# CHAPTER 30 -- Industrial Microbiology

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30.1 Industrial Microorganisms and Their Products p. 942

• Industrial microbiology: small and large scale production of commercial products, and also to carry out complex chemical transformations. Microbes become biocatalysts Fig. 30.1.

Biocatalysts are very valuable and specific producing strains are stored in “national” collections.
ATTC -American Type Culture Collection
DSMZ – Deutsche Sammlung Von Mikroorganismen und Zellkulturen
AN INDUSTRIAL MICROORGANISM MUST:

1. Produce the product of interest in high yield

2. Grow rapidly on inexpensive culture media available in bulk quantity (corn steep liquor, whey [NON-animal (Mad Cow Disease – BSE)] – a major proportion of the production cost of commodity chemicals is the substrate.

   **Commodity chemicals** are inexpensive chemicals produced in bulk, including ethanol, citric acid, and many others - DEE antibiotics).

3. Be amenable to genetic manipulation – mutation, genetically engineered - yet stable

4. Be non-pathogenic

30.2 Primary and Secondary Metabolites p. 943

- **Primary metabolites** are produced during active cell growth (Fig. 30.2). [lag, exponential, stationary]
- **Secondary metabolites** (p.943) are produced near the onset of stationary phase
• Primary metabolites
  - Ethanol
  - Amino-acids

• Secondary metabolites
  - Antibiotics
  - “Statins” cholesterol lowering agents

*Secondary metabolites:
  • not essential for growth
  • dependent on growth conditions (repression)
  • over-production often achievable
    (not growth related)
  • often produced as a series of closely related compounds

• Inter-relationship between the primary metabolism (aromatic amino acid synthesis and 2ndry metabolism (antibiotics - Fig. 30.3)
30.3 Characteristics of Large-Scale Fermentations, p. 945

- **Fermentation** (generic term) is carried out in a **Fermentor** (a vat) by a **Fermenter** (microbe).
- Scale – laboratory 5 – 10 l -- Production up to 500,000 l

<table>
<thead>
<tr>
<th>Size of fermentor (liters)</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–20,000</td>
<td>Diagnostic enzymes, substances for molecular biology</td>
</tr>
<tr>
<td>40–80,000</td>
<td>Some enzymes, antibiotics</td>
</tr>
<tr>
<td>100–150,000</td>
<td>Penicillin, aminoglycoside antibiotics, proteases, amylases, steroid</td>
</tr>
<tr>
<td></td>
<td>transformations, amino acids, wine, beer</td>
</tr>
<tr>
<td>200,000–500,000</td>
<td>Amino acids (glutamic acid), wine, beer</td>
</tr>
</tbody>
</table>

- Large-scale industrial **fermentation** presents several engineering problems while the microbial process must be continuously monitored to ensure yield and no contamination.

- **Fermentation scale-up** (p.946) is an art in itself – optimization of laboratory scale to production scale.

- Industrial fermentations will be anaerobic and aerobic processes (Figure 30.4), aeration in the latter being critical, and requires special attention to stirring (impellor)/bubbling (sparger). Both require heat removal systems.

**Ethanol – Kyowa Hakko**

240 m³
At Cook College, New Brunswick Scientific Company originated in part through repair of small shaking apparatus.
Computer control – yields 100g/l dry wt (10g glucose ➔ 5g cells)

II. Products for the Health Industry p. 947
30.5 Antibiotics: Isolation & Characterization

An antibiotic is of microbial origin which inhibits or kills other micro-organisms (at low concentration) Selman Waksman - see p. 688.

