Modern era of industrial fermentation technology

Introduction: 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.

Fermentation is a process of chemical change caused by organisms or their products, usually producing effervescence and heat. Fermentation technology is the oldest of all biotechnological processes. The term is derived from the Latin verb *fevere,* to boil--the appearance of fruit extracts or malted grain acted upon by yeast, during the production of alcohol. Industrial fermentation is the intentional use of fermentation by microorganisms such as bacteria and fungi to make products useful to humans. Fermentation process is a biological process and, therefore, has requirements of sterility and use of cellular enzymatic reactions.

History and development of modern fermentation Industry:

Traditional fermentation processes, such as those involved in the production of fermented dairy products and alcoholic beverages, have been performed for thousands of years. However, it is less than 150 years ago that the scientific basis of these processes was first examined. The birth of industrial microbiology largely began with the studies of **Pasteur**. In 1857 he finally demonstrated beyond doubt that alcoholic fermentation in beer and wine production was the result of microbial activity, rather than being a chemical process. During the early part of the 20th century, further progress in this field was relatively slow. Around the turn of the century there had been major advancements in the large-scale treatment of sewage, enabling significant improvement of public health in urban communities. However, the first novel industrial-scale fermentation process to be introduced was the acetone–butanol fermentation, developed by **Weizmann** (1913–15) using the bacterium *Clostridium acetobutylicum*. In the early 1920s an industrial fermentation process was also introduced for the manufacture of citric acid, employing a filamentous fungus (mould), *Aspergillus niger*. Further innovations in fermentation technology were greatly accelerated in the 1940s through efforts to produce the antibiotic penicillin, stimulated by the vital need for this drug during World War II.

The knowledge of media and microbial strain development had a great impact on the successful development of many other fermentation industries. More recent progress includes the ability to produce **monoclonal antibodies** for analytical, diagnostic, therapeutic and purification purposes, pioneered by **Milstein** and **Kohler** in the early 1970s. Since last 20 years because of development of genetic engineering principles, the fermentation industries have altogether entered into a modern era of recombinant DNA technology.

Dear students, we will discuss this topic under these five g=heading mentioned below,

I. Requirements of a fermentation industry

II. Types of fermentation systems

III. Objectives of industrial fermentation

IV. Microbial fermentation in industries: phases and process

V. Important fermentation products

Let us start the discussion with each of the following,

I. REQUIREMENTTS OF A FERMENTATION INDUSTRY:

A. REQUIREMENTS FOR ARTIFICIAL CULTURE

The growth of organisms involves complex energy based processes. The rate of growth of micro-organisms is dependent upon several **culture conditions,** which should provide for the energy required for various chemical reactions. The production of a specific compound requires very precise cultural conditions at a particular growth rate.

The rate of growth of micro-organisms and hence the synthesis of various chemical compounds under artificial culture, requires organism specific chemical compounds as the **growth (nutrient) medium**. The kinds and relative concentrations of the ingredients of the medium, the pH, temperature, purity of the cultured organism, etc., influence microbial growth and hence the production of **biomass** (the total mass of cells or the organism being cultured), and the synthesis of various compounds. The nutrient sources for industrial fermentation are given in Table 1.

B. Fermenters and Bioreactors

A fermenter is the set up to carry out the process of fermentation. The fermenters vary from laboratory experimental models of one or two litres capacity, to industrial models of several hundred litres capacity, which refers to the volume of the main fermenting vessel.

A **bioreactor** differs from a fermenter in that the former is used for the mass culture of plant or animal cells, instead of micro-organisms. The chemical compounds synthesised by these cultured cells, such as therapeutic agents, can be extracted easily from the cell biomass.

