1

History of Industrial Microbiology

Industrial microbiology came into existence, primarily, based on a naturally occurring microbiological process called **fermentation**. There are many evidences which clearly shows that ancient man knew fermentation process and practiced it more as an art rather than as a science. Early fermentation process practiced by man included the leavening of bread, retting of flax, preparation of vinegar from wine, production of various alcoholic beverages like beer, wine, mead and the production of various fermented foods and milk. Due to invention of microscope, discovery of microorganisms and understanding of their metabolic processes, lead to clear understanding of the fermentation, which paved the way for the development of Industrial Microbiology.

The history of industrial microbiology can be divided into five phases, which are précised in table 1.1 Phase I up to 1900 Alcohol fermentation period, Phase II 1900-1940 Antibiotic period, Phase III 1940-1964 Single cell protein period, Phase IV 1964-1979 Metabolite production period, and Phase V 1979 onward Biotechnology period.

Table 1.1: The phases in the history of Industrial Microbiology

Phase	Main products	Fermenters	Process control	Culture method	Quality control	Pilot plant facilities	Strain selection
I Period before 1900	Alcohol	Wooden upto 1500 barrels capacity	Use of thermo-meters, hydrometer and heat exchangers	Batch	Prac-tically nil	Nil	Pure yeast culture used at some of the breweries
	Vinegar	Barrels-shallow trays-trickle filters	---	Batch	Practi-cally nil	Nil	Process inoculated with good vinegar

contd...

Phase	Main products	Fermenters	Process control	Culture method	Quality control	Pilot plant facilities	Strain selection
	Bakers yeast, glycerol, citric acid, lactic acid and acetone/ butanol	Steel vessels upto 200 m^3 for acetone / butanol. Air sprayers used for bakers yeast.	pH electrodes with off-line control. Temperature control	Batch and fed-batch systems	Practi-cally nil	Nil	Pure cultures used
II Period bet-ween 1900-1940	Penicillin, streptomycin other antibiotics	Mechanical stirring used in small vessels, mechanically aerated vessels	Sterilizable pH and oxygen electrodes	Batch and fed-batch common	Very impor-tant	Becomes common	Mutations and selection program-me essential
III Period bet-ween 1940-1964	Gibberellins, amino acids, nucleotides, enzymes, transformations	Vessels operated aseptically, true fermentations	Use of control loops which were later compu-terised	Contin-uous culture introduced for brewing and some primary metabolites	Very impor-tant	Becomes common	Mutation and selection program-me essential
IV Period bet-ween 1964-1979	Single cell protein using hydrocarbons and other feed stocks	Pressure cycle and pressure jet vessels developed to overcome gas and heat exchange problems	Use of computer linked control loops	Continuous culture with medium recycle	Very impor-tant	Very impor-tant	Genetic engineer-ing of producer strain attempted
V 1979-onward	Production of heterogenous proteins by microbial and animal cells; Monoclonal antibodies produced by animal cells	Fermenters developed in phase 3 and 4. Animal cell reactors developed	Control and sensors developed in phases 3 and 4	Batch, fed-batch or continuous fermenta-tion developed for animal cell processes	Very impor-tant	Very impor-tant	Introduc-tion of foreign genes into microbial and animal cells. In vitro recombi-nant DNA techniques used in the improve-ment of phase 3 products

1.1 ALCOHOL FERMENTATION PERIOD (BEFORE 1900)

The period before 1900 is marked by the production of primarily alcohol, vinegar and beer, although without the knowledge of biochemical processes involved in it. Though beer, which

represents the phase-I in fermentation process, was produced by ancient Egyptians, large scale brewing in large wooden vats of 1500-barrel capacity was started in the early 1700. An attempt was also made for process control by the use of thermometers and heat exchangers in these early breweries.

In the middle of 18th century, the chemist **Liebig** considered fermentation purely as a chemical process. He believed fermentation as a disintegration process in which molecules present in the starter substance like starch or sugar underwent certain changes resulting in the production of alcohol. Other eminent chemists of this period like **Berzelius** (1779–1848) and **Bertholet** (1827–1907) have also supported this view. **Cagniard Latour, Schwan** and **Kutzilog** while working independently concluded that alcoholic fermentation occurs due to action of yeast which is an unicellular fungus. But, it was **Louis Pasteur** who eventually convinced the scientific world that the fermentation is a biological process. By conducting series of experiments, **Louis Pasteur** conveniently proved that yeast is required for conversion of sugars into alcohol. In 1857, he discovered the association of different organisms other than yeasts in the conversion of sugars into lactic acid. These observations led **Pasteur** to conclude that different kinds of organisms are required for different fermentations.