Penicillin Alexander Fleming 1928 – Howard Florey & Ernst Chain 1939
(UK see p. 686)
Tyrothricin René Dubos 1939 (@ Rockefeller Institute using NJ bog soil)

Actinomycin Selman Waksman and H. Boyd Woodruff 1940
Streptomycin Selman Waksman, Albert Schatz & Elizabeth Bugie 1944
Neomycin Selman Waksman and Hubert Lechevalier 1949
17 antibiotics including candicidin (antifungal)

The New Jersey Experiment Station is recognized as the original American site of Soil Bacteriology (Amer. Soc. Microbiology) and also the site of Discovery of Actinomycete Antibiotics (Amer. Chem. Soc.)
COMMERCIAL DEVELOPMENT

Is the antibiotic new?
Chemical characterization
Animal trials (sulfonamide discovery)
Increasing yields – strains/physiology (rate limiting steps)
mutation
genetic engineering
Industrial Production of Penicillins & Tetracylines

- Major antibiotics of clinical significance include:
  - β-lactams penicillin (Fig 30.9) & cephalosporin Also the tetracyclines (Fig. 30.11).
- Production Notes: 1. Natural 2. Precursor 3. Enzyme /Chemical
  Semi-synthetic – side chains

Overview

I. Actinomycetes “n” (diverse)
   Fungi - greatest usage
   Bacteria

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Producing microorganism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacitracin</td>
<td><em>Bacillus licheniformis</em> (EFB)</td>
</tr>
<tr>
<td>Cephalosporin</td>
<td><em>Cephalosporium spp.</em> (F)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>Chemical synthesis (formerly produced microbiologically by Streptomyces venezuelae) (A)</td>
</tr>
<tr>
<td>Cycloheximide</td>
<td><em>Streptomyces griseus</em> (A)</td>
</tr>
<tr>
<td>Cycloserine</td>
<td><em>Streptomyces ochraceoaurantios</em> (A)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td><em>Streptomyces erythraeus</em> (A)</td>
</tr>
<tr>
<td>Griseofulvin</td>
<td><em>Penicillium griseofulvum</em> (F)</td>
</tr>
<tr>
<td>Kanamycin</td>
<td><em>Streptomyces kanamyceticus</em> (A)</td>
</tr>
<tr>
<td>Lincomycin</td>
<td><em>Streptomyces lincomycins</em> (A)</td>
</tr>
<tr>
<td>Neomycin</td>
<td><em>Streptomyces fradiae</em> (A)</td>
</tr>
<tr>
<td>Nystatin</td>
<td><em>Streptomyces noursei</em> (A)</td>
</tr>
<tr>
<td>Penicillin</td>
<td><em>Penicillium chrysogenum</em> (F)</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td><em>Bacillus polymyxa</em> (EFB)</td>
</tr>
<tr>
<td>Streptomycin</td>
<td><em>Streptomyces griseus</em> (A)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td><em>Streptomyces rimosus</em> (A)</td>
</tr>
</tbody>
</table>

II. Cephalosporins and others – (low toxicity)
   broad-spectrum antibiotics - attacks a wide variety of bacterial pathogens
   (Gram positive & Gram negative).
30.7 Vitamins p. 953

- Vitamins have major usage as food and feed supplements. Due to their complex nature, two are microbially produced - vitamin \( \text{B}_12 \) and riboflavin (Figure 30.12).

Vitamin \( \text{B}_12 \) is only produced by microbes and we benefit via uptake from our guts. Deficiency leads to pernicious anemia. Production is mainly from Propionibacteria and Pseudomonas. Cobalt is essential and its addition enhance fermentative yields.

Riboflavin (a parent compound of flavins – FAD and FMN co-enzymes. The fungus Ashbya gossypii is a prodigious producer (7g/l), yet the fermentation and chemical synthesis are highly competitive.

Amino Acids

- MSG – monosodium glutamate (glutamic acid) – flavor enhancer
- Lysine – essential nutritional amino acid
- Methionine – essential nutritional amino acid
- Aspartic acid & phenylalanine – non-nutritive sweetener - (artificial ?)