Table: 1. **Nutrient sources for Industrial fermentation**

Nutrient sources for industrial fermentation	
Nutrient	**Raw material**
Carbon source	
Glucose	Corn sugar, Starch, Cellulose
Sucrose	Sugarcane, Sugar beet molasses
Lactose	Milk whey
Fats	Vegetable oils
Hydrocarbons	Petroleum fractions
Nitrogen source	
Protein	Soybean meal, Cornsteep liquor, Distillers' solubles
Ammonia	Pure ammonia or ammonium salts
Nitrate	Nitrate salts
Nitrogen	Air
Phosphorous source	Phosphate salts

C. Design Of Industrial Fermentation Process

The fermentation process requires the following:

a) A pure culture of the chosen microorganism, in sufficient quantity and in the correct physiological state

b**)** Sterilised, carefully composed medium for growth of the organism with all necessary raw materials.

c) A seed fermenter, a mini-model of production fermenter to develop an inoculum to initiate the process in the main fermenter.

d) A production fermenter, the functional large model for large scale culturing of desired organism.

e) Equipments for i) drawing the culture medium in steady state, ii) cell separation, iii) collection of cell free supernatant, iv) product purification, and v) effluent treatment.

f). Fermenters/bioreactors are equipped with an aerator to supply oxygen in aerobic processes, a stirrer to keep the concentration of the medium uniform, and a thermostat to regulate temperature, a pH detector and similar control devices.

II. TYPES OF FERMENTATION SYSTEMS:

Industrial fermentation processes may be divided into two main types, with various combinations and modifications. These are batch fermentations and continuous fermentations.

a) <u>Batch fermentations</u>

A tank of fermenter is filled with the prepared mash of raw materials to be fermented. The temperature and pH for microbial fermentation is properly adjusted, and occassionally nutritive supplements are added to the prepared mash. The mash is steam sterilized in a pure culture process. The inoculum of a pure culture is added to the fermenter, from a separate pure culture vessel. Fermentation proceeds, and after the proper time the contents of the fermenter, are taken out for further processing. The fermentor is cleaned and the process is repeated. Thus each fermentation is a discontinuous process divided into batches.

b) Continuous fermentation

Growth of microorganisms during batch fermentation confirms to the characteristic growth curve, with a lag phase followed by a logarithmic phase. This, in turn, is terminated by progressive decrements in the rate of growth until the stationary phase is reached. This is because of limitation of one or more of the essential nutrients. In continuous fermentation, the substrate is added to the fermneter continously at a fixed rate. This maintains the organisms in the logarithmic growth phase. The fermentation products are taken out continuously. The design and arrangements for continuous fermentation are somewhat complex.

III. OBJECTIVES OF INDUSTRIAL FERMENTATION:

A vast range of important products, many of which were formerly manufactured by chemical processes, are now most economically produced by microbial fermentation and biotransformation processes. Microorganisms also provide valuable services. They have proved to be particularly useful because of the ease of their mass cultivation, speed of growth, use of cheap substrate that in many cases are wastes, and the diversity of potential products. In addition, their ability to readily undergo genetic manipulation has opened up almost limitless possibilities for new products and services from the fermentation industries. In general, fermentations are carried on for following Objectives:

- Production of biomass (viable cellular material)
- Production of extracellular metabolites (chemical compounds)
- Production of intracellular components (enzymes and other proteins)
- Transformation of substrate (in which the transformed substrate is itself the product)

IV. MICROBIAL FERMENTATION IN INDUSTRIES: PHASES AND PROCESS

A microbial fermentation can be viewed as a **three-phase system**, involving liquid-solid, gas-solid, and gas-liquid reactions.

- The **liquid phase** contains dissolved nutrients, dissolved substrates and dissolved metabolites.
- The **solid phase** consists of individual cells, pellets, insoluble substrates, or precipitated metabolic products.
- The **gaseous phase** provides a reservoir for oxygen supply and for CO_2 removal.

