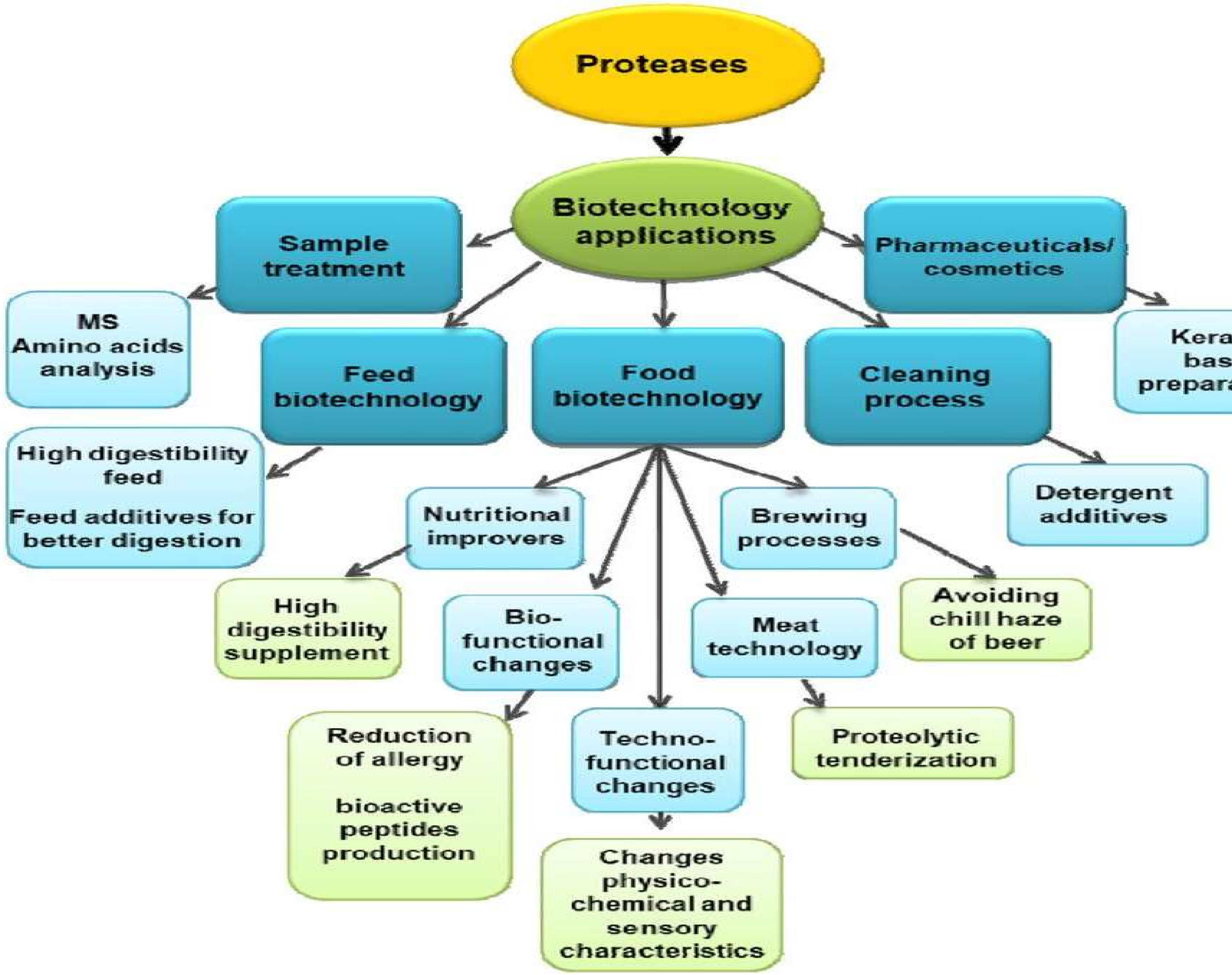
- Proteases execute a large variety
extending from the cellular level to the org
level, to produce cascade systems such as
inflammation.

