Industrial Microbiology

4th year Biology

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Introduction to Industrial Microbiology:

Industrial microbiology or microbial biotechnology: encompasses the use of microorganisms (bacteria, yeast, fungi, or actinomycete) in the manufacture of food or industrial products (Vitamins, Nucleic acids, Amino acids, Organic acids, Alcohols, Drugs, Enzymes, Interferons and hormones) . The use of microorganisms for the production of food, either human or animal, is often considered a branch of food microbiology. The microorganisms used in industrial processes may be natural isolates; laboratory selected or genetically engineered organisms.

History of Industrial Microbiology: can be divided into five phases:

I- Alcohol fermentation period (before1900):

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. 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.

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.

Louis Pasteur who eventually convinced the scientific world that the fermentation is a biological process. By conducting series of experiments, conversion sugars into alcohol.

He 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 .1861 working on butyric acid fermentation :The rod shaped organisms responsible for butyric acid fermentation, remains active in the absence of oxygen(anaerobic microorganisms)

was later on identified as butyric acid bacterium .Pasteur saved the wine industry of France from heavy losses by Pasteurization (kills all the bacteria but does not alter the desirable qualities of juice).

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.

II-Antibiotic period (1900–1940):

Important advances made in the progress of Industrial Microbiology were the development of techniques for the mass production of baker's yeast and solvent fermentations , the growth of yeast cells in alcoholic fermentation was controlled by the addition of Wort(*An infusion of malt that is fermented to make beer)* periodically in small amounts (fed batch culture) 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 (aseptic and anaerobic fermentation) which facilitated in the production of glycerol, citric acid and lactic acid. Another remarkable milestone in the industrial microbiology was production of an antibiotic called Penicillin .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. 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): Some antibiotics produced by microorganisms

Antibiotics	Producing microorganism
Penicillin	*Penicillium chrysogenum*
Cephalosporin	*Cephalosporium spp.*
Chloramphenicol	*Streptomyces venezuelae*
Erythromycin	*Streptomyces erythreus*
Neomycin	*Streptomyces fradiae*

III-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 fermentors ranging from 80,000 to 150 000 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

IV- Metabolite production period (1964-1970):

During this period, numerous processes for enzyme production, which were required for industrial, analytical and medical purposes. 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. Other processes that were developed during this period includes the use of microorganisms for tertiary oil recovery

V- Biotechnological period (1980 onward):

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. Erythropoietin 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.

Fermentation:

Industrial microbiology deals with the production of microbial biomass or microbial products by a process called fermentation. Microbiologists use the term fermentation in two different Contexts:

First: fermentation refers to energy-generating processes where organic compounds act as both electron donor and acceptor.

Second: refers to the growth of large quantities of cells under aerobic or anaerobic conditions, within a vessel referred to as a fermentor or bioreactor.

Fermentation process consist of three parameters include:

A- Microbes:

It is necessary first to identify a suitable M.Os that carries out the desired process in the most efficient manner. This M.Os then is used, either in a controlled environment such as a fermentor or in complex systems such as in soils or waters to achieve specific goals. The major sources of microbial cultures for use in industrial microbiology were natural materials such as soil samples, waters and spoiled bread and fruits…..etc.

Microbes thus serve a dual purpose: **Firstly**, they are good agents of disposal of these wastes, and **secondly** the resultant end products of their breakdown are useful commercial products.

Characteristics of using M.O. in industrial microbiology:

1.They possess a broad variety of enzymes making an array of chemical conversions possible.

2.They have relatively high metabolic activity which allows conversions to take place rapidly.

3. They possess a large surface area for quick absorption of nutrients and release of end products.

4. They have a high rate of multiplication.

5. They should be easily cultivated and maintained.

6. They should have genetic stability with infrequent mutation.

7. Safety, non-pathogenic (except when used for the production of vaccine & toxin) & no cause any allergic reaction.

8. Limited or no need for vitamins and additional growth factors.

B- Fermentation media:

Most fermentation requires broth &solid-substrate must satisfy all the nutritional requirements of the microorganism to promote the synthesis of the target product, either cell biomass or a specific metabolite. General media requirements include a carbon source, which in virtually all industrial fermentations provides both energy and carbon units for biosynthesis, and sources of nitrogen, phosphorus and sulphur. Other minor and trace elements must also be supplied, and some microorganisms require added vitamins, such as biotin and riboflavin. Aerobic fermentation are dependent on a continuous input of molecular oxygen, and even some anaerobic fermentations require initial aeration of media .Certain media nutrients or environnemental conditions may affect not only the physiology and biochemistry, but also the morphology of the microorganism.

The main factors that affect the final choice of individual raw materials are as follows:

1. Cost and availability: ideally, materials should be inexpensive, and of consistent quality and year round availability.

2. Ease of handling in solid or liquid forms, along with associated transport and storage costs, e.g. requirement for temperature control.

3. It should not affect the sterilization requirements and any potential denaturation.

4. It should not affect the agitation, aeration and foaming during fermentation and downstream processing stages.

5. It should produce maximal amount of biomass & bioproduct.

6. It should produce a little amount of undesirable products during the process.

7. Overall health and safety implications.

C- Fermentor:

Is an instrument which is used to provide a suitable environment in which an organism can efficiently produce a target product that may be cell biomass, a

metabolite or bioconversion product (Fig.:1).The performance of any fermentor depends on many factors, but the key physical and chemical parameters that must be controlled are agitation rate, oxygen transfer, pH, temperature and foam production. Laboratory fermentations may be performed in bottles or conical flasks which are normally plugged with cotton wool to prevent microbial contamination, but this can lead to evaporation losses and restricted exchange of gases.

