INDUSTRIAL MICROBIOLOGY (MCB 406)

By

OLAITAN, Faith Jesupemi

Microbiology Unit, Department of Biological Sciences

Thomas Adewumi University, OKO, KWARA STATE

INTRODUCTION

- Industrial microbiology is a branch of microbiology that focuses on the use of microorganisms to produce useful products and carry out specific industrial processes.
- Significant advancements that has shaped Industrial microbiology:
- 1. Fermentation Processes: the use of microorganisms in fermentation processes to produce food and beverages
- 2. Discovery of Microorganisms: The development of the microscope in the 17th century by pioneers like Antonie van Leeuwenhoek led to the discovery and characterization of microorganisms.
- 3. Pasteur and Germ Theory: Louis Pasteur's work disproved the theory of spontaneous generation, provided evidence for the germ theory of disease and coined the word “pasteurization”.
- 4. Early Industrial Applications: In the late 19th and early 20th centuries, industrial microbiology found applications in using microorganisms on large-scale fermentation processes to convert raw materials into valuable products.
- 5. Antibiotics and Pharmaceutical Industry: The discovery of antibiotics, starting with penicillin by Alexander Fleming in 1928, revolutionized medicine and the pharmaceutical industry. Microorganisms such as bacteria and fungi were screened for their ability to produce antibiotics, leading to the development of life-saving drugs.
- 6. Genetic Engineering and Biotechnology: The advent of genetic engineering techniques in the 1970s opened new frontiers in industrial microbiology.
- 7. Industrial Enzymes: Enzymes such as amylases, proteases, lipases, and cellulases are produced by microorganisms and used as biocatalysts in industrial applications.
- 8. Bioremediation and Environmental Applications: Microorganisms are used to degrade pollutants, detoxify hazardous wastes, and clean up contaminated sites, contributing to sustainable environmental management.

Important characteristics of industrial microbes

- The major organisms used in industrial microbiology are fungi (yeasts and molds) and bacteria.
- **i**. The organism must be able to grow in a simple medium and should preferably not require growth factors (i.e. pre-formed vitamins, nucleotides, and acids) outside those which may be present in the industrial medium in which it is grown.
- **ii**. The organism should be able to grow vigorously and rapidly in the medium in use.
- **iii**. The organism should produce the desired materials, whether they be cells or metabolic products, in as short a time as possible for reasons given above.
- **iv.** Its end products should not include toxic and other undesirable materials, especially if these end products are for internal consumption.
- **v.** The organism should have a reasonable genetic and physiological stability. An organism which mutates easily is an expensive risk.
- **vi.** The organism should be compatible with an efficient method for product extraction at the conclusion of fermentation.
- **vii**. Wherever possible, organisms which have physiological requirements which protect them against competition from contaminants should be used.

Key roles of microorganisms in industrial processes

- 1. Fermentation Processes
- 2. Enzyme Production
- 3. Antibiotic Production
- 4. Bioremediation
- 5. Biofuel Production
- 6. Biopharmaceuticals

STRAIN SELECTION AND DEVELOPMENT

- **The following points highlight the five main steps involved in developing producer strains. The steps are:**
- **1. Isolation of Industrial Microorganisms:** The first step in developing producer strains is the isolation of concerned microorganisms from their natural habitats. Alternatively, microorganisms can be obtained as pure cultures from organisations, which maintain culture collections, e.g.. American Type Culture Collection (ATCC).
- **2. Screening for New Products:** The next step in developing producer strains after isolation of microorganisms is their screening. A set of highly selective procedures, which allows the detection and isolation of microorganisms producing the desired metabolite constitutes primary screening.
- **3. Identification of Metabolites:** This step allows the identification of desirable isolates and the suitable culture conditions for them. these cultures provide the material necessary for developing a suitable procedure for isolation of the active principle or compound of interest by trying out various solvents-extraction procedures over a wide range of pH.
- **4. Maintenance of Microbial Isolates:** Industrial microbiology continuously uses specific microorganism isolates/strains as research, assay, development and production cultures. These strains are highly valuable and must be preserved under certain conditions over long periods without any genetic and, as a result, phenotypic changes.
- **5. Strain Improvement: it is based on the following three-approaches:**

  (i) Mutant selection and selective isolation of mutants (secondary screening),

  (ii) Recombination, and

  (iii) Recombinant DNA technology

Fermentation Media

- **Fermentation media** is simply defined as those preparations that support the growth of microorganisms used in a fermentation process. The fermentation media used for the operation of fermentation processes in the industry are usually in the liquid phase or solid state.

- However, liquid media are mostly used in fermentation processes because they require less space compared to solid media; and they are cheaper to work with – since no additional cost of procuring agar or solid agents will be accrued.

- To fully harness the innate productive capacity of the microorganism, the fermentation media must be adequate enough to meet all the nutritional requirements of the microbe(s) used for fermentation.

Fermentation Media Constituents

- For optimal growth, these microorganisms require a source of energy, water, oxygen, carbon, nitrogen, hydrogen, sulphur, phosphorus and salts like potassium, calcium and sodium.
- Some trace elements including but not limited to nickel, molybdenum and cobalt may also be important for microbial growth and thus must be provided in the fermentation media during its compounding.
- Some microbes also require vitamins such as biotin for growth, and this must be provided for in the fermentation medium.
- Buffers or acids and bases may also be added to the media to control the pH of the system;
- Anti-forming agents and precursor, inducer or inhibitor compounds may also be added at certain stages of the fermentation process.
- These compounds (i.e. precursors, inducers and inhibitor compounds) are added to the fermentation medium to regulate the production of specific metabolite or end-product by the fermentation microbe(s).

Qualities of a good fermentation media

- The fermentation media must produce the appropriate amount of the desired end-product.
- It must produce the required cell biomass.
- It should be of consistent quality.
- It should always be available for fermentation process throughout the year.
- The fermentation media should produce little or no toxic substance.
- The fermentation media must not affect the physiochemical parameters of the fermentation vessel such as temperature and aeration supply.
- It should not interact negatively with other constituents of the fermentation medium.
- Fermentation media must not affect the cleaning and sterilization process of the vessel.

CRITERIA FOR RAW MATERIALS IN INDUSTRIAL MEDIA

- In deciding the raw materials to be used in the production of given products using
- designated microorganism(s) the following factors should be taken into account.
- ***(a) Cost of the material***
- ***(b) Ready availability of the raw material***
- ***(c) Transportation costs***
- ***(d) Ease of disposal of wastes resulting from the raw materials***
- ***(e) Uniformity in the quality of the raw material and ease of standardization***
- ***(f) Adequate chemical composition of medium***
- ***(g) Presence of relevant precursors***
- ***(h) Satisfaction of growth and production requirements of the microorganisms***

RAW MATERIALS USED IN COMPOUNDING INDUSTRIAL MEDIA

- The raw materials to be discussed are used because of the properties mentioned above:
- cheapness, ready availability, constancy of chemical quality, etc.
- (a) Corn steep liquor
- (b) Pharmamedia
- (c) Distillers solubles
- (d) Soy bean meal
- (e) Molasses
- (f) Sulfite liquor
- (g) Other substrates used as raw materials in fermentations are alcohol, acetic acid, methanol,
- methane, and fractions of crude petroleum.

Types of Fermentation Media

- **Defined (Synthetic) Media**: Composed of chemically pure and known quantities of all ingredients. Consistency and reproducibility, ideal for studying the specific nutritional requirements of microorganisms.
- **Complex Media:** Contain complex ingredients like yeast extract, peptone, and meat extract, whose exact chemical composition is not fully known. Generally supports robust microbial growth and are easier to prepare.
- **Selective Media**: Contain specific substances that favor the growth of particular microorganisms while inhibiting others.
- **Differential Media:** Contain indicators that help distinguish between different types of microorganisms based on their metabolic activities.
- **Enriched Media:** Supplemented with extra nutrients such as blood, serum, or special growth factors to support the growth of fastidious organisms.
- **Minimal Media:** Contain only the minimal nutrients necessary for the growth of a microorganism. Useful for studying the metabolic requirements and genetic traits of microorganisms.
- **Production Media:** Optimized for the production of a specific metabolite, such as antibiotics, enzymes, or biofuels. Maximizes the yield of the desired product.

Fermentation Media Optimization

- Optimizing media for specific microbial processes is crucial for maximizing microbial growth, productivity, and the production of desired metabolites or products. Here's a step-by-step guide on how to optimize media for specific microbial processes:
- 1. Understand Microbial Requirements: Identify the specific carbon, nitrogen, phosphorus, sulfur, vitamins, and mineral requirements of the microorganism. Analyze the metabolic pathways involved in the production of the desired product or metabolite.
- 2. Initial Medium Formulation: Start with a standard or commonly used medium suitable for the microorganism. Choose carbon and nitrogen sources that support optimal growth and product formation. Include essential minerals and vitamins required for microbial growth.
- 3. Experimental Design and Optimization Techniques: Design of Experiments (DoE): Use statistical methods like factorial designs, response surface methodology (RSM), and central composite designs (CCD) to systematically investigate the effects of multiple variables.
- 4. Monitoring and Analyzing Results: Measure microbial growth (e.g., optical density, cell dry weight), pH, and other critical parameters during fermentation. Quantify the desired product or metabolite using appropriate analytical methods. Use techniques like HPLC, GC-MS, or NMR to profile metabolites and understand metabolic fluxes.
- 5. Optimization Strategies: experiment with different carbon sources, nitrogen sources (e.g., ammonium salts, urea). Supplement the medium with essential minerals and vitamins based on microbial requirements. Optimize pH and buffering capacity, ensure adequate oxygen supply for aerobic processes through proper agitation and aeration.

FERMENTOR

- A fermentor (or fermenter) is a vessel for the growth of microorganisms which, while not permitting contamination, enables the provision of conditions necessary for the maximal production of the desired products.
- Fermentors are therefore also known as bioreactors as they are vessels in which biological reactions take place
- Fermentations may be liquid(submerged) or solid state (surface).
- Most fermentations used in the industry are submerged processes, because they save space and are more amenable to engineering control and design.
- On the basis of the agent used, fermentors are grouped into two broad classes:

  (i) those based on living cells and, (ii) those employing enzymes.
- But in terms of process requirements, they are of the following types:

  (i) aerobic, (ii) anaerobic, (iii) solid state, and (iv) immobilized cell bioreactors.

