Study material for DSE II (Industrial Microbiology)
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INDUSTRIAL MICROBIOLOGY

1.0 Scope of Industrial Microbiology

Industrial Microorganisms and Their Products

The major organisms used in industrial microbiology are fungi (yeasts and molds) and certain prokaryotes, in particular species of the genus Streptomyces. Industrial microorganisms can be thought of as metabolic specialists, capable of synthesizing one or more products in high yield. Industrial microbiologists often use classical genetic methods to select high-yielding microbial variants, with the goal being to increase the yield of the product to the point that an economically feasible process is possible. Thus the behaviour of the actual production strain may be far removed from that of the original wild-type strain.

The use of microbes to obtain a product or service of economic value constitutes industrial microbiology. *Any process mediated by or involving microorganisms in which a product of economic value is obtained is called fermentation*. The terms industrial microbiology and fermentation are virtually synonymous in their scope, objectives and activities. The microbial product may be

- microbial cells (living or dead), microbial biomass, and components of microbial cells,
- microbial metabolites,
- intracellular or extracellular enzymes
- chemicals produced by the microbes utilizing the medium constituents or the provided substrate
- modified compound that has been microbiologically transformed
- Recombinant products through the DNA recombinant technology.

The services generated by microorganisms range from the

- degradation of organic wastes, detoxification of industrial wastes and toxic compounds,
- the degradation of petroleum to manage oil spills, etc.
- Industrial microbiology also encompasses activities like production of biocontrol agents, inoculants used as biofertilizers, biofuel: etc.

The activities in industrial microbiology begin with the isolation of microorganisms from nature, their screening for product formation, improvement of product yields, maintenance of cultures, mass culture using bioreactors, and usually end with the recovery of products and their purification.

Properties of a Useful Industrial Microorganism A microorganism used in an industrial process must have other features besides just being able to produce the substance of interest in high yield.

1. First and foremost, the organism must be capable of growth and product formation in large-scale culture.

2. It should produce spores (if fungi or yeast) or some other reproductive cell form so that it can be easily inoculated into the large vessels used to grow the producing organism on an industrial scale.

3. It must also grow rapidly and produce the desired product in a relatively short period of time.

4. It must also be able to grow in a liquid culture medium obtainable in bulk quantities at a low price. Many industrial microbiological processes use waste carbon from other industries as major or supplemental ingredients for large-scale culture media. These include corn steep liquor (a product of the corn wet-milling industry that is rich in nitrogen and growth factors) and whey (a waste liquid of the dairy industry containing lactose and minerals).

5. An industrial microorganism should not be pathogenic, especially to humans or economically important animals or plants. Because of the high cell densities in industrial microbial processes and the virtual impossibility of avoiding contamination of the environment outside the growth vessel, a pathogen would present potentially disastrous problems.

6. Finally, an industrial microorganism should be amenable to genetic manipulation because increased yields are often obtained by means of mutation and classical genetic selection techniques. A genetically stable and easily engineered microorganism is thus a clear advantage for an industrial process.

Problems often associated with Industrial Microbial Processes

1. Finding the ***least expensive medium*** in which to grow the microbe so as to **maximize** yield and profits.

Often this is a **waste product** from another industrial process, such as corn steep liquor, sugar processing wastes or whey.

2. Maintaining strain purity and developing better strains for **improving the yield**.

A single mutation may decrease the yield by a significant percentage or result in undesirable substances being produced. The industrial research laboratories constantly seek better strains for the production of their product.

3. Preventing contamination by other microbes and by viruses (phage) that live on the microbe involved.

The media must be sterilized prior to being inoculated with the desired organism and purity must be maintained throughout the production process. A small quantity of a contaminant may produce an enzyme that can destroy the product in thousands of gallons of medium. For many microbes, viruses present a constant danger as a single virus can infect and destroy the desired microbe in an entire tank. The sterilization of large containers and huge quantities of media represent both an engineering and microbial challenge.

4. Developing rapid and efficient methods for purification of the desired produce in a stable form that is safe to use.

The products of many fermentations are often unstable in the IMPURE FORM or subject to unwanted modifications if they are not purified quickly. The final growth mixture may contain dangerous substances from which the desired product must be separated. As every step in the purification results in a loss of the product, the search for more efficient purification procedures is never ending.

5. Always striving to improve yield by modifying the strain, nutrients or environmental conditions.

As product yields are **sensitive** to subtle modifications in the nutrient and the environmental conditions, these are constantly monitored. For example, the pH, oxygen content, nitrogen/phosphorous ratio etc. may be adjusted during the production process.

6. Safe and inexpensive disposal of the massive quantities of **waste products** remaining after the product is formed. The waste products of these large fermentations present
major waste disposal problems as they are rich in organic matter that are highly polluting if released untreated into the environment. However, the cost of treatment cuts into the profit margin and increases the cost of the product.