While working on butyric acid fermentation in 1861, **Pasteur** made another important discovery that the fermentation process can proceed in the absence of oxygen. The rod shaped organisms responsible for butyric acid fermentation, remains active in the absence of oxygen. This organism was later on identified as butyric acid bacterium. This observation subsequently lead to the emergence of a new concept of anaerobic microorganisms and a classification of three organisms broadly into two categories, viz., **aerobic** and **anaerobic** microorganisms.

During this period, wine Industry in France was incurring heavy losses due to soaring of wine. Pasteur was requested by the Government of France to study this problem. After careful study, he reported that the soaring of wine was due to the growth of other unwanted microorganisms, other than yeast, which invaded the wine and changed its chemical and physical properties leading to soaring. He showed that these unwanted organisms could be eliminated from the wine by partially sterilizing the juice from which wine is produced, below the boiling point. This process is now called as **Pasteurization**. Pasteurization kills all the bacteria but does not alter the desirable qualities of juice. This proposition of ***Pasteur*** saved the wine industry of France from heavy losses. Later on ***Pasteur*** has also studied the fermentation of acetic acid and beer. He disproved the concept of chemical basis of fermentation.

During the late 19th century ***Hansen***, working at Carlsberg Brewery, developed methods for production of pure cultures of yeast and techniques for production of starter cultures. Thus, by the end of nineteenth century, the concept of involvement of microorganisms in fermentation process and its control were well established in brewing industry.

1.2 ANTIBIOTIC PERIOD (1900–1940)

Important advances made in the progress of industrial microbiology were the development of techniques for the mass production of bakers yeast and solvent fermentations. However, the growth of yeast cells in alcoholic fermentation was controlled by the addition of Wort periodically in small amounts. This technique is now called as ***fed batch culture*** and is widely used in the fermentation industry specially to avoid conditions of oxygen limitation. The aeration of early yeast cultures was also improved by the introduction of air through sparging tubes.

The other advancement during this period was the development of acetonebutanol fermentation by ***Weisman***, which was considered to be truly aseptic and anaerobic fermentation. The techniques developed for the production of these organic solvents were major advances in fermentation technology, which led to the successful introduction of aseptic aerobic processes, which facilitated in the production of glycerol, citric acid and lactic acid.

Another remarkable milestone in the industrial microbiology was the large-scale production of an antibiotic called penicillin, which was in great demand to save lives of thousands of wounded soldiers of Second World War. The production of penicillin is an aerobic process which is carried out by submerged culture technique under aseptic conditions. The inherent problems of contamination, requirement of large amount of liquid medium, sparging the culture with large volume of sterile air, mixing of highly viscous broth were solved. The technology established for penicillin fermentation paved the way for the development of a wide range of new processes such as production of other antibiotics, vitamins, amino acids, gibberellins, enzymes and steroid transformations.

At about the same time ***Dubos*** at Rockfeller Institute, discovered a series of microbial products which showed antimicrobial properties and hence useful in treating certain human diseases. ***Waksman***, a soil microbiologist, and his associates have discovered many antibiotics produced by species of *Streptomyces*, soil inhabiting, which is now widely used (**table 1.2**).