- All fermentors deal with heterogeneous systems having two or more phases, e.g., liquid, gas, solid.
- Fermentors can be designed for laboratory purpose (small as 1 liter or up to about 20 liters) or for production (from 100,000 liters to 500,000 liters).
- Only about 75% of the volume is usually utilized for actual fermentation, the rest being left for foam and exhaust gases.
- Several types of fermentors are known which may be grouped in several ways: shape or configuration, whether aerated or anaerobic, and whether they are batch or continuous.
- The most commonly used type of fermentor is the Aerated Stirred Tank Batch Fermentor.

Optimization of fermentation processes

- Optimal conditions for fermentation necessitate efficient transfer of mass, heat and momentum from one phase to the other. A fermentor should provide for the following:
- (i) agitation (for mixing of cells and medium),
- (ii) aeration (aerobic fermenters; for O 2 supply),
- (iii) regulation of factors like temperature, pH, pressure, aeration, nutrient feeding, liquid level, etc.,
- (iv) sterilization and maintenance of sterility, and
- (v) withdrawal of cells/medium (for continuous fermenters).
- Modern fermenters are usually integrated with computers for efficient process monitoring, data acquisition, etc.

Optimization of fermentation processes contn'd

- In fermentations where aerobic organisms are used, oxygen must be dissolved in aqueous solution along with the nutrients.
- The air used in most fermentation is sterile but if sterility is not necessary, such as in yeast fermentation, the air is merely scrubbed by passing it through glycerol.
- Many fermentation processes release heat, which must be removed so as to maintain the optimum temperature for the productivity of the organism.
- In small laboratory fermentors, temperature control may be achieved by immersing the tank in a water bath;
- In medium-sized ones, control may be achieved by a jacket of cold water circulating outside the tank or merely by bathing the unjacketed cylinder with water.
- In large fermentors, temperature is maintained by circulating refrigerated water in pipes within the fermentor and sometimes outside it as well.
- A heating coil is also provided to raise the temperature when necessary.
- Foams in industrial fermentations are controlled either by chemical or mechanical means.
- Chemicals controlling foams have been classified into antifoams, which are added in the medium to prevent foam formation, and defoamers, which are added to knock down foams once these are formed.

Fermentor designs

- **1. Stirred Tank Bioreactor (STR)**: For production of enzymes, vaccines, therapeutic proteins, and biofuels.
- **2. Airlift Bioreactor:** For cultivation of animal and plant cells, production of secondary metabolites.
- **3. Bubble Column Bioreactor: For w**astewater treatment, fermentation processes for alcohol and organic acids.
- **4. Fluidized Bed Bioreactor (FBB):** For Wastewater treatment, biotransformations, production of high-value biochemicals.
- **5. Packed Bed Bioreactor:** For Production of pharmaceuticals, biofuels, and biocatalysts.

Fermentor designs continued

- **6. Photobioreactor:** For production of biofuels, bioplastics, and high-value compounds (e.g., pigments, antioxidants).
- **7. Membrane Bioreactor (MBR):** For wastewater treatment, production of therapeutic proteins and peptides.
- **8. Wave Bioreactor:** For production of monoclonal antibodies, vaccines, and other biopharmaceuticals.
- **9. Trickling Filter Bioreactor:** For wastewater treatment, biodegradation of pollutants.
- **10. Anaerobic Digester:** For wastewater treatment, biogas production, organic waste management.

Factors affecting efficiency of the fermentation process

- **Physical Parameters**
- 1. Temperature: Affects enzyme activity, cell membrane fluidity, and overall metabolic rates.
- 2. pH: Affects enzyme activity, nutrient solubility, and transport across cell membranes.
- 3. Dissolved Oxygen (DO): Critical for aerobic microorganisms; affects respiration and energy production.
- 4. Agitation: Ensures uniform distribution of nutrients, gases, and cells; prevents settling and clumping.
- 5. Pressure: High pressures can enhance gas solubility but may also stress cells.

Factors affecting efficiency of the fermentation process continued

- **Chemical Parameters**
- 1. Nutrient Concentration: Provides the building blocks for cell growth and product formation.
- 2. Substrate Type and Concentration: Primary carbon and energy source; affects cell growth rates and metabolic pathways.
- 3. Product and By-product Concentration: Accumulation of products/by-products can inhibit microbial growth and metabolism.
- 4. Inhibitors and Toxins: Presence of inhibitory compounds can reduce microbial growth and product yield.
- **Biological Parameters**
- 1. Microbial Strain: Genetic makeup determines metabolic capabilities and efficiency.
- 2. Inoculum Size and Quality: Determines the initial cell density and health, impacting the lag phase and overall fermentation kinetics.
- 3. Cell Viability and Physiological State: Healthy, viable cells with high metabolic activity are essential for efficient fermentation.

Downstream processing

- Downstream processing refers to the series of operations required to isolate, purify, and formulate a desired product from the fermentation broth or cell culture. This phase is critical in bioprocessing and can significantly impact the overall yield, purity, and quality of the final product.
- **Steps in downstream processing:**
- 1. Solid-liquid separation is the first step in downstream processing and is essential for recovering the desired product from the culture broth. This stage involves removing whole cells (cell biomass) and other insoluble components from the fermentation or cell culture broth.
- Several methods are in used for solid-liquid separation, these are:

1. Flotation
2. Flocculation
3. Filtration
4. Centrifugation
5. sedimentation

Downstream processing continued

- **Stage 2. Release of Intracellular Products:** which requires breaking open the cells to extract the desired biomolecules such as proteins, enzymes, nucleic acids, or other metabolites. This stage is critical for products that are located within the cell rather than secreted into the culture medium.
- The methods used for cell disruption can be mechanical and non-mechanical.

- **Mechanical Methods**
- 1. Bead Milling: Cells are agitated with small beads that collide with the cells, causing them to break open.
- 2. High-Pressure Homogenization: Cell suspension is forced through a narrow valve at high pressure, causing cells to rupture due to shear forces.
- 3. Sonication: Uses ultrasonic waves to create cavitation bubbles in the liquid, which collapse and cause cell disruption.
- 4. Mechanical Shearing: Cells are subjected to high shear forces using rotor-stator devices or blenders.

Downstream processing continued

- **Non-Mechanical Methods**
- **1. Chemical Lysis:** Uses chemicals such as detergents, solvents, or alkalis to disrupt cell membranes.
- **2. Enzymatic Lysis:** Uses specific enzymes (e.g., lysozyme for bacterial cells) to break down cell walls or membranes.
- **3. Osmotic Shock:** Exposes cells to a sudden change in osmotic pressure, causing them to burst.
- **4. Thermal Lysis:** Uses rapid temperature changes to disrupt cell membranes.

Downstream processing continued

- **Stage 3. Concentration:**
- • The filtrate that is free from suspended particles usually contains 80-98% of water. The desired product is a very minor constituent. The water has to be removed to achieve the product concentration.
- • The commonly used techniques for concentrating biological products are:
- 1. Evaporation
- 2. Liquid-liquid extraction
- 3. Membrane filtration
- 4. Precipitation
- 5. Adsorption.

Downstream processing continued

- **Stage 4: Purification by Chromatography:** for isolating and purifying biological products from fermentation. This technique is highly effective for separating proteins, pharmaceuticals, diagnostic compounds, and research materials based on their physical and chemical properties.
- Chromatography usually consists of a stationary phase and mobile phase.
- **Types of Chromatography Used in Purification**

  Ion Exchange Chromatography (IEX)

  Affinity Chromatography

  Gel Filtration (Size Exclusion) Chromatography

  Hydrophobic Interaction Chromatography (HIC)

Downstream processing continued

- **Stage 5: Formulation**
- Formulation encompasses the strategies employed to preserve the activity and stability of biotechnological products throughout their storage and distribution. This crucial stage ensures that the product maintains its efficacy and integrity from production to end-use.
- **Objectives of Formulation:** Preservation of Activity, Stability Enhancement, Prevention of Degradation, Facilitating Storage and Distribution
- **Formulation Strategies:**

  Drying

  Spray drying:

  Buffering.

  Stabilizers and Excipients

  Lyophilization (Freeze-Drying)