Microbes History

- Microbes have been employed for product generation, e.g., wines, bread, etc., since thousands of years, but these activities were purely art.
- The science of industrial microbiology is only about 150 years old.
- The first observations of microorganisms by Anthony Leeuwenhoek were published in 1677.
- The experiments of Spallanzani in 1799 and of Schwan in 1837 not only disproved the idea of spontaneous generation of microorganisms, but also provided a means of sterilization of liquids (by heat) and air (by heat), respectively.
- Schwan's findings also suggested that alcoholic fermentation was due to a fungus or mold, i.e., yeast, and inoculation resulted in quicker fermentation.
- But microbiology is widely considered to have begun in 1857 when Luis Pasteur reported his studies on lactic acid fermentation, including the microscopic features of the microorganisms and a suitable medium for the process; the scientific basis of industrial microbiology began with this paper.
- In 1860, Pasteur reported the first synthetic medium for microorganisms, and used it to study alcohol fermentation.
- In 1861, Luis Pasteur showed that growth and physiology of yeast (and hence the accumulation of fermentation product, alcohol) differs depending on the presence or absence of CO2. This phenomenon is known as Pasteur effect and is applicable to other microorganisms as well.
- In 1878, Lister described the dilution technique for obtaining the first pure microbial culture of lactic acid bacterium.
- A simpler and more effective technique for obtaining pure cultures from isolated separate colonies developed on solidified medium was described by Robert Koch in 1881; this technique is widely followed even today
- In 1876, Cohn showed that bacterial spores have a high level of heat resistance and developed the technique of 'intermittent sterilization' for their inactivation.

In 1897, Buchner demonstrated alcohol fermentation by cell-free yeast juice; he suggested that a proteinaceous enzyme was responsible for fermentation.

- Wildiers demonstrated in 1901 that yeast required growth factors (vitamins) for growth, especially at low inoculum levels; vitamins are used in fermentation even today.
- In 1929, Alexander Fleming accidentally discovered penicillin produced by *Penicillium* growing as contaminant in a Petri plate of *Staphylococcus*. Fleming developed the technique for assay of antibacterial activity of penicillin using bacteria and showed its low toxicity to man and animals. This was followed by an intensive search for antibiotics during the Second World War leading to the discovery of streptomycin, chloramphenicol, tetracyclines, etc. Later developments have resulted in the use of metabolically blocked mutants of microorganisms, which accumulate large amounts of metabolic intermediates.

2.0. Strain selection and development

Isolation of Microorganisms

The first step in developing a producer strain is the isolation of concerned microorganisms from their natural habitats. Alternatively, microorganisms can be obtained as pure cultures from organisation, which maintain culture collections, e.g., **American Type Culture Collection** (**ATCC**) Rockville, Maryland, U.S.A.; **Commonwealth Mycological Institute** (CMI), Kew, Surrey, England; **Fermentation Research Institute** (FERM), Tokyo, Japan; U.S.S.R. **Research Institute for Antibiotics** (RIA), Moscow, U.S.S.R., etc.

The *microorganisms of industrial importance* are, generally, bacteria, actinomycetes, fungi and algae. These organisms occur virtually everywhere, e.g., in air, water, soil, surfaces of plants and animals, and plant and animals tissues. But most common sources of industrial microorganisms are soils, lake and river mud. Often the ecological habitat from which a desired microorganism is more likely to be isolated will depend on the characteristics of the product desired from it, and of process development. For example, if the objective is to isolate a source of enzymes, which can withstand high temperatures, the obvious place to look will be hot water springs.

Screening of Microorganisms for New Products

The next step after isolation of microorganisms is their screening.

A set of highly selective procedures, which allows the detection and isolation of microorganisms producing the desired metabolite, constitutes primary screeningRapid and effective screening techniques have been devised for a variety of microbial products, which utilize either a property of the product or that of its biosynthetic pathway for detection of desirable isolates.

Inoculum Development

- The preparation of a population of microorganisms from a dormant stock culture to an active state of growth that is suitable for inoculation in the final production stage is called inoculum development. As a first step in inoculum development, inoculum is taken from a working stock culture to initiate growth in a suitable liquid medium. Bacterial vegetative cells and spores are suspended, usually, in sterile tap water, which is then added to the broth. In case of non-sporulating fungi and actinomycetes the hyphae are fragmented and then transferred to the broth. Inoculum development is generally done using flask cultures; flasks of 50 ml to 12 litres may be used and their number can be increased as per need.

3.0 Media Formulation and Economics

Culture Media

Inoculum preparation media are quite different from production media. These media are designed for rapid microbial growth, and little or no product accumulation will normally occur. Many production processes depend on inducible enzymes. In all such cases, the appropriate inducers must be included either in all the stages or at least in the final stages of inoculum development. This will ensure the presence of the concerned inducible enzymes at high levels for the production to start immediately after inoculation.