Conditions in the fermentor are carefully monitored to regulate cell growth, and all pipe work must be sterile before fermentation begins. This is usually achieved by flushing the whole system with superheated steam before the production begins. Process if frequently aerobic so fermentor has to be well aerated .The aeration will be sufficient to mix many cultures. If the culture is thick or sticky, additional stirring is required by a motor driven paddle called an impeller, while initially the culture may need warming to start of the process – once it has started a cooling system is vital. Cooling is achieved by either a water jacket or cooling coils inside the fermentor.

Industrial fermentations comprise both upstream processing (USP) and downstream processing (DSP) stages (Fig.:2).

USP : involves all factors and processes leading to the fermentation, and consists of three main areas.

a- Producer microorganism: Include obtaining a suitable microorganism, industrial strain improvement to enhance productivity and yield, maintenance of strain purity, preparation of a suitable inoculum and the continuing development of selected strains.

b- Fermentation media: Selection of suitable cost-effective carbon and energy sources, along with other essential nutrient.

c- Fermentation process: For optimal production of metabolites.

DSP: encompasses all processes following the fermentation, include recovery of products (its concentration & purification) & effluent waste.

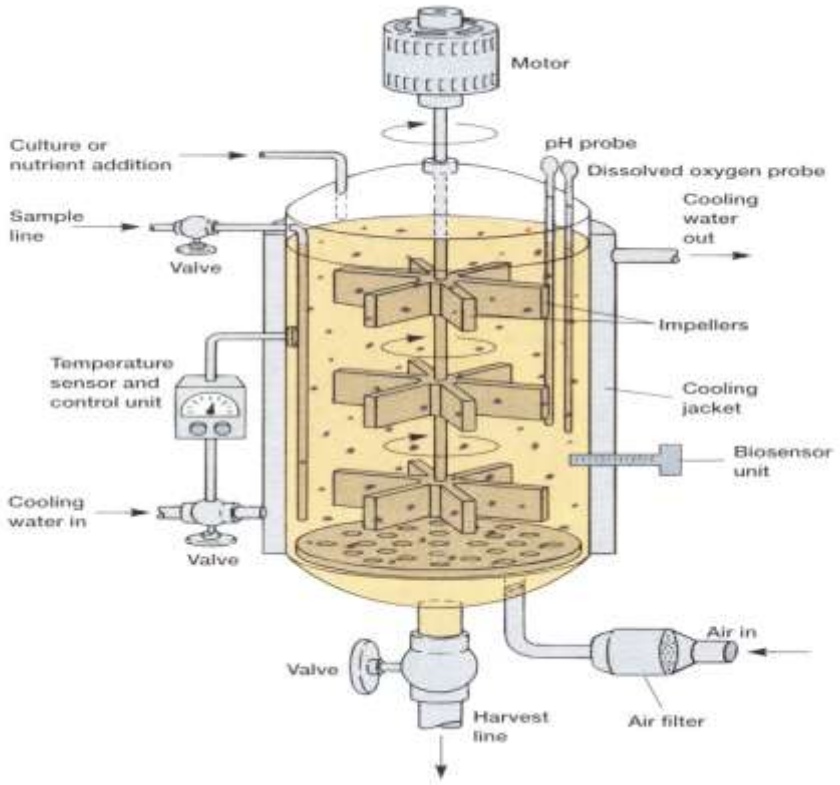

Fig.1: Details of a fermentor unit

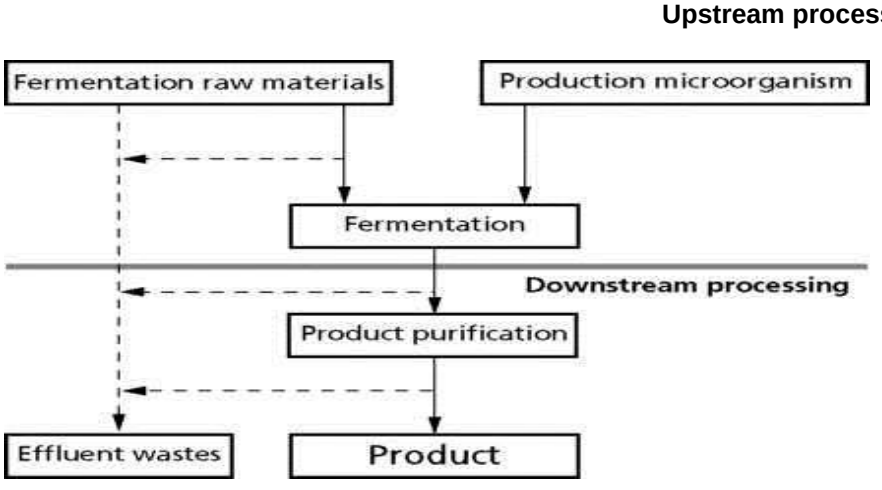

Fig.2: Outline of a fermentation process