Table 1.2: List of antibiotics and the year of their discovery

Name of the antibiotic	Name of the discoverer	Year of discovery	Producing organism
Penicillin	Alexander Fleming	1929	Penicillium Chrysogenum
Tyrothricin	–	1939	Bacillus
Griseofulvin	–	1939	Penicillium griseofulvum
Streptomycin	S.A. Waksman *et al.*	1943	Bacillus licheniformis
Bacitracin	Johnson *et al.*	1945	Streptomyces griseus
Chloramphenicol	Ehrlich	1947	St. Venezuelae
Polymyxin	–	1947	Bacillus polymyxa
Chlortetracycline	Duggar	1948	St. aureofacieus
Cephalosporin, C, N, P	Brolzu	1948	Cephalosporium acremonium
Neomycin	Waksman *et al.*	1949	St. fradiae
Oxytetracycline	Finley *et al.*	1950	St. rimosus
Nystatin	–	1950	St. noursei
Erythromycin	Clark	1952	St. erythreus
Novobiocin	–	1955	St. niveus
Kanamycin	–	1957	St. kanamyceticus
Fusidic Acid	–	1960	Furidium calcineurin
Ampicillin	–	1961	Semi synthetic
Cephalothin	–	1962	Semi synthetic
Lincomycin	–	1962	St. lincolensis
Gentamycin	–	1963	Micromonospora purpurea
Carbenicillin	–	1964	Semi synthetic
Cephalexin	–	1967	Semi synthetic
Clindamycin	–	1968	Semi synthetic

1.3 SINGLE CELL PROTEIN PERIOD (1940–1964)

This period is marked by the production of proteinaceous food from the microbial biomass. As the cost of the resultant product was very low there was a need for large-scale production of microbial biomass. This led to the development of largest mechanically stirred fermenters ranging from 80,000 to 1,50,000 liters or even more in diameter, which were to be operated continuously for several days, if they were to be economical. Thus, a new fermentation process called continuous culture fermentation came into existence. The most long-lived continuous culture fermentation was the ICI Pruteen animal feed process employing the culture of *Methylophillus methylotrophus.*

1.4 METABOLITE PRODUCTION PERIOD (1964–1979)

During this period, new microbial processes for the production of amino acids and 5^1- nuclosides as flavour augmenters were developed in Japan. Numerous processes for enzyme production, which were required for industrial, analytical and medical purposes, were perfected. Techniques of immobilization of enzymes and cells were also developed. Commercial production of microbial biopolymers such as Xanthan and dextran, which are used as food additives, had been also started during this period. Other processes that were developed during this period includes the use of microorganisms for tertiary oil recovery.

1.5 BIOTECHNOLOGICAL PERIOD (1980 ONWARDS)

Rapid strides in industrial microbiology have taken place since 1980, primarily because of development of new technique like genetic engineering and hybridoma technique. By genetic engineering it was made possible to *in vitro* genetic manipulations which enabled the expression of human and mammalian genes in microorganisms so thereby facilitating large scale production of human proteins which could be used therapeutically. The first such product is the human insulin used for treating the ever growing disease, diabetes. This was followed by the production of human growth hormone, erythropoietin and myeloid colony stimulating factor (CSFs), which control the production of blood cells by stimulating the proliferation, Erythro-poietin used in the treatment of renal failures, anemia and platelet deficiency associated with cancer, gametocyte colony stimulating factor (GCSF) used in cancer treatment and several growth factors used in wound healing processes. The hybridoma technique, which is employed for the production of monoclonal antibodies which aid in medical diagnosis and therapeutics, is also developed during this period.

Perfection of production of microbial secondary metabolites related fermentation processes and their large-scale production is the other major development of this period. Some of such secondary metabolites released into the market includes:

1. Cyclosporine, an immunoregulant used to control rejection of transplanted organs.
2. Imipenem, a modified carbapenem used as a broad-spectrum antibiotic.
3. Lovastatin, a drug used for reducing blood cholesterol levels.
4. Ivermectin, an antiparasitic drug used to prevent African River Blindness disease.

This brief account of history of development of industrial microbiology justifies the statement of Foster (1949), “Never underestimate the power of microbes”.

REVIEW QUESTIONS

I. Essay Type Questions

1. Trace the history of use of microorganisms in industry.
2. Discuss the role of microorganisms in food industry.
3. Discuss milestones in the development of industrial microbiology.

II. Write short notes on:

(a) Antibiotic era
(b) Alcoholic beverage period
(c) Microbial metabolites era
(d) Biotechnology era
(e) Single cell protein concept
(f) Monoclonal antibody era
(g) Pasteurization
(h) cyclosporin
(i) lovastatin

FURTHER READING

1. Bader, F.G. (1992). Evolution in fermentation facility design from antibiotics to recombinant proteins in Harnessing Biotechnology for the 21st century (eds. Ladisch, M.R. and Bose, A.) American Chemical Society, Washington DC pp. 228–231.
2. Bushell, M.E. (1998). Application of the principles of industrial microbiology to biotechnology (ed. Wiseman, A.) Chapman and Hall, New York pp. 5–43.
3. Rehm, H.J. and Reed, G. (1993), Biotechnology (2nd edition) Vol. 1–12, VCH, Weinheim.