An overall scheme of a fermentation process can be described as follows:

- Stage 1: inoculum preservation
- Stage 2: inoculum build-up
- Stage 3: fermentor culture

Stage 1: Inoculum Preservation

The objective of preservation is to maintain strains as long as possible without cell division. The optimal method of preservation must be worked out for each strain. The following three techniques are most commonly used:

- Storage at low temperatures (2-6 degrees Celcius)
- Frozen storage (-18, -80 or -196 degrees Celcius)
- Lyophilization

Stage 2: Growth of Inoculum

The preserved culture is initially revived by growth in a erlenmeyer flask on a biological shaker or on a solid medium (if spore formation is needed). In order to obtain sufficient inoculum for small fermentors, a second series of shake cultures is usually made in more flasks. Out from lyophilized strains the growth of inoculum takes around 4-10 days, out from frozen cultures the growth of inoculum takes 4-48 hours for bacteria and 1-7 days for fungi. Finally out of refrigerated cultures the growth of inoculum takes 4-24 hours for bacteria and 1-5 days for fungi.

Stage 3: Fermentor Culture

The nutrient media for production must be optimized not only in the ingredients used but also how the medium is prepared and sterilized, pH value before and after sterilization.

The most important parameters during the fermentation are temperature, Aeration and Stirring.

V. IMPORTANT FERMENTATION PRODUCTS

The overall economics of fermentation processes are influenced by the costs of raw materials and consumables, utilities, labour and maintenance, along with fixed charges, working capital charges, factory overheads and operating outlay. Of several significant fermented products, following products are noteworthy.

Food, beverages, food additives and supplements

A wide range of fermented foods and beverages have been produced throughout recorded history. They continue to be major fermentation products worldwide and are of vast economic importance. Fermented dairy products, for example, result from the activities of lactic acid bacteria in milk, which modify flavour and texture, and increase long-term product stability. Yeasts are exploited in the production of alcoholic beverages, notably beer and wine, due to their ability to ferment sugars, derived from various plant sources, to ethanol. Most processes use strains of one species, *Saccharomyces cerevisiae*, and other strains of this yeast are used as baker's yeast for bread dough production. Several organic acids derived from microbial action are employed in food manufacture and for a wide range of other purposes. The first human use was for acetic acid, as vinegar, produced as a result of the oxidation of alcoholic beverages by acetic acid bacteria by name *Acetobacter aceti*. In chemical industries working on fermentation as basic principle, **citric acid** production is carried on by the filamentous fungus, *Aspergillus niger*, which has become a major industrial fermentation product, as it has numerous food and non-food applications. Also, most of the **amino acids** and **vitamis** used as supplements in human food and animal feed are produced most economically by microorganisms, particularly if high-yielding overproducing strains are developed. In addition, some microorganisms contain high levels of protein with good nutritional characteristics suitable for both human and animal consumption. This so-called '**single-cell protein' (SCP)** can be produced from a wide range of microorganisms such as a blue green algae *Spirulina*.

Chemical and pharmaceutical products

In terms of providing human benefit, antibiotics are probably the most important compounds produced by industrial microorganisms. Most are secondary metabolites synthesized by filamentous fungi and bacteria, particularly the actinomycetes. Well over 4000 antibiotics have now been isolated, but only about 50 are used regularly in antimicrobial chemotherapy. The best known and probably the most medically useful antibiotics are the b-lactams, penicillins and cephalosporins, along with aminoglycosides (e.g. streptomycin) and the tetracyclines. Other important pharmaceutical products derived from microbial fermentation and/or biotransformations are alkaloids, steroids and vaccines. More recently, therapeutic recombinant human proteins such as insulin, interferons and human growth hormone have been produced by a range of microorganisms. This is a rapidly expanding field and many more recombinant therapeutic products are likely to come on to the market over the coming years.

Industrial chemicals and fuels

Industrial feedstock chemicals supplied through fermentation include various alcohols, solvents such as acetone, organic acids, polysaccharides, lipids and raw materials for the production of plastics. Some of these fermentation products also have applications in food manufacture. Currently, methane and ethanol are the main products, although other potential fuels can be generated using microorganisms, including hydrogen, ethane, propane and butanol.

CONCLUSION

Fermentation technology is a very vibrant and fast growing area of biotechnology, absorbing an ever increasing processes and products. With a longer history than any area of biological sciences, fermentation technology has a longer and brighter future, in the service of mankind, covering such important areas as food and medicine.