  Microencapsulation

  Packaging Materials

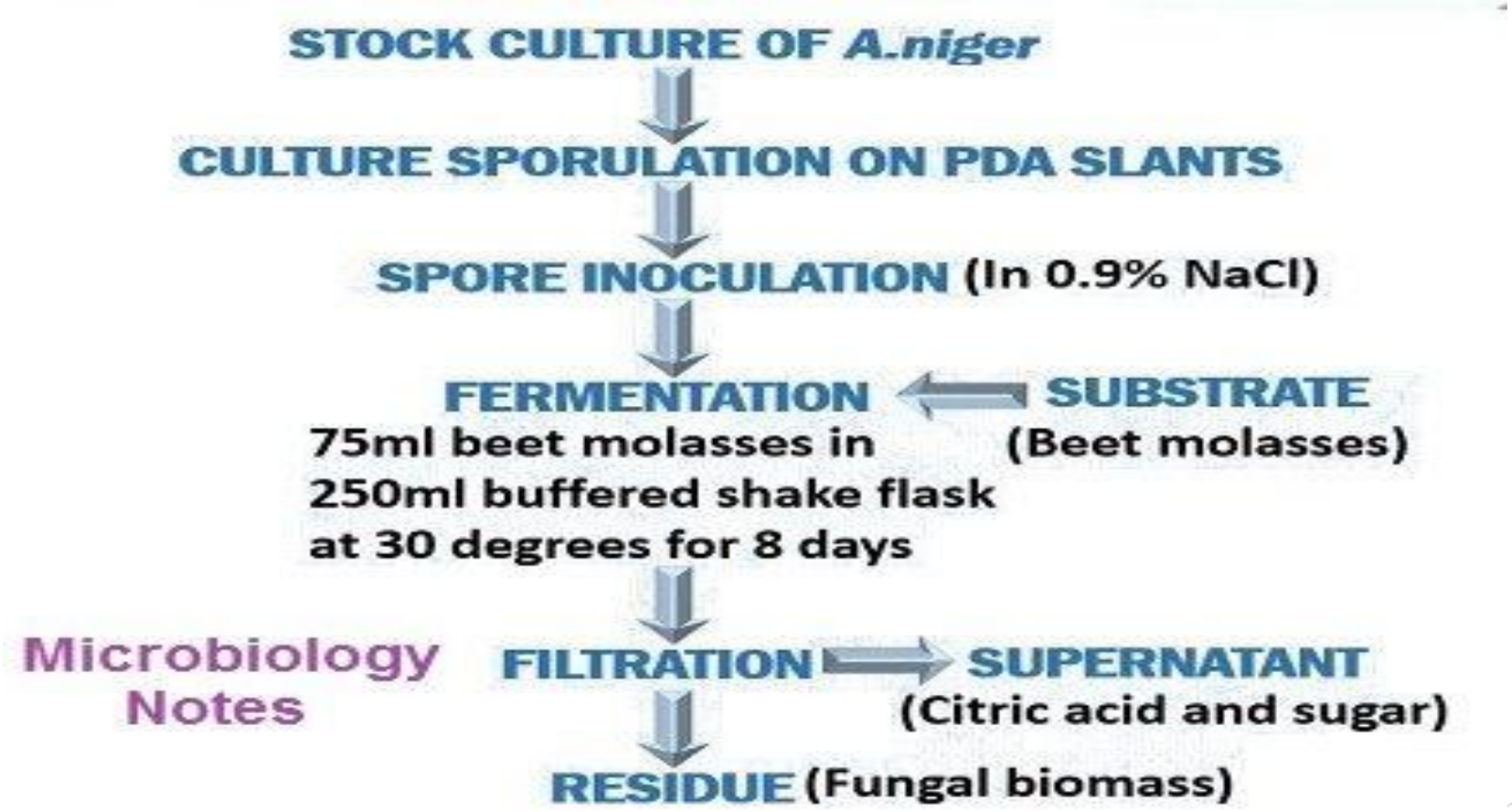
  Controlled Release Systems

Mcrobial production of Industrial Products (Organic acids)

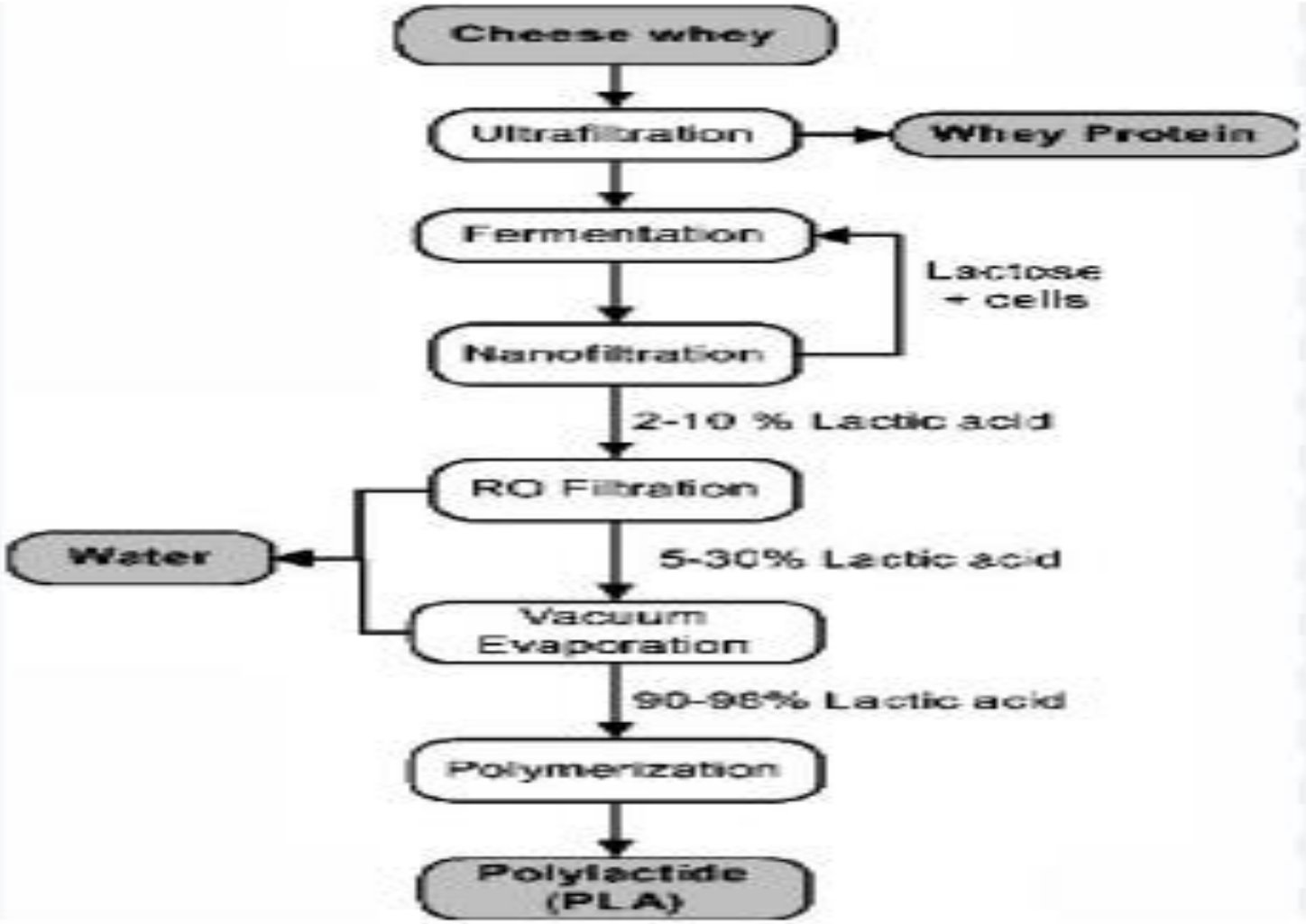
- **Citric acid**
- For a long time the production of citric acid has been based on the use of molasses and various strains of *Aspergillus niger* and occasionally *Asp. wenti*. In recent times, yeasts, especially *Candida* spp. And *Candida guilliermondii*, have been used to produce the acid from sugar.
- Bacteria and yeasts have been used. Among the bacteria were *Arthrobacter paraffineus* and corynebacteria; the yeasts included *Candida lipolytica* and *Candida oleiphila*.
- Fermentation with molasses and other sugar sources can be either surface or submerged.
- Fermentation with paraffins however is submerged.

- **Lactic acid**
- Although many organisms can produce lactic acid, the amounts produced are small; the organisms which produce adequate amounts and are therefore used in industry are the homofermentative lactic acid bacteria, *Lactobacillus* spp., especially *L. delbrueckii*.
- In recent times, *Rhizopus oryzae* has been used. Both organisms produce the L-form of the acid, but
- *Rhizopus* fermentation has the advantage of being much shorter in duration; furthermore, the isolation of the acid is much easier when the fungus is used

Citric acid production



Lactic acid production

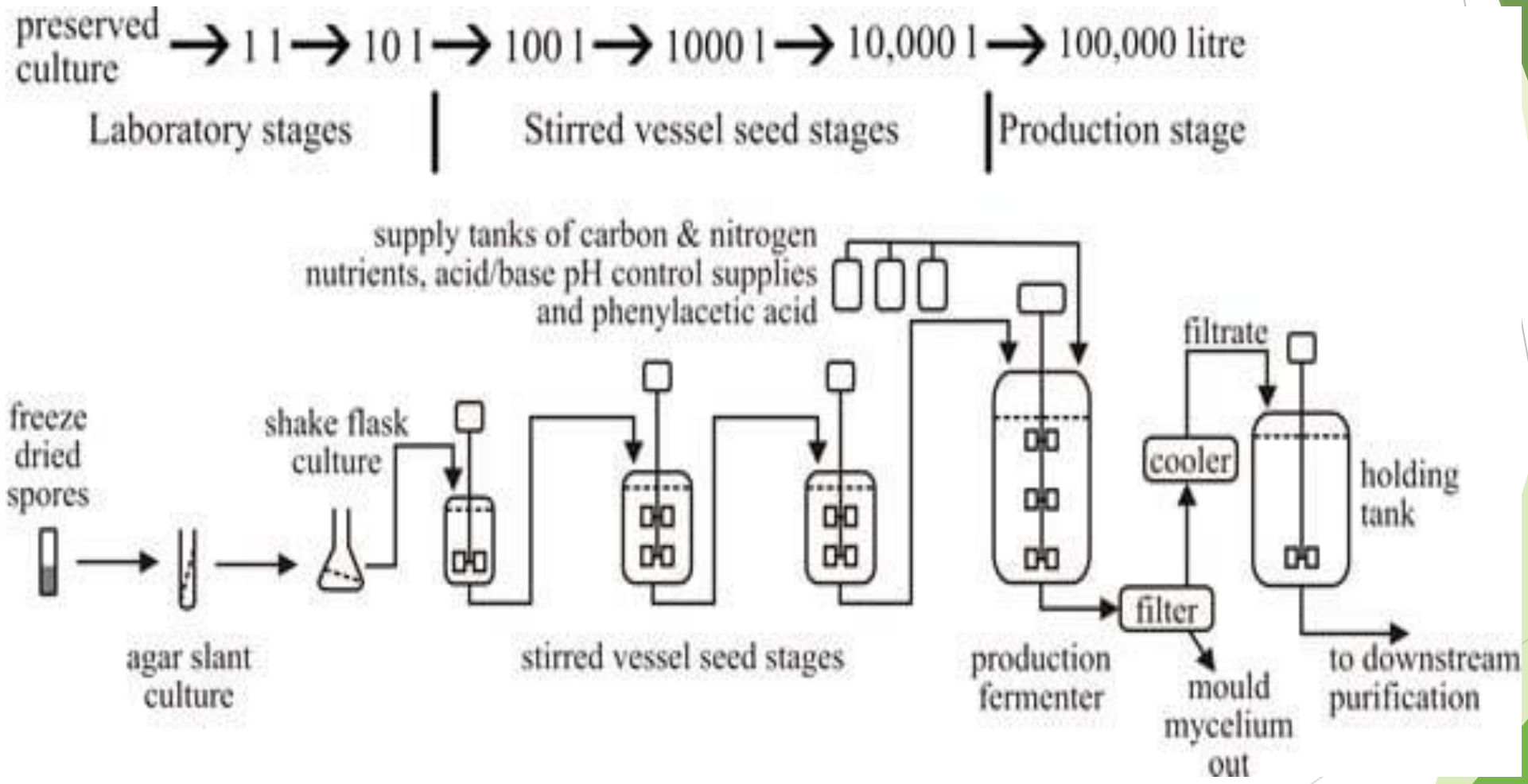


Microbial production of Industrial Products (Antibiotics)