Enzyme Production



PROTEASE Production

ROLE


Proteases
Biotechnology applications
Sample treatment
Pharmaceuticals/ cosmetics
MS Amino acids analysis
Feed biotechnology
Food biotechnology
Cleaning process
High digestibility feed
Feed additives for better digestion
Nutritional improvers
Brewing processes
Detergent additives
High digestibility supplement
Bio-functional changes
Meat technology
Avoiding chill haze of beer
Reduction of allergy
bioactive peptides production
Techno-functional changes
Proteolytic tenderization
Changes physico-chemical and sensory characteristics

Microbial protease

Major fungi producing alkaline proteases

- Aspergillus candidus , A. flavus , A. fumigatus , A. melleus , A. niger , A. oryzae , A. soja, Cephalosporium sp.

Major bacteria producing alkaline proteases

- Streptomyces microflavus , Streptomyces moderatus , Streptomyces rectus, Pseudomonas aeruginosa , Pseudomonas maltophilia , Pseudomonas sp. SJ320

Classification Based upon the
which the Proteases are Ac

- Alkaline serine Proteases
- Acid Proteases
- Neutral Proteases

Classification of Protease

By source organism:

1. Animal : chymosin, trypsin, pepsin
2. Plant : bromelain, papain, ficin
3. Bacterial: subtilisin(Bacillus subtilis), bacillopeptidases
4. Fungal : Aspergillopepsin

By proteolytic mechanism:

a. Serine proteases
b. Threonine proteases
c. Cysteine proteases
d. Aspartic proteases
e. Metallo proteases
f. Glutamic acid proteases

Serine proteases (EC 3

- The serine proteases contribute major indu therapeutic protease where serine serves a amino acid.
- In this class of enzyme chymotrypsin/tryp are commercially available serine protease
- With their tremendous scope in industry a recombinant serine protease have been pro commercial use.

Alkaline Protease

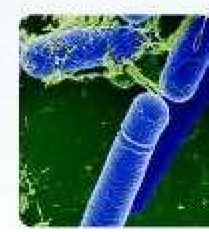
- One of the class of protease enzyme.
- An **extracellular** enzyme.
- Performs **proteolysis**, that is, protein catabolism by hydrolysis of the peptide bonds.
- Active at alkaline **pH 8 to 12** and at temperature **30^{0}-80^{0}C.**
- Molecular weight is about **20,000 to 45,000 Dalton.**
- The structure is determined by **X-ray crystallography.**
- EC (Enzyme Commission) Number: **3.4.21–24.99**
- In **1971**, Japanese scientist **Koki Horikoshi** first reported the production of alkaline protease from bacteria.

Sources of Alkaline P

Bacteria	Fungi
Bacillus subtilis	*Aspergillus flavus*
Bacillus pumilus	*Aspergillus fumigatus*
Bacillus licheniformis	*Aspergillus melleus*
Bacillus altitudinis	*Aspergillus niger*
Bacillus firmus	*Fusarium graminearum*
Bacillus amyloliquefaciens	*Penicillium griseofulvim*
Bacillus proteolyticus	*Penicillium lilacinus*
Thermomonospora fusca	*Scedosporium apiosermum*