2

Fermentation Process

Fermentation term for the first time was coined by **Louis Pasteur** for a phenomenon of bubbling of sugar solution. Later on, it has been applied for the phenomenon of production of different chemicals involving microorganisms. Presently, the term is used solely to any phenomenon involving microorganisms. Many products are made by large-scale fermentation including amino acids, enzymes, organic acids, vitamins, antibiotics, solvents and fuels. The typical fermentation process is depicted in Fig. 2.1.

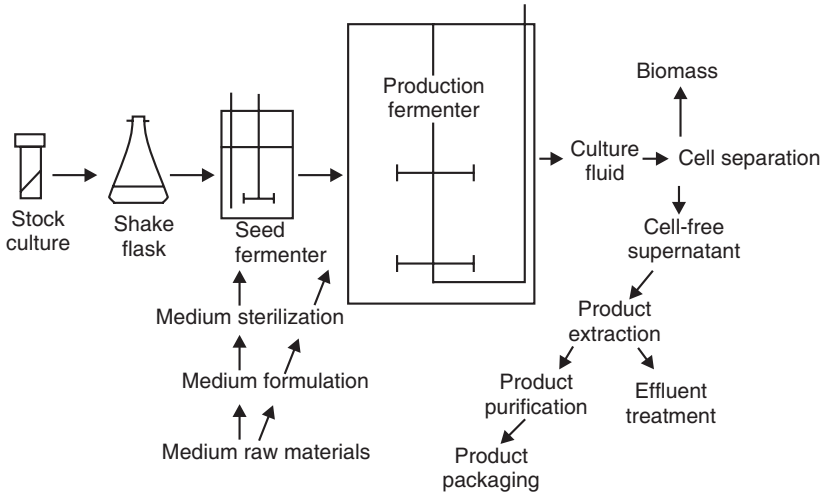

Fig. 2.1: A schematic representation of a typical fermentation process

The advantages in producing materials by fermentation are as follows:

1. Complex molecules such as antibiotics, enzymes and vitamins are impossible to produce chemically.
2. Optically active compounds such as amino acids and organic acids are difficult to prepare chemically.

3. Though some of the products that can be economically derived by chemical processes, but for food purpose they are better produced by fermentation such as beverages, ethanol and vinegar (acetic acid).
4. Fermentation usually uses renewable feed stocks instead of petrochemicals.
5. Reaction conditions are mild, in aqueous media and most reaction steps occur in one vessel.
6. Byproducts of fermentation are usually chemicals. The cell mass and other major by products are highly nutritious and can be used in animal feeds.

However, it is beset with some drawbacks, which are as follows:

1. The products are made in complex solutions in low concentrations as compared to chemically derived compounds.
2. It is difficult and expensive to purify the product.
3. Microbial processes are much slower than chemical processes, increasing the fixed cost of the process.
4. Microbial processes, are subjected to contamination by competiting microorganisms, requires the sterilization of the raw materials and the containment of the process to avoid contamination.
5. Most microorganisms do not tolerate wide variation in temperature, pH and are also sensitive to upsets in the oxygen and nutrient levels. Such upsets not only slow the process, but fatal to microorganism. Thus careful control of pH, nutrients, air and agitation require close monitoring and control.
6. Although nontoxic, waste products have high BOD and requires extensive sewage treatment.

Though microorganism belonging to bacteria, fungi and yeasts are extensively used in these fermentation, few fermentations are also based on algae, plants and animal cells. Several cellular activities contribute to fermentation products such as:

1. **Primary metabolites:** Ethanol, lactic acid and acetic acid.
2. **Energy storage compounds:** Glycerol, polymers and polysaccharides.
3. **Proteins:** SCP, enzymes of both extra and intracellular nature and foreign protein.
4. **Intermediate metabolites:** Amino acids, citric acid, vitamins and malic acid.
5. **Secondary metabolites:** Antibiotics.
6. **Whole cell products:** SCP, bakers yeast, *brewers* yeast, bioinsecticides.