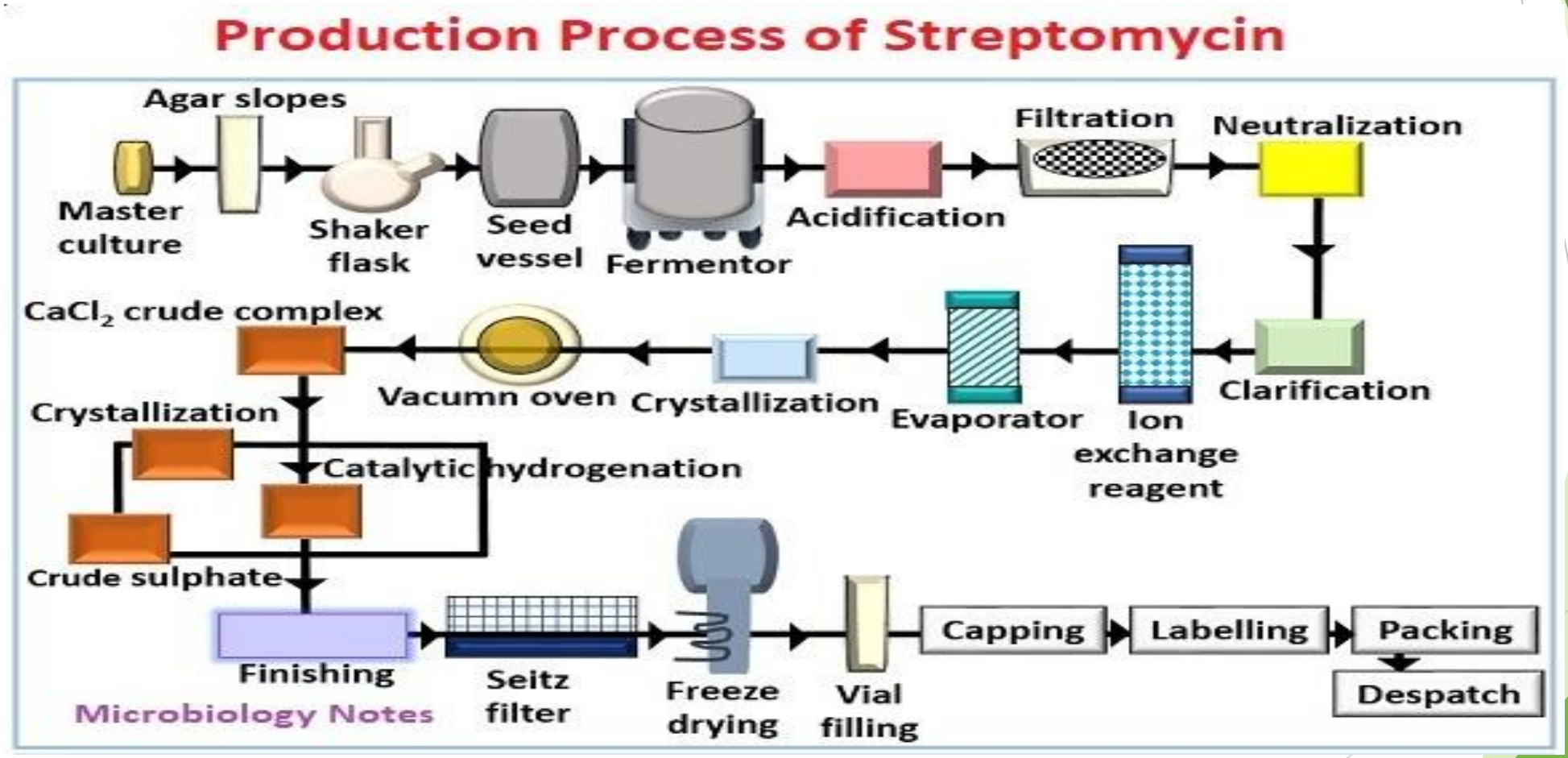
- Antibiotics produced by microbes are regarded was one of the most significant discoveries of the twentieth century and have made major contributions towards the welfare of human society.

- Many antibiotics are produced by microorganisms, predominantly by Actinomycetes in the genus Streptomycin (e.g. Tetracycline, Streptomycin, Actinomycin D) and by filamentous fungi (e.g. Penicillin, Cephalosporin)

Microbial production of Penicillin



Microbial production of Streptomycin



Microbial production of Industrial Products (Enzymes)

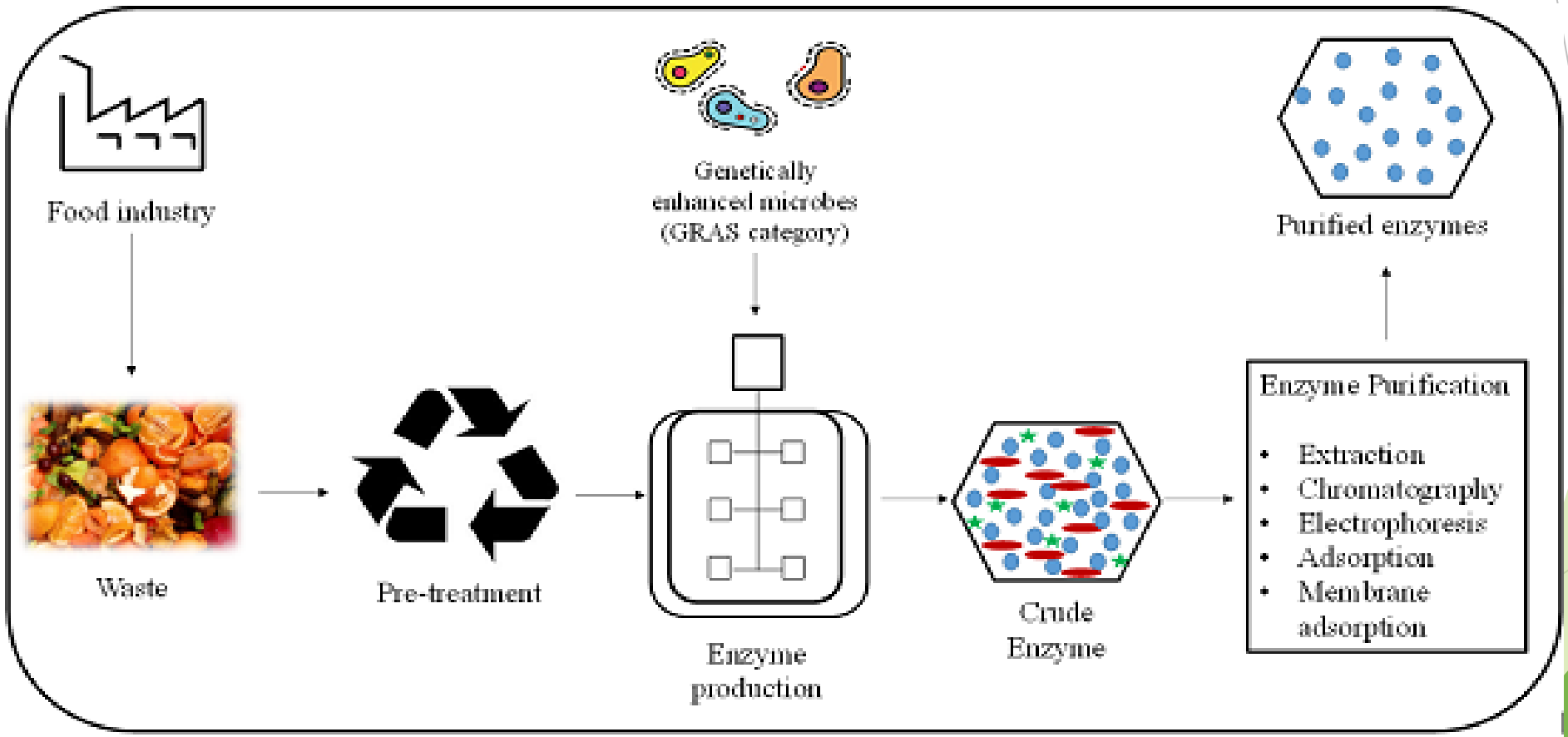
- Many microbes synthesize and excrete large quantities of enzymes into the surrounding medium. Using this feature of these tiny organisms, many enzymes have been produced commercially.
- These include Amylase, Cellulase, Protease, Lipase, Pectinase, Streptokinase, and many others. Enzymes are extensively used in food processing and preservation, washing powders, leather industry, paper industry and in scientific research.

Microbial Enzyme Production

Surface culture technique:
amylase (from *Aspergillus* sp.),
protease (from *Aspergillus* sp.) and
pectinase (from *Penicillium* sp. and *Aspergillus* sp.).