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Annual production worldwide (metric tons)</th>
<th>Uses</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Glutamate (monosodium glutamate, MSG)</td>
<td>370,000</td>
<td>Various foods</td>
<td>Flavor enhancer, meat tenderizer</td>
</tr>
<tr>
<td>( \text{L}-\text{Aspartate and alanine} )</td>
<td>5,000</td>
<td>Fruit juices</td>
<td>“Bowed off” taste</td>
</tr>
<tr>
<td>Glycine</td>
<td>6,000</td>
<td>Sweetened foods</td>
<td>Improves flavor; starting point for organic syntheses</td>
</tr>
<tr>
<td>L-Cysteine</td>
<td>700</td>
<td>Bread</td>
<td>Improves quality</td>
</tr>
<tr>
<td>( \text{L}-\text{Tryptophan } + \text{ L-Histidine} )</td>
<td>400</td>
<td>Fruit juices</td>
<td>Antioxidant</td>
</tr>
<tr>
<td>Aspargose (made from ( \text{L}-\text{phenylalanine } + \text{ L-aspatic acid} )</td>
<td>7,000</td>
<td>Vegetable foods, dried milk</td>
<td>Antioxidant, prevent rancidity, nutritive additives</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>70,000</td>
<td>Soft drinks, chewing gum, many other “sugar-free” products</td>
<td>Low-calorie sweetener</td>
</tr>
<tr>
<td>( \text{L}-\text{Methionone} )</td>
<td>70,000</td>
<td>Bread (dough), feed additives</td>
<td>Nutritive additive</td>
</tr>
</tbody>
</table>


The structures of these amino acids are shown in Figure 3.12.
• High yields of amino acids are obtained by modifying (eliminating?) regulatory signals that control synthesis

► Resultant overproduction occurs.

Lysine – overproduction via de-control of regulation
Over production is controlled by
FEEDBACK REGULATION (Sect. 8.2) “ALLOSTERY”

Lysine feeds back and inhibits aspartokinase
(ALLOSTERIC site cf. aspartate and lysine)
But a mutant aspartokinase insensitive to lysine would give over production

Such mutants can be selected for by resistance to S-aminoethylcysteine - AEC. AEC binds to AK and shuts it down.
Mutants AECᵣ have an AK not subject to feedback inhibition, i.e. allosteric negative

Yields 60g/l
30.8 Steroids & Biotransformation p. 955

- Microbial **biotransformation** employs microorganisms to biocatalyze a specific step or steps in an otherwise strictly chemical synthesis (Figure 30.14).

  In the production of cortisones, *Rhizopus nigricans* (bread mold) is used to perform a stereo-specific hydroxylation (circumvents a several step synthesis) –

  Over 800 tons/year of major corticosteroids are produced.

30.9 Enzymes as Industrial Products p. 955

Microorganisms are ideal for the large-scale production of enzymes (cf. plant and animal enzymes). There is also the ease of production as the enzymes are secreted *(extracellular)* and recovery is from culture broth. Enzymes have diverse uses (Table 30.4). The major production is for use for:

- Starch modification *(amylases)*

- In laundry detergents (stain removal – **proteases**, plus **amylases** and **lipases**: and brighteners – **cellulase** for removal of pilling). The detergent enzymes are selected for their heat stability and activity at high pH (pH 9-10) – esp. *Bacillus.*
Amylases and Glucose Isomerase

- Amylases are used to convert starches to sugars –
  - $\alpha$ Amylases yield short oligomers
  - Glucamylase nibbles off glucose units
Glucose is converted Glucose Isomerase $\rightarrow$ FRUCTOSE
- Fructose is roughly twice as sweet as glucose
- Thus $\alpha$ amylase, glucamylase and glucose isomerase $\rightarrow$
  - HFCS - High Fructose Corn Syrup
    10 billion Kg/yr – soft drinks, bakery

Immobilized Enzymes (Fig. 30.16):