Some of the products such as ethanol, lactic acid and cell mass products are generally growth associated, while secondary metabolites, energy storage compounds, and polymers are non-growth associated. Other products, such as protein depends on the cellular or metabolic function. Unlike primary metabolites which are essential for growth and reproduction, secondary metabolites are not essential for the growth and development of reproducing organism and are produced only in luxuriant conditions (**Bu Lock, 1961**). The secondary metabolites are basically are:

1. Secondary metabolites are produced only by few organisms.
2. Secondary metabolites are needed depending on environmental conditions.
3. Secondary metabolites are produced as a group of closely related structures.
4. Some organisms forms a variety of different classes of substances such as secondary metabolites.
5. The regulation of biosynthesis of secondary metabolites differs significantly from that of primary metabolites.
6. Secondary metabolites are mostly produced in iodophase (Fig. 2.3)

   Origin and production of different secondary metabolites are depicted in Fig. 2.2 and 2.2 a.

Fermentative products are in use by man since ancient times. Fermentation of grains or fruit produce, bread, beer and wine that retained much of the nutrition of raw materials, while keeping the product from spoiling. The natural yeasts that caused fermentation added some vitamins and other nutrients to the bread or beverage. Lactic acid producing bacteria ferment milk to *yogurt* and *cheese* and extend the life of milk products. Other food products such as pickles, vegetables and the fermentation of tea leaves and coffee beans were preserved or enhanced in flavor by fermentation.

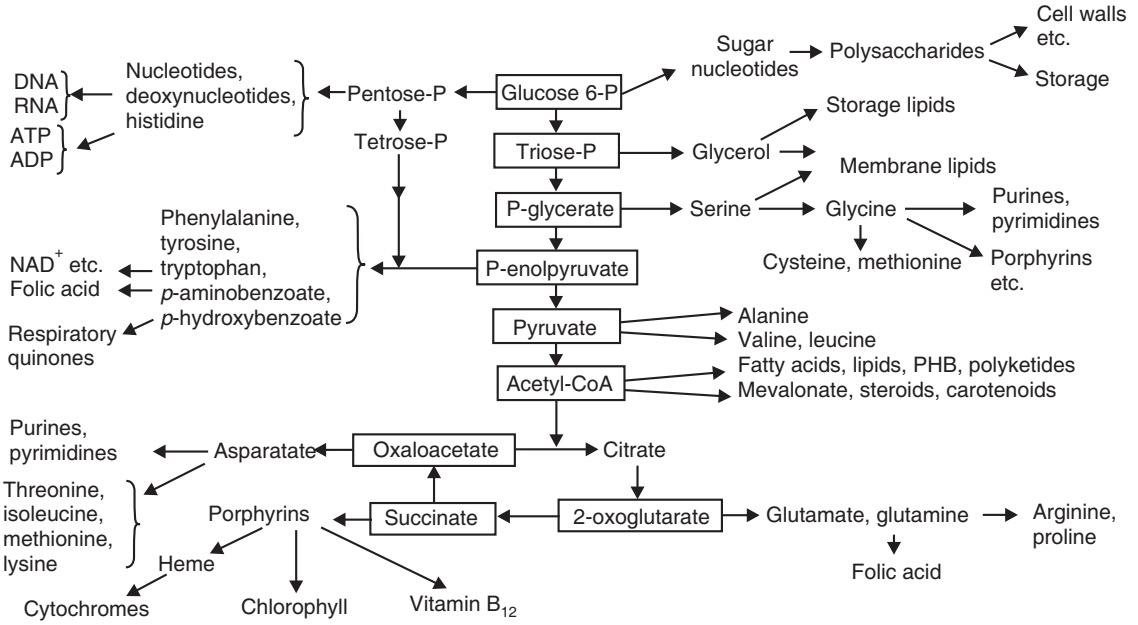

Fig. 2.2: Primary metabolites giving rise to variety of cell substances

Fermentation was an art until the second half of the 19th century. A batch was begun with either a starter, a small portion of previous culture, or with culture residing in the products or vessel. **Pasteur** (1775) made it clear that fermentation needs, heat treatment to improve storage quality and thus formed the basis for sterilization of medium. **Emil Christian Hansen** (1883) used for the first time pure culture of yeast for production of yeast in Denmark. During 1920–30

the emphasis in fermentation shifted to organic acids primarily lactic acid and citric acid. The discovery of penicillin in 1929 and commercialized in 1942, gave a boost to fermentation industry and led to the development of big fermenters and submerged cultivation. Success of penicillin inspired pharmaceutical companies to launch massive efforts to discover and develop many other antibiotics. In 1960s amino acid fermentations were developed in Japan. Commercial production of enzymes for use in industrial process began on a large scale in 1970. The discovery of the tools of genetic engineering expanded the possibilities for products made by fermentation *in situ,* and the first genetically engineered fermentation product was developed and commercialized in 1977. The historical events developed in the progress of fermentations are précised in table 2.1.