Submerged culture method:
Continuous culture technique - cellulase production by *Trichoderma*

Microbial production of Industrial Products (Enzymes)



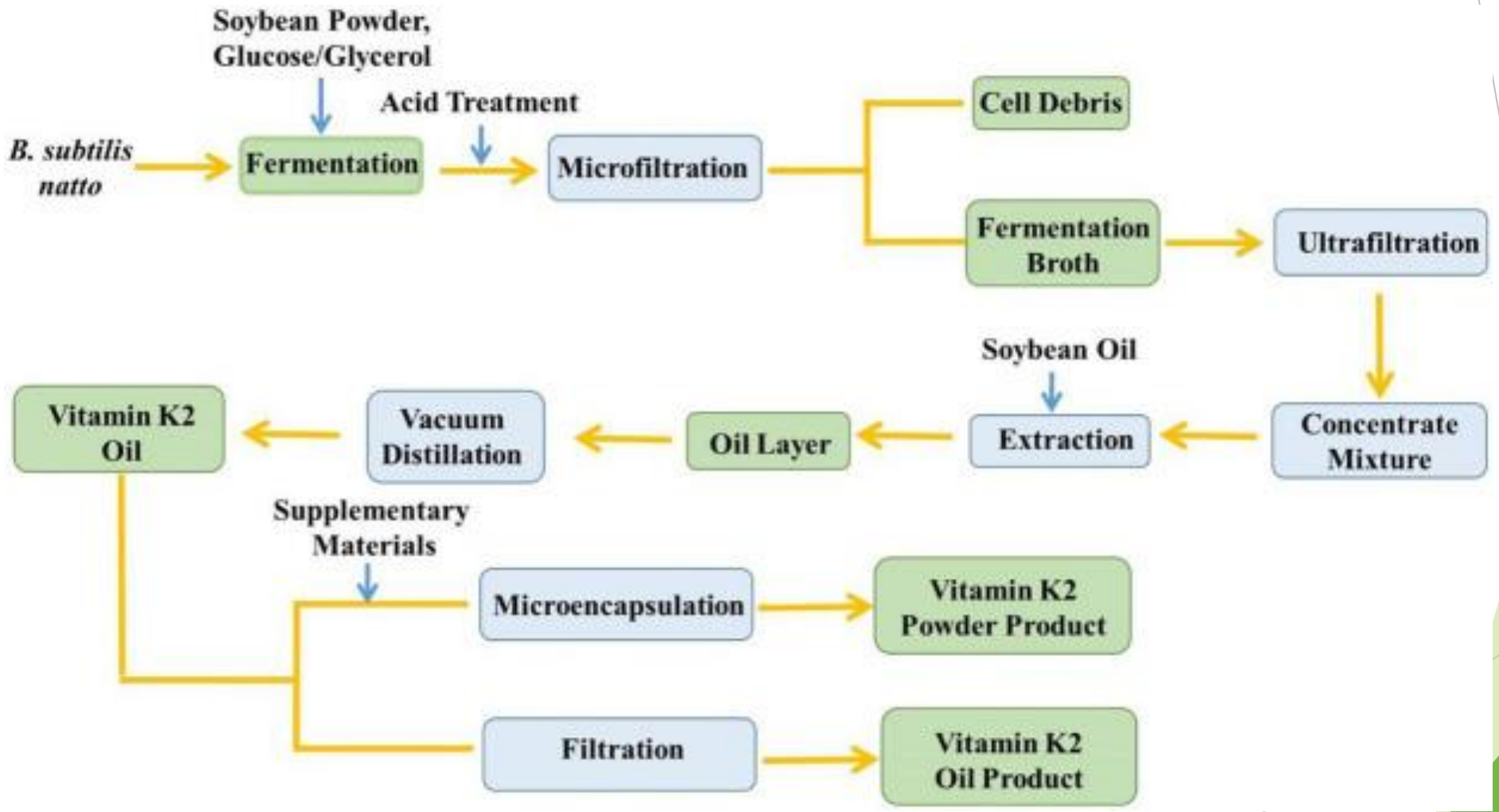
Microbial production of Industrial Products (Enzymes)

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

Microbial production of Industrial Products (Vitamins)

- Vitamins are some organic compounds which are capable of performing many life-sustaining functions inside our body. These compounds cannot be synthesized by humans and therefore they have to be supplied in small amounts in the diet.
- Microbes are capable of synthesizing the vitamins and hence they can be successfully used for the commercial production of many of the vitamins e.g. thiamine, riboflavin, pyridoxine, folic acid, pantothenic acid, biotin, vitamin B12, ascorbic acid, beta-carotene (pro-vitamin A), ergosterol (provitamin D)

Microbial production of Vitamin B12



Microbial production of Industrial Products (Single cell protein)

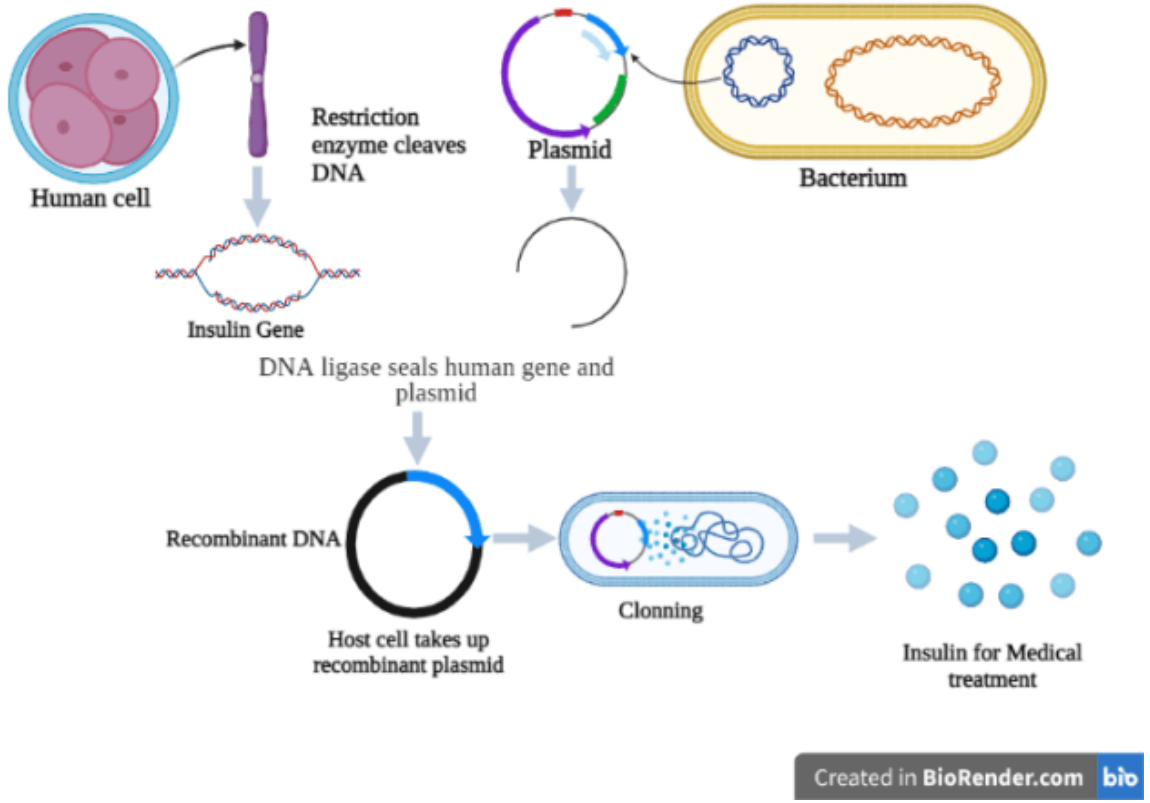
- Single Cell Protein (SCP) refers to sources of mixed protein extracted from pure or mixed cultures of algae, yeasts, fungi or bacteria that are grown from agricultural wastes.
- In some bacteria, the protein content is as high as 80%. Along with protein, the biomass also contains other essential nutrients so that it is an ideal supplement to conventional food supply.
- SCP are dried cells of microorganisms which can be used as dietary protein supplement.
- They are used as animal feed & can be used for human feed as protein supplement.
- 60-80% dry cell weight; contains nucleic acids, fats, CHO, vitamins and minerals.
- Single Cell Protein (SCP) can serve as an alternate source of energy when a larger portion of the world is suffering from hunger and malnutrition.
- Microbes like Spirulina can be grown easily on materials like waste water from potato processing plants (containing starch), straw, molasses, animal manure, and even sewage, to produce large quantities and can serve as food rich in protein, minerals, fats, carbohydrate, and vitamins.

Microbial production of Industrial Products (Single cell protein)

Microbial production of Industrial Products (Steroids)

- Steroids: These are a very important group of chemicals, which are used as anti-inflammatory drugs and as hormones such as estrogens and progesterone, which are used in oral contraceptives.
- Steroids are widely distributed in animals, plants, and fungi like yeasts.
- But, producing steroids from animal sources or chemically synthesizing them is difficult, but microorganisms can synthesize steroids from sterols or from related, easily obtained compounds.
- Mostly *Mycobacterium* sp are used frequently.