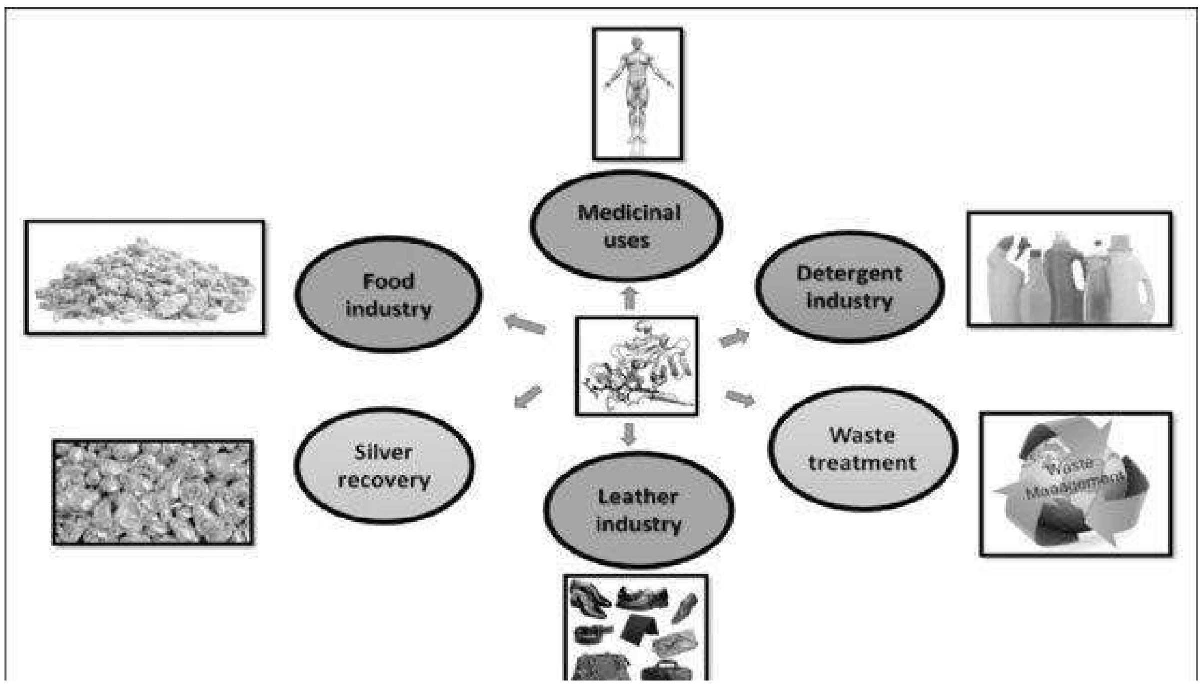
Genetic E

- More than 5
enzymes are
engineered
- **Methods Us**
(UV or chem
DNA technol



Applications of Alkaline Proteases

Industry	Protease	Application
Baking	Neutral protease	Dough conditioner
Beverage	Papain	Chill proofing, removal of haze in beverages
Dairy	Fungal proteases, chymosin, other proteases	Replacement of calf rennet, whey protein processing, production of enzyme modified cheese (EMC)
Detergent	Alkaline protease, subtilisin	Laundry detergents for protein stain removal
Food processing	Several proteases	Modification of protein rich material i.e., soy protein or wheat gluten
Leather	Trypsin, other proteases	Bating of leather, dehairing of skins
Meat and fish	Papain, other proteases	Meat tenderization, recovery of protein from bones and fish waste
Medicine	Trypsin	Dead tissue removal, blood clot dissolution
Photography	Several proteases	Recovery of silver from used X-ray and photographic films
Sweetner	Thermolysin	Reverse hydrolysis in aspartame synthesis

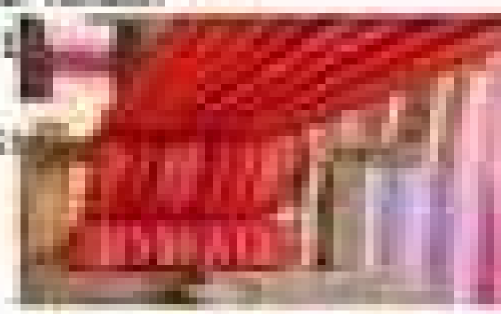


Protease in textile industry

- Another most significant area of commercial application of protease is textile industry where a final elegant texture is provided by enzyme treatment.
- Silk is processed by thermostable protease to remove gum and other impurities lies of core protein fibre on silk.
- Silk degumming is growing industry, enormously utilize protease and results in high quality silk.
- Synthetic fabric also subjected to protease treatment for elegant and smooth finish.
- Fungal proteases are key enzyme in fabric industry since decade and growing tremendously.
- Indian sericulture has grown double in last one decade and consumption of protease also enhances in several folds.
- Use of such method not only offer good quality fab[illegible] mechanical strength to fibre.
- Use of protease minimizes chemical detergent as[illegible]