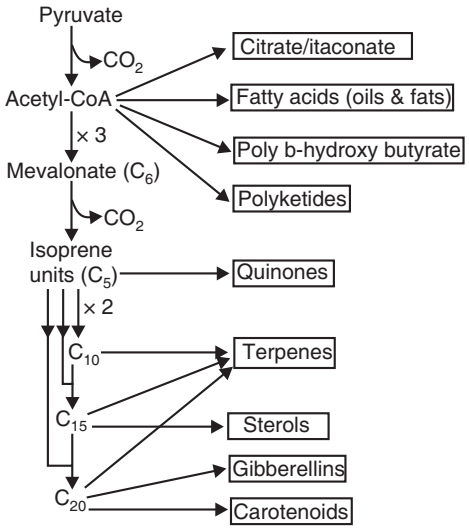

Fig. 2.2(a): Production of secondary metabolites

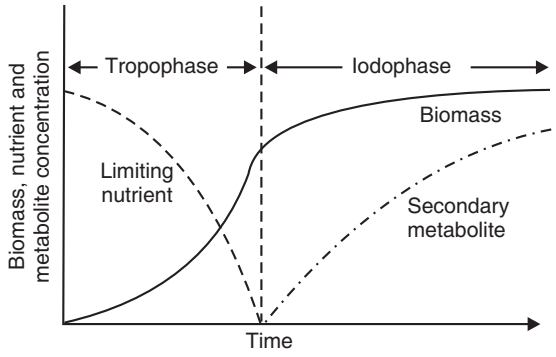

Fig. 2.3: The growth phases of biomass production and secondary metabolite production

Table 2.1: Historical events in the progress of fermentation

Year of commer-cialization	Fine Chemicals	Enzymes	Therapeutic substances	Microbial transformations	Other
1880-1900	Lactic acid	-	-	-	-
1900-1910	Ethanol Glycerol	Amylases	-	-	-
1910-1920	Acetone n-butanol	Invertase	-	-	-
1920-1930	Citric acid	-	-	-	-
1930-1940	Gluconic acid	Proteinases	Riboflavin	Sorbose Acyloions	-
1940-1950	Itaconic acid 2-keto-D-gluconic acid	Cellulases Pectinases	Vitamin B_{12} Penicillin G Other antibiotics	-	-
1950-1960	Kojic acid L-glutamic acid lysine	Oxidase Glucose Catalase	Ampholericin B Erythromycin Semi-synthetic penicillins	Steroid oxidation	Gibberellins Dextran SCP
1960-1970	Valine	Glucose Isomerase Glucamylase Lipases Lactase	Amphomycin Cephalosporins Fusidic acid Linomycin Some more antibiotics	Acetone dihydroxy	Xanthan 5'nucleosides bioinsecticide
1970-1980	Comenic acid	Microbial enzyme Rennet Replacement Melibiase Dextranase	Salinomycin Validanycin A	Sterol cleavage Xylitol, malic acid	Ribose zearalenol

Fermentation may be aerobic if it is operated in the presence of oxygen, while it may be anaerobic if carried out in the absence of oxygen. Anaerobic fermentations can be carried out either by use of fresh medium, covered with an inert gas such as nitrogen or argon or accumulation of CO_2 or foam (Fig. 2.4).

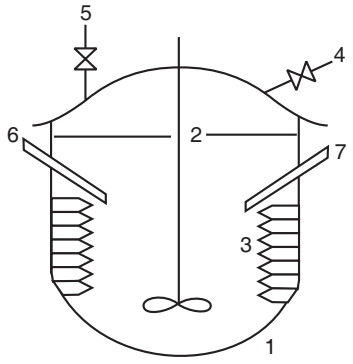

Fig. 2.4: Anaerobic fermenter