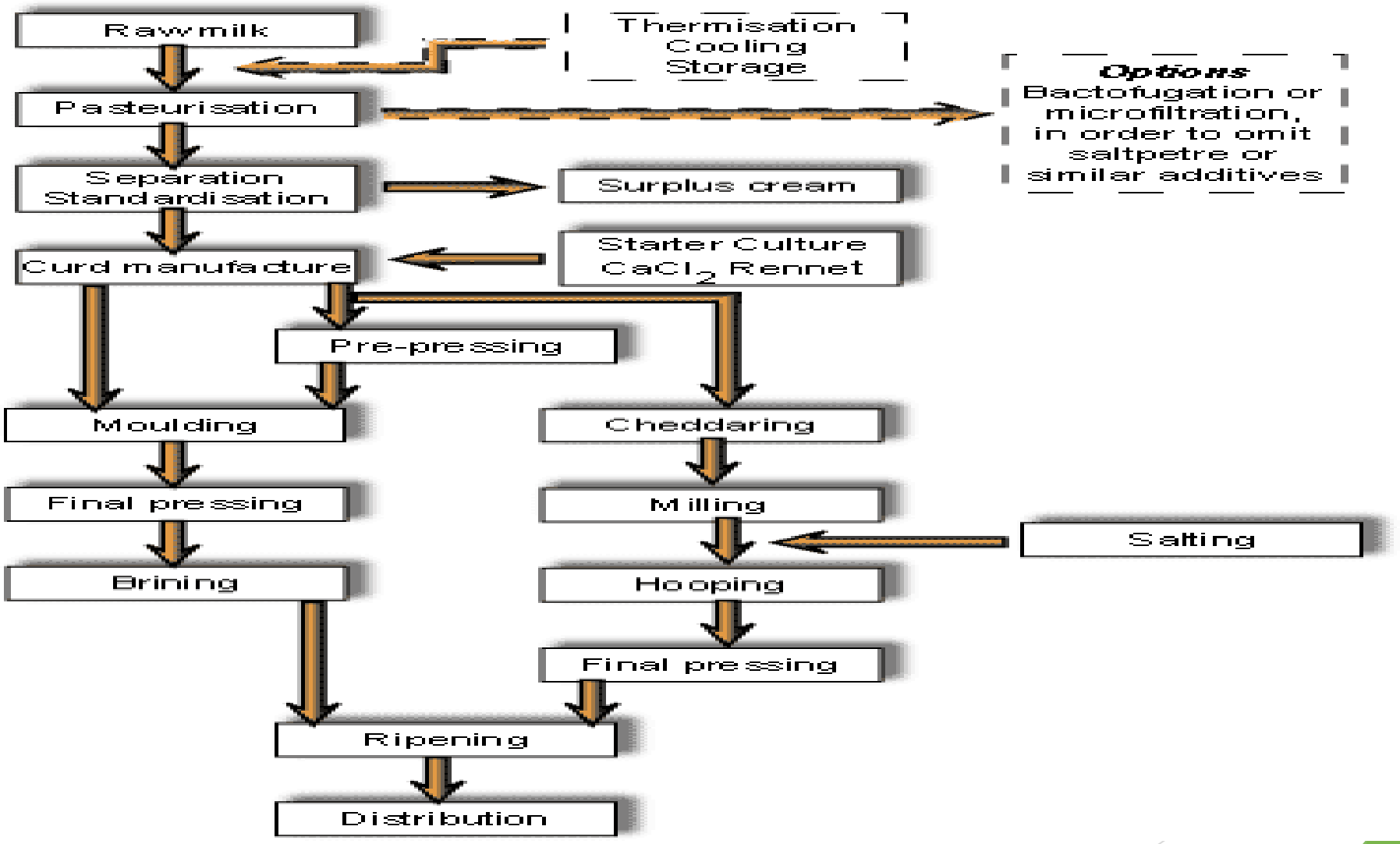
Industrial production of hormones



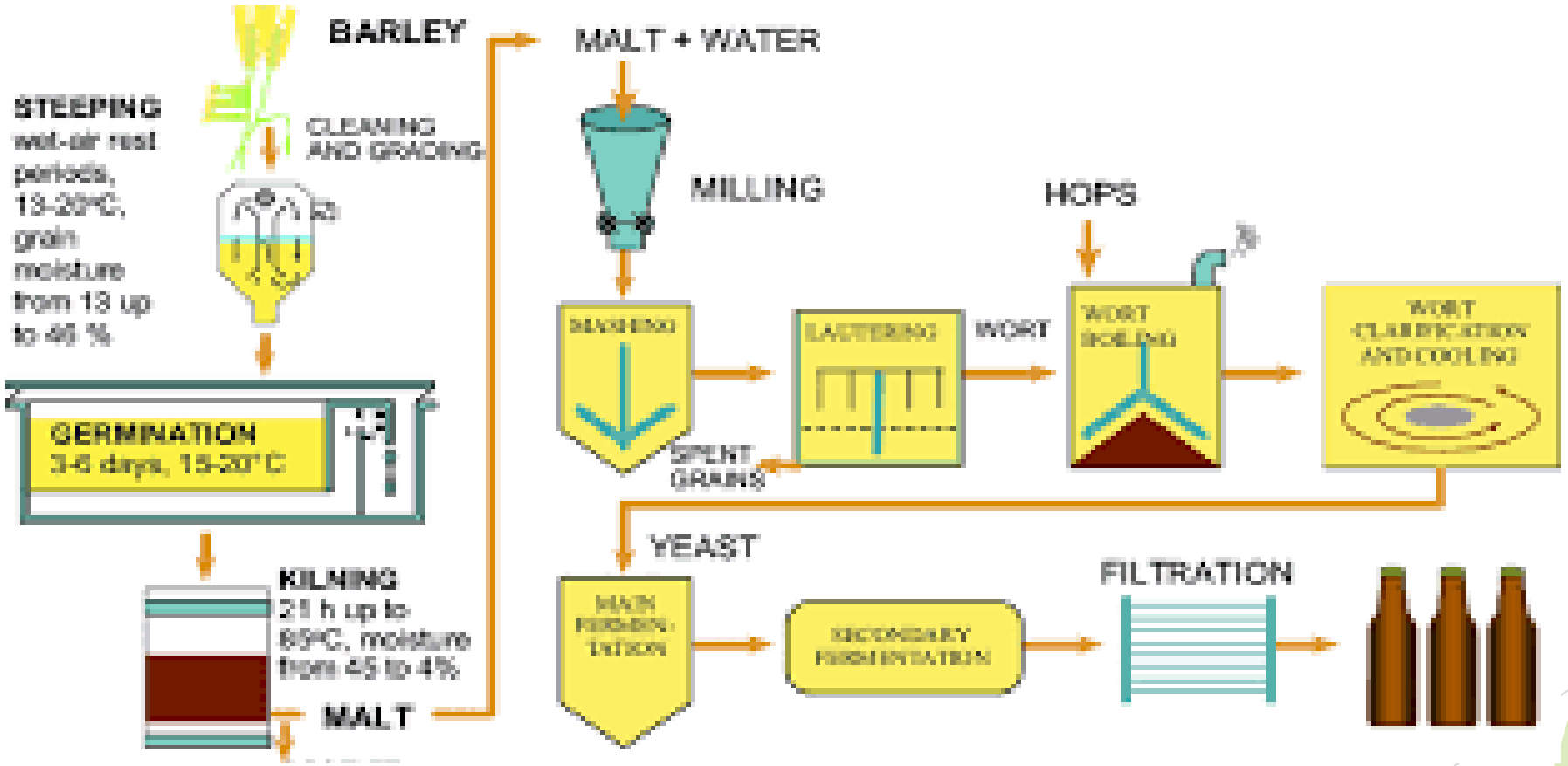
Microbial production of Industrial Products (Dairy food)

- Microbes are used in dairy industry to make dairy product such as curd, yogurt, cheese, kefir , kumiss, bread and various types of milk product . *Saccharomyces cerevisiae* , *Streptococcus* sp , *Penicillium roqueforti*, *P. camemberti* , *Streptococcus thermophilus* , *Lactobacillus bulgaricus* , *Lactobacillus* sp , *Candida* sp.

Microbial production of cheese



Microbial production of malt



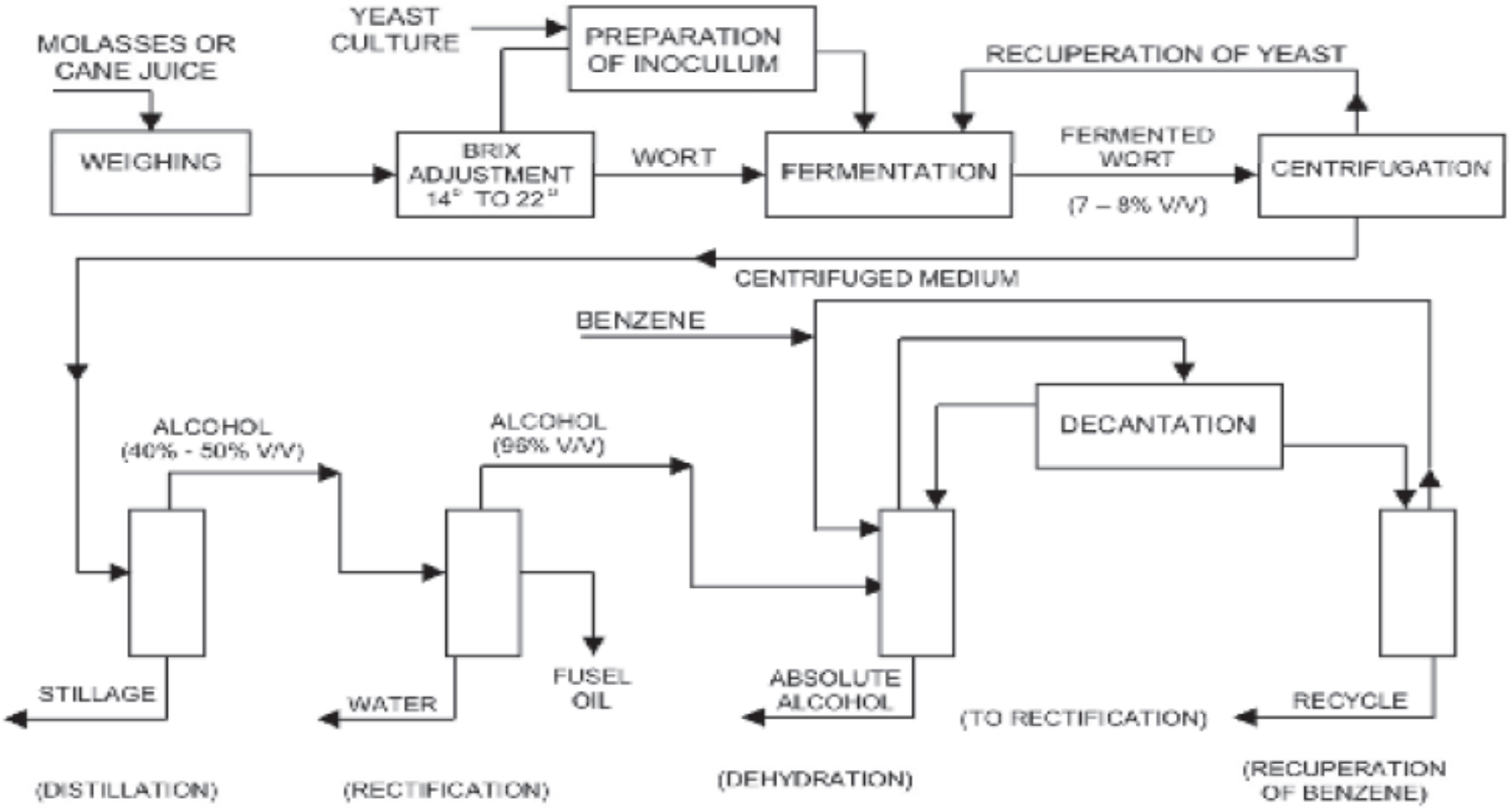
BIOFUEL

- Fossil energy is non-renewable. Well-established processes for fermentation of sugars to ethanol and
- esterification of fatty acids in lipids and oils, with alcohols such as methanol, ethanol, and
- propanol laid foundation for production of first generation liquid transportation biofuel.