Table 30.4 Microbial enzymes and their applications

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Source</th>
<th>Application</th>
<th>Industry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amylase (starch-digesting)</td>
<td>Fungi</td>
<td>Bread</td>
<td>Baking</td>
</tr>
<tr>
<td></td>
<td>Bacteria</td>
<td>Starch coatings</td>
<td>Paper</td>
</tr>
<tr>
<td></td>
<td>Fungi</td>
<td>Syrup and glucose manufacture</td>
<td>Food</td>
</tr>
<tr>
<td></td>
<td>Bacteria</td>
<td>Cold-swelling, laundry starch</td>
<td>Starch</td>
</tr>
<tr>
<td></td>
<td>Fungi</td>
<td>Digestive aid</td>
<td>Pharmaceutical</td>
</tr>
<tr>
<td></td>
<td>Bacteria</td>
<td>Removal of coatings (desizing)</td>
<td>Textile</td>
</tr>
<tr>
<td>Protease (protein-digesting)</td>
<td>Fungi</td>
<td>Bread</td>
<td>Baking</td>
</tr>
<tr>
<td></td>
<td>Bacteria</td>
<td>Spot removal</td>
<td>Dry cleaning</td>
</tr>
<tr>
<td></td>
<td>Bacteria</td>
<td>Meat tenderizing</td>
<td>Meat</td>
</tr>
<tr>
<td></td>
<td>Bacteria</td>
<td>Wound cleansing</td>
<td>Medicine</td>
</tr>
<tr>
<td></td>
<td>Bacteria</td>
<td>Devizing</td>
<td>Textile</td>
</tr>
<tr>
<td></td>
<td>Bacteria</td>
<td>Household detergent</td>
<td>Laundry</td>
</tr>
<tr>
<td>Invertase (sucrose-digesting)</td>
<td>Yeast</td>
<td>Soft-center candies</td>
<td>Candy</td>
</tr>
<tr>
<td>Glucose oxidase</td>
<td>Fungi</td>
<td>Glucose removal, oxygen removal</td>
<td>Food</td>
</tr>
<tr>
<td>Glucose isomerase</td>
<td>Bacteria</td>
<td>Test paper for diabetes</td>
<td>Pharmaceutical</td>
</tr>
<tr>
<td>Pectinase</td>
<td>Fungi</td>
<td>Pressing, clarification</td>
<td>Soft drink</td>
</tr>
<tr>
<td>Renin</td>
<td>Fungi</td>
<td>Coagulation of milk</td>
<td>Wine, fruit juice</td>
</tr>
<tr>
<td>Cellulase</td>
<td>Bacteria</td>
<td>Fabric softening, brightening; detergent</td>
<td>Cheese</td>
</tr>
<tr>
<td>Lipase</td>
<td>Fungi</td>
<td>Breaks down fat</td>
<td>Laundry</td>
</tr>
<tr>
<td>Lactase</td>
<td>Fungi</td>
<td>Breaks down lactose to glucose and galactose</td>
<td>Dairy, laundry</td>
</tr>
<tr>
<td>DNA polymerase</td>
<td>Bacteria</td>
<td>DNA replication in polymerase chain</td>
<td>Dairy, health foods</td>
</tr>
<tr>
<td></td>
<td>Archaea</td>
<td>DNA reaction (PCR) technique (Fig. 7.9)</td>
<td>Biological research</td>
</tr>
</tbody>
</table>

Table 30.4 Brock Biology of Microorganisms 11/e © 2006 Pearson Prentice Hall, Inc.
The term *extremozyme* has been coined to describe enzymes that function at some environmental extreme, such as high temperatures or low pH (Figure 30.15; Table 2.1). Enzymes from extremophiles are desirable for biocatalyses under extreme conditions.

III 30.10 Alcohol and Alcoholic Beverages  

p. 958

• Alcoholic beverages are produced by yeast from the fermentation of sugar to ethyl alcohol and CO₂.

• Wine is produced from grape juice (Figure 30.18)

• Beer from fermentation of malted grain (*brewing*)

• Distilled beverages from the distillation of fermented solutions.

