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قسم التقنيات الاحيائية الطبية

INDUSTRIAL MICROBIOLOGY

Lec. 4

Microbial Production of Enzymes

by

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1. Introduction

Microbial enzyme production is one of the most significant applications of industrial microbiology. Microorganisms — bacteria, fungi, and yeasts — are used to synthesize enzymes that catalyze biochemical reactions in various industries, including pharmaceuticals, food, textiles, paper, and detergents.

Definition:

Enzymes are biological catalysts — mostly proteins — that accelerate chemical reactions without being consumed in the process.

Advantages of Microbial Enzymes:

- High yield in short time.
- Easy to manipulate genetically.
- Can grow on inexpensive substrates.
- Stable at extreme conditions (pH, temperature, salinity).
- Consistent supply and quality.

2. Types of Enzymes and Their Industrial Importance

Type of Enzyme	Source (Microbe)	Major Application
Amylases	*Bacillus subtilis*, *Aspergillus oryzae*	Starch processing, brewing, baking
Proteases	*Bacillus licheniformis*, *Aspergillus niger*	Detergents, leather, pharmaceuticals
Lipases	*Candida rugosa*, *Pseudomonas fluorescens*	Food, biodiesel, detergents

Type of Enzyme	Source (Microbe)	Major Application
Cellulases	*Trichoderma reesei*, *Aspergillus* spp.	Paper, textiles, biofuel production
Pectinases	*Aspergillus niger*	Juice clarification, textile
Laccases / Peroxidases	*Pleurotus* spp., *Phanerochaete chrysosporium*	Biobleaching, wastewater treatment
Glucose Isomerase	*Streptomyces* spp.	High-fructose corn syrup production

3. Microorganisms Used for Enzyme Production

A. Bacteria

- *Bacillus subtilis*, *B. licheniformis*, *B. amyloliquefaciens*: extracellular enzyme producers.
- *Streptomyces* spp.: source of oxidative and hydrolytic enzymes.

B. Fungi

- *Aspergillus niger*, *Trichoderma reesei*: produce cellulase, amylase, pectinase.
- *Penicillium* spp.: diverse enzyme profiles.

C. Yeasts

- *Candida rugosa*, *Saccharomyces cerevisiae*: lipases, invertase.

4. Fermentation Methods for Enzyme Production

Enzyme production typically uses **biotechnological fermentation** under controlled conditions.

A. Submerged Fermentation (SmF)

- Microbes grow in **liquid nutrient medium**.
- Ideal for bacteria and yeasts.
- Easier control of pH, temperature, and aeration.
- Common for **amylase, protease, lipase**.

B. Solid-State Fermentation (SSF)

- Growth on moist solid substrates without free-flowing water (e.g., wheat bran, rice husk).
- Suitable for **fungi**.
- Economical and produces concentrated enzymes.

C. Fed-Batch Fermentation

- Nutrients added gradually to avoid repression or substrate inhibition.
- Used for **high-yield enzyme production** and **metabolic control**.

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5. Factors Affecting Enzyme Production

1. **Carbon Source:** Glucose, starch, maltose (inducers or repressors).
2. **Nitrogen Source:** Peptone, yeast extract, ammonium salts.
3. **pH and Temperature:** Optimal for each microbe (e.g., *Bacillus* prefers pH 7–9, 37–45°C).
4. **Dissolved Oxygen:** Aeration and agitation important in aerobic fermentations.
5. **Inducers:** Substrates that trigger enzyme synthesis (e.g., starch for amylase).
6. **Inhibitors:** Catabolite repression (e.g., glucose inhibiting amylase).
7. **Incubation Time:** Enzyme production peaks during late exponential or stationary phase.

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6. Strain Improvement for Enhanced Enzyme Yield

To increase enzyme productivity, microbial strains are genetically or mutationally enhanced.

A. Mutation and Selection

- **Physical mutagens:** UV radiation.
- **Chemical mutagens:** Nitrosoguanidine (NTG), EMS.
- Mutants are screened for higher enzyme output.

B. Recombinant DNA Technology

- **Gene cloning:** Inserting enzyme-encoding genes into high-expression vectors.
- Example: Cloning *amyE* gene (amylase) into *E. coli* or *Bacillus*.
- **Expression optimization:** Using strong promoters and codon optimization.

C. CRISPR/Cas9 Gene Editing

- Allows **precise modification** of regulatory regions.
- Enhances secretion pathways or enzyme stability.
- Example: CRISPR-mediated overexpression of cellulase genes in *Trichoderma reesei*.

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7. Downstream Processing of Enzymes

After fermentation, enzymes must be separated and purified.

Steps:

1. **Cell Separation:** Centrifugation or filtration.
2. **Extraction:** For intracellular enzymes, cell disruption is needed.
3. **Concentration:** Ultrafiltration or precipitation (ammonium sulfate).
4. **Purification:** Chromatography (ion exchange, affinity).
5. **Stabilization:** Addition of stabilizers (glycerol, Ca^{2+}).

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8. Enzyme Formulation and Immobilization

- **Drying methods:** Spray drying or freeze drying for storage stability.
- **Immobilization:** Fixing enzymes onto solid supports (e.g., agarose beads, silica) to allow reuse.
- Used in continuous reactors (e.g., glucose isomerase columns).

9. Applications of Microbial Enzymes

Industry	Application	Examples
Food & Beverages	Brewing, baking, juice clarification	Amylase, Pectinase
Textile	Fabric softening, desizing	Cellulase, Amylase
Detergent	Stain removal	Protease, Lipase
Paper & Pulp	Biobleaching	Xylanase, Laccase
Pharmaceutical	Drug modification	Lipase, Protease
Biofuel	Biomass hydrolysis	Cellulase, Amylase