Generation	*Examples*	*Feedstock*	*Process*
First generation	Bioethanol	**Starch** from corn, wheat, milo (sorghum) and cassava **Sugar** from sugar cane, sugar beet	Starch requires digestion to sugar using amylases, before fermentation of sugar to ethanol
	Biodiesel	**Oil** from seeds such as rapeseed, Soybean, oil palm, sunflower, groundnut, canola	Esterification of fatty acids of oil with alcohol such as methanol and ethanol
Second generation	Bioethanol Biobutanol	**Agricultural residues** such as wheat straw, corn stover (leaves, stalks, and cobs), bagasse (sugarcane waste), **Forestry residues** such as logging residues and wood, wood-processing mill residues	Hydrolysis of cellulose and hemicellulose to fermentable sugars and fermentation to alcohol. Requires pretreatment to remove lignin
Third generation	Biodiesel	**Oil** from microalgal species such as *Botryococcus* and *Chlorella*	Esterification of fatty acids of oil with alcohol such as methanol and ethanol
	Bioethanol	**Carbohydrates** from macroalgae such as *Spirulina* platensis and *Chlamydomonas* sp. as well as *Cyanobacteria*	Hydrolysis of carbohydrates to fermentable sugars and fermentation to alcohol. Requires pretreatment to remove lignin

Constructed from Ho et al. 2014

Flow Diagram of Conventional Alcohol Production



ENZYME IMMOBILIZATION

- Enzyme immobilization is confinement of enzyme to a phase (matrix/support) different from the one for substrates and products. Inert polymers and inorganic materials are usually used as carrier matrices.
- For large extent commercialization of bio-derived catalysts, their reusability factor becomes mandatory. Maintenance of their structural stability during any biochemical reaction is highly challenging. Hence, immobilized enzymes with functional efficiency and enhanced reproducibility are used as alternatives. Immobilized biocatalysts can either be enzymes or whole cells.
- An ideal matrix must be inert, have physical strength, stability and regenerability, ability to increase enzyme specificity/activity and reduce product inhibition, nonspecific adsorption and microbial contamination. Immobilization generates continuous economic operations, automation, high investment/capacity ratio and recovery of product with greater purity.

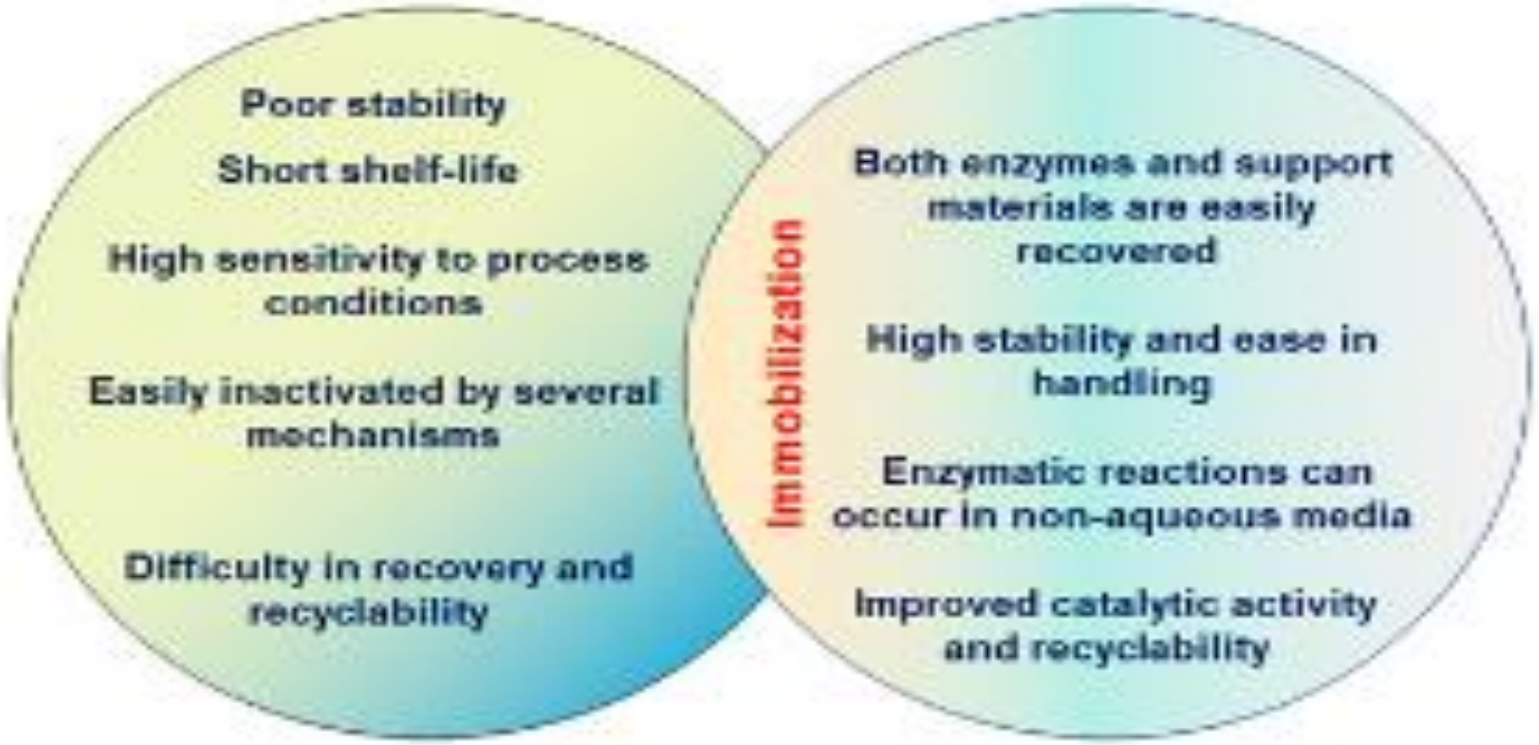


Fig. 2: showing the difference between enzymes and immobilized enzymes.

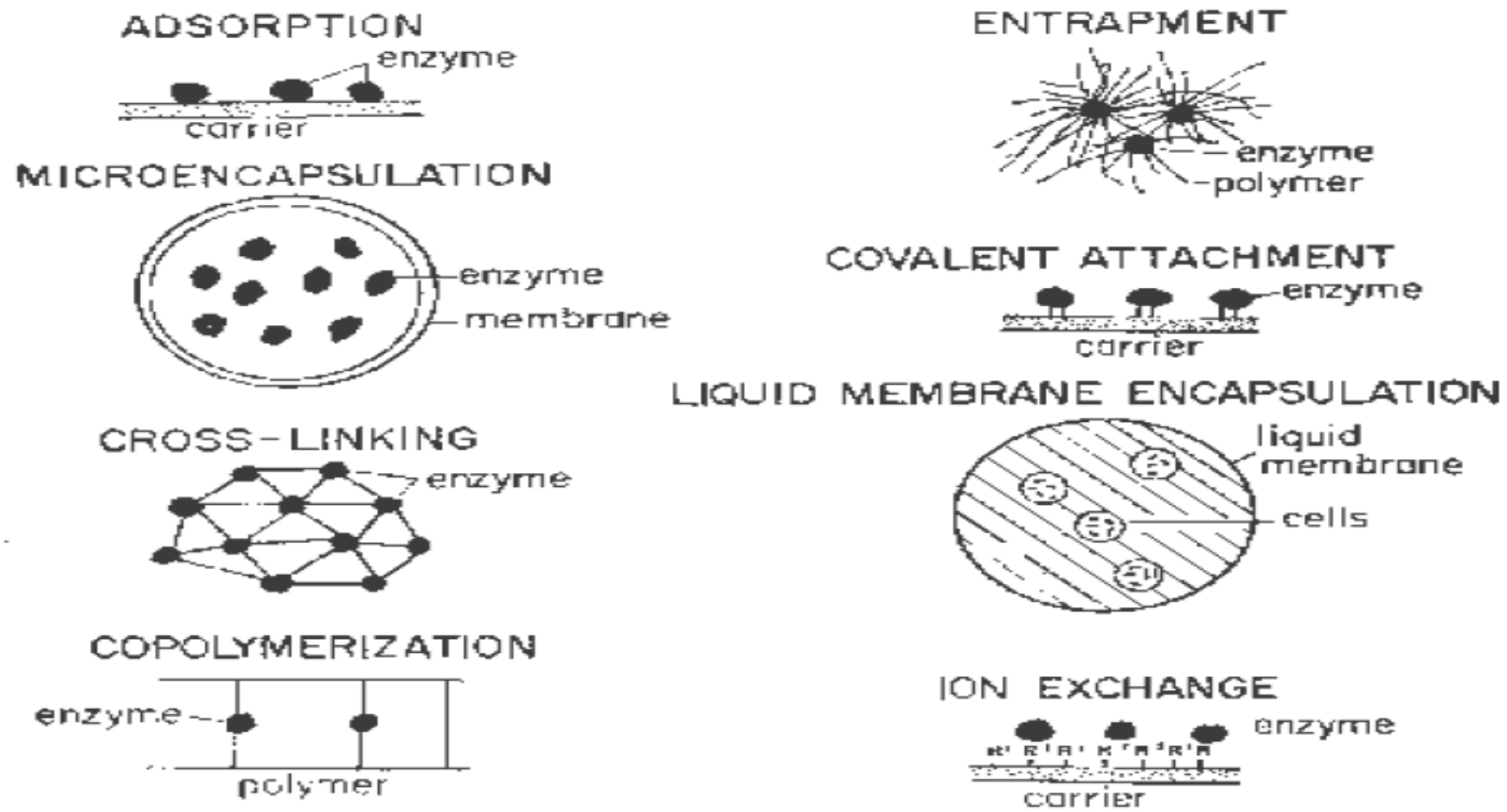


Fig 2: Diagram illustrating major enzyme immobilization methods.
Reversible methods include adsorption, ionic bonding, and affinity bonding.
Irreversible methods include covalent binding, entrapment, encapsulation, and cross-linking