• Commodity alcohol is used as a gasoline additive (*gasohol*) and an industrial solvent.
30.11 Vinegar Production p. 963

Vinegar is acetic acid which is produced by acetic acid bacteria (Gluconobacter and Acetobacter) oxidizing an alcohol-containing fruit juice. Distilled vinegar comes from use of pure ethanol and refers to its prior distillation.

Although a strict aerobe, Gluconobacter oxidizes ethanol partially - only to acetic acid and NOT to carbon dioxide and water (Fig. 30.22).

3 Processes:

1. **Open vat (Orleans)** – shallow vats / slime/ lots of air

2. **Trickle Generator**: beechwood shavings covered by a trickle of alcoholic substrate, with air entry at the base (Fig. 30.23). Recycle the product until 4% acetic acid is obtained.

3. **Bubble method**: Large scale fermentor with massive air input (and heat removal). Efficiency 90-98% conversion.
Itaconic, gluconic, lactic acids

**CITRIC ACID** – food, beverages, leavening of bread, metal treatment, as a detergent.

Over 550,000 tons per year (approx. $1 billion).

**Probably the earliest real fermentation**

**Production is using Aspergillus niger** (Figure 30.24).

*Production is very sensitive to iron – iron has to be scavenged from the medium.*

*Even glass-lined fermentors are used.*

Deep tank (much aeration), plus starch hydrolysates, molasses, sugarcane syrup.

*Ppt as Ca Citrate. Plus sulfuric acid*  
  + calcium sulfate (remove).  
  *Citric acid then crystallizes.*
Commercial yeast, *Saccharomyces cerevisiae*, is produced in large-scale aerated fermentors using molasses with controlled continuous feeding of the sugar substrate.

(Figure 30.25).

**Industrial uses of yeast and yeast products**

<table>
<thead>
<tr>
<th>Production of yeast cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baker’s yeast, for bread making</td>
</tr>
<tr>
<td>Dried food yeast, for food supplements</td>
</tr>
<tr>
<td>Dried feed yeast, for animal feeds</td>
</tr>
</tbody>
</table>

**Yeast products**

- Yeast extract, for microbial culture media
- B vitamins, vitamin D
- Enzymes for food industry: invertase (sacchase), galactosidase
- Biochemicals for research: ATP, NAD, RNA

**Fermentation products from yeast**

- Ethanol, for industrial alcohol and as a gasoline extender
- Glycerol
- Beverage alcohol
  - Beer, wine
- Distilled beverages
  - Whiskey, brandy, vodka, rum

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*Microbial Sidebar, The Products of Yeast Fermentation, Chapter 5 and Sections 5.10 and 5.13.*

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**Yeast production**

1. Dewatering — Compressing — Packaging — Cold storage
2. Dewatering — Extruding — Gentle drying — Packaging — Cool storage
3. Pasteurizing — Drum drying — Grinding — Packaging — Dry storage
30.14 Mushrooms as a Food Source p. 966

- Mushrooms are a major food produced with focus on flavor rather than protein *per se*.
- The classic is *Agaricus bisporus*. Mushrooms (fruit bodies) develop from a spreading mycelium in the soil (the fungus that ate “Ohio” – fairy rings).
  - Commercially this development is followed, first by inoculating rich soil with mushroom spawn – pure cultures grown on solid substrate substrates on enriched straw. This bed after a few weeks is cased with a thin layer of soil to induced mushroom formation – mushroom flushes appear and continue perhaps through 3 flushes.

Novel “Mushrooms”

EXOTIC MUSHROOMS:

**Shiitake (Lentinus edulis).** Oriental culture on deciduous logs. Inoculum placed in small holes in the log and then sealed. Logs are stacked up, and after about a year the shiitake fruits – Gene Varney, NJMS.

Variety of species in the super-market via plastic bag culture – inoculum is added to cellulosic wastes plus nitrogenous wastes.

**QUORN** (UK) from a filamentous fungus is promoted as a health food – no cholesterol, low carbohydrate, high protein. It is manipulated (shape and flavor) and sold widely in Britain since 1980s.