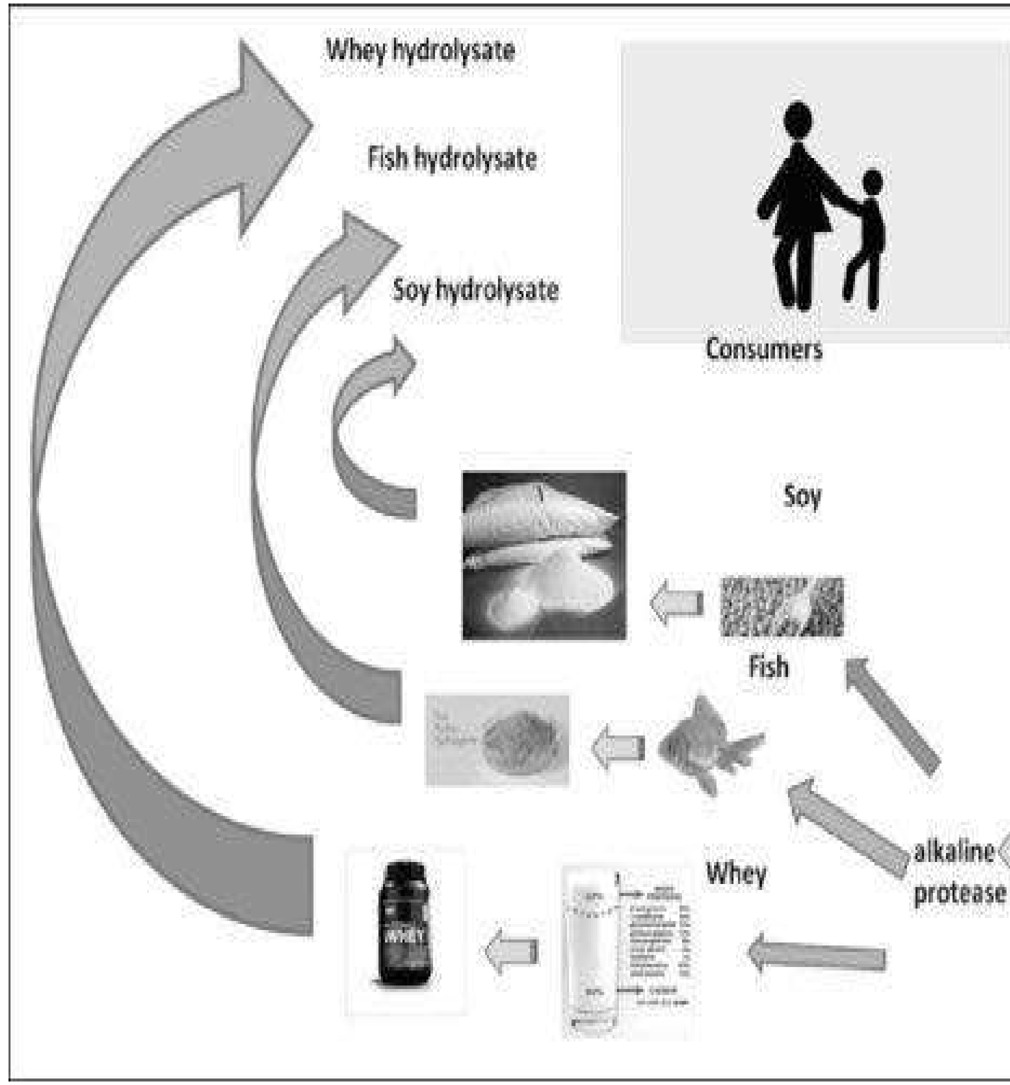
with large scale of

environment pollution.



Applications of Protease

- **Other Applications:**
- Besides their industrial and medicinal applications, proteases play an important role in **basic research**.
- Their selective peptide bond cleavage is used in the elucidation of structure-function relationship, in the synthesis of peptides, and in the sequencing of proteins.
- In essence, the wide specificity of the hydrolytic action of proteases finds an extensive application in the food, detergent, leather, and pharmaceutical industries, as well as in the structural elucidation of proteins, whereas their synthetic capacities are used for the synthesis of proteins.



Production steps :