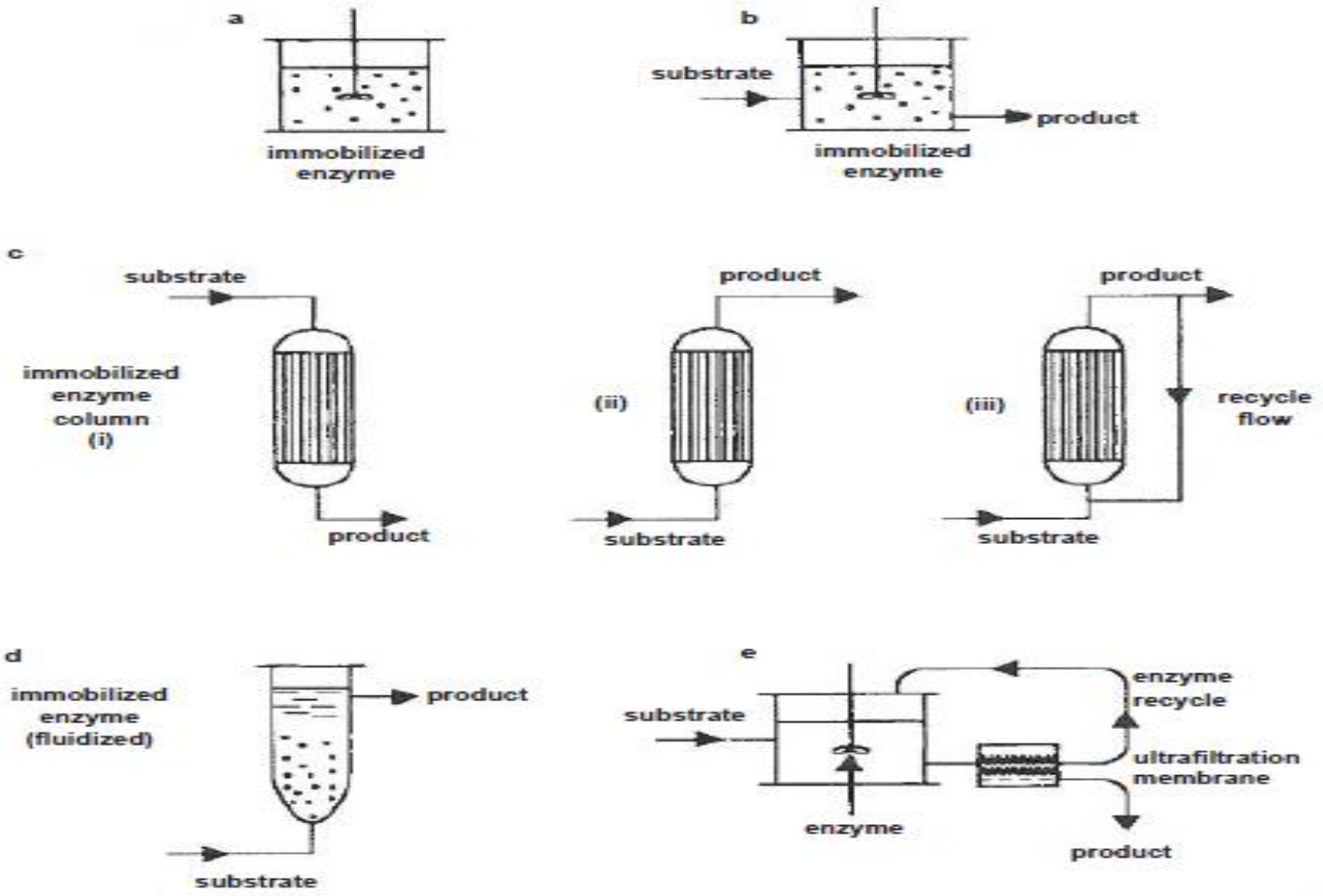


Fig 3: Various Designs of Bioreactors for Use in Biocatalysis.

Application of Immobilized Enzymes

- **1. Food industry**

Fermentation: Immobilized yeast cells are employed in continuous fermentation processes for beer, wine, and bioethanol production.

Flavor Enhancement: Enzymes such as lipases are used for flavor enhancement and aroma development in cheese and dairy products.

- **2. Pharmaceuticals and Biotechnology:**

Drug Synthesis: Immobilized enzymes catalyze key steps in the synthesis of pharmaceutical intermediates and active pharmaceutical ingredients (APIs).

Biocatalysis: Immobilized enzymes enable chemoenzymatic synthesis, biotransformation, and resolution of chiral compounds for drug discovery and development.

Enzyme Replacement Therapy: Immobilized enzymes are used for enzyme replacement therapy in the treatment of metabolic disorders, such as phenylketonuria (PKU) and lysosomal storage diseases.

Application of Immobilized Enzymes

- **3. Environmental Biotechnology:**

Bioremediation: Immobilized enzymes and microbial consortia are employed for the degradation of organic pollutants, xenobiotics, and hazardous chemicals in wastewater treatment and environmental remediation.

Biosensing: Immobilized enzymes serve as bio-recognition elements in biosensors for the detection and quantification of environmental contaminants, pathogens, and toxins.

- **4. Bioenergy and Biorefineries:**

Biofuel Production: Immobilized enzymes are used in enzymatic hydrolysis of lignocellulosic biomass for bioethanol and biofuel production.

Bioproducts: Immobilized enzymes catalyze the production of biochemicals, biopolymers, and biomaterials from renewable feedstocks in biorefinery processes.

- **5. Textile and Leather Industry:**

Enzymatic Treatment: Immobilized enzymes are utilized for biofinishing, desizing, scouring, bleaching, and stone washing of textiles, enhancing fabric properties and reducing environmental impact.

Leather Processing: Immobilized enzymes aid in enzymatic dehairing, bating, degreasing, and softening of leather hides, improving processing efficiency and waste management.

Application of Immobilized Enzymes

- 6. Fine Chemicals and Specialty Chemicals:

Chemical Synthesis: Immobilized enzymes catalyze selective chemical transformations, such as oxidation, reduction, esterification, and hydroxylation, for the synthesis of fine chemicals, flavors, fragrances, and specialty compounds.

Biocatalytic Resolution: Immobilized enzymes are used for enantioselective resolution of racemic mixtures to produce optically pure compounds for pharmaceuticals, agrochemicals, and fine chemicals.

- 7. Personal Care and Cosmetics:

Enzyme-Based Formulations: Immobilized enzymes are incorporated into cosmetic and personal care products for exfoliation, skin rejuvenation, anti-aging, and hair conditioning.

Biocatalytic Processes: Immobilized enzymes are used for enzymatic synthesis of cosmetic ingredients, emulsifiers, and surfactants, reducing reliance on chemical additives and preservatives.

Application of Immobilized Enzymes

- **8. Agriculture and Agrochemicals:**

Biological Control: Immobilized enzymes and microbial formulations are used for biological control of plant pathogens, pests, and diseases in agriculture and horticulture.

Soil Amendments: Immobilized enzymes contribute to soil health and fertility as biofertilizers, soil conditioners, and plant growth promoters, enhancing nutrient availability and plant growth.

- **9. Paper and Pulp Industry:**

Pulp Bleaching: Immobilized enzymes, such as xylanases and cellulases, are applied in enzymatic pretreatment and bleaching of wood pulp to reduce chlorine usage and environmental pollution in papermaking.

- **10. Dairy and Animal Feed Industry:**

Feed Enzymes: Immobilized enzymes, such as phytases, proteases, and carbohydrases, are used as feed additives in animal nutrition to enhance nutrient digestibility, feed efficiency, and animal performance.

Application of Immobilized Enzymes

- **11. Agrochemicals and Agriculture:**

Biological Control: Immobilized enzymes and microbial formulations are used for biological pest control, soil conditioning, and plant growth promotion in agriculture, reducing reliance on chemical pesticides and fertilizers.

12. Biosensing and Diagnostics:

- **Biomedical Applications**: Immobilized enzymes serve as bio-recognition elements in biosensors and diagnostic devices for the detection and quantification of biomarkers, pathogens, toxins, and environmental pollutants in healthcare, food safety, and environmental monitoring.

IMMOBILIZATION CASE STUDIES

- Glucose isomerase (GI) serves as an interesting model for studying structure–function relationships by advanced biochemical and genetic engineering techniques.
- Besides its academic importance, it has received increased attention by industries for its use in producing high-fructose corn syrup (HFCS) and for its potential application in the production of ethanol from hemicelluloses.
- The use of GI is expensive because it is an intracellular enzyme, and large quantities are needed to compensate for the high K_m for glucose. Therefore, it is important to immobilize GI for its industrial applications.
- Recently, several methods for immobilizing GI have been developed. However, only a few are economical and yield enzyme preparations with properties that are suitable for commercial production of HFCS and ethanol.
- Two main methods are used for immobilization of GI: cell-free enzyme immobilization and whole-cell immobilization.

IMMOBILIZATION CASE STUDIES

- **Cell-free immobilization:** Soluble enzymes that are immobilized to a support structure have excellent flow characteristics suitable for continuous operations, in contrast to whole-cell immobilized supports, and offer considerable savings in terms of capital equipment.
- **GI**s from *Streptomyces phaeochromogenes* and *Lactobacillus breviswere* immobilized on DEAE-cellulose. The Streptomyces GI immobilized on DEAE-cellulose is being used to produce HFCS in a semi continuous plant by the Clinton Corn Processing Company.
- **Whole-cell immobilization:** Because GI is an intracellular enzyme, whole-cell immobilization is the method of choice foremost of the commercially available immobilized GIs. Whole cells containing GI were spray-dried and used in the first industrial process to produce HFCS by the Clinton Corn Processing Company.
- Addition of inorganic salts such as magnesium hydroxide to the fermentation broths of ***Streptomyces*** or ***Arthrobacter*** species followed by
- Filtration and drying of the cake provided a straightforward method to immobilize cells containing GI.

Production of High-Fructose Corn Syrup

- Physical entrapment of whole cells in polymeric materials was used as an immobilization method by Novo Industries, whereas chemical entrapment of cells in a membrane followed by cross-linking with glutaraldehyde was used to prepare an immobilized GI to be used on a commercial scale.
- The most common raw material used for the production of HFCS is the corn-starch manufactured by the wet milling process.
- The production of HFCS from starch comprises three major processes:

  (i) liquefaction of starch by amylase,

  (ii) saccharification of starch by the combined action of amylo glucosidase and a debranching enzyme, and

  (iii)isomerization of glucose by GI.
- The final product is a corn syrup containing a mixture of glucose and fructose and hence with a greater sweetening capacity than that of sucrose.
- Other sources of starch such as wheat, tapioca, and rice are used to a minor extent in other parts of the world.