Contamination

- The inoculum used for production tanks must be contamination free. But the risk of contamination is always present during inoculum development. Therefore, every effort must be made to detect as well as prevent contamination.

Sterilization

- Sterilization is the process of inactivating or removing all living organisms from a substance or surface. In concept, it is regarded as absolute in all living cells must be inactivated / removed, usually in a single step at the given time. But in practice, the success of sterilization procedures is only a probability. Therefore, the probability of a cell escaping inactivation/filtration does exist although it is usually very small. When a closed system is sterilized once, it remains so indefinitely since it has no openings for the entry of microorganisms. But most fermentation vessels are open systems; such systems are initially sterilized and must be kept sterilized by ensuring the removal of living cells at their entry points, e.g., the cotton plug of a culture flask.

Common Contaminants

- The most common contaminants of different industrial processes are considerably different. Some examples are given below

1. In canning industry, *Clostridium butylicum* is the chief concern. This obligate anaerobe can grow in sealed cans, and produce heat resistant spores and a deadly toxin. However, it is not a problem for catsup (too acidic), jam and jellies (too high sugar concentration) and milk (stored at low temperature).
2. Organisms like lactobacillus are a problem in production of wine.
3. In antibiotic industry, potential contaminants are many, e.g., molds, yeast, and many bacteria, including *Bacillus*

Sterilization Procedures

- Sterilization involves either inactivation or removal of living organisms. This may be achieved by

(i) heating,
(ii) irradiation.
(iii) Chemicals or
(iv) filtration; these are briefly discussed below.

Heating.

- It is the most commonly used and the least expensive sterilizing agent.
- Dry heat is used in ovens and is suitable for sterilization of solids, which can withstand the high temperatures needed for sterilization, e.g., laboratory glassware, talc, etc.

Steam, i.e., moist or wet heat, is used for sterilization of media and fermenter vessels. An autoclave uses steam for sterilization (at 121°C and 15p.s.i.). the period of time at this temperature pressure depending on medium volume, e.g., 12-15 min for 200 ml. 17-22 min for 500 ml, 20-25 min for 1 L and 30-35 min for 2 L.

- Bacterial spores are the most heat resistant, e.g., spores of thermophilic bacteria can survive steam at 30p.s.i. at 134°C for 1-10 min and dry heat at 180°C for up to 15 min.

Radiation.

High energy X-rays are used for sterilization of a variety of lab ware and of food. In general, vegetative cells are much more susceptible than bacterial spores (Clostridium spores can resist nearly 0.5 M rad). But *Deinococcus radiodurans* vegetative cells can survive 6 M rad. Viruses areusually similar to bacterial spores but some viruses, e.g., encephalitis virus require up to 4.5 M rad. In practice, 2.5 M rad is used for sterilizing pharmaceutical and medical products. X-rays cause inactivation by inducing single and double strand DNA breaks, and by producing free radicals and peroxides, to which -SH enzymes are particularly susceptible.

Chemicals

. The chemicals used for sterilization cause inactivation by oxidation or alkylation; these are formaldehyde, H2O2, ethylene oxide, propylene oxide etc. H2O2 (10-25% w/v) is being increasingly used in the sterilization of milk and of containers for food products. It is a powerful oxidizing agent, kills both vegetative cells and spores and is very safe. Ethylene oxide is used for sterilizing equipment, which are likely to be damaged by heat, and is very effective, but highly toxic and violently explosive if mixed with air.

Filtration.

Aerobic fermentation requires a very high rate of air supply often amounting to 1 vol of air (equal to medium volume) every minute. Air contains both fungal spores and bacteria, which are ordinarily removed by filtration using either a depth filter or a screen filter. Depth filters are made from fibrous or powdered materials pressed or bonded together in a relatively thick layer; the materials used are fiberglass, cotton, mineral wool, cellulose fibers, etc. in form of mats, wads or cylinders. Modern depth filters are cylinders of bonded borosilicate microfibers. Depth filters allow higher filtration rates and efficiencies than screen filters, but are not suitable for filtration of moist air. Screen filters are membranes of cellulose esters or other polymers with pores of 0.45 μm or smaller (bacterial contaminants are 0.5 μm or larger). Usually, a microfibers profiler is used with such filters to remove gross contamination. All filters themselves must be sterilized before they can be used to sterilize the air. Filters are also used to sterilize the effluent gases from fermenters, especially in case of pathogenic microorganisms.

4.0 Strain Selection and Development

Strain Improvement

- After an organism producing a valuable product is identified, it becomes necessary to increase the product yield from fermentation to minimise production costs. Product yields can be increased by

(i) developing a suitable medium for fermentation,
(ii) refining the fermentation process and
(iii) improving the productivity of the strain

Generally, major improvements arise from the last approach; therefore, all fermentation enterprises place a considerable emphasis on this activity. The techniques and approaches used to genetically modify strains, to increase the production of the desired product are called strain improvement or strain development. Strain improvement is based on the following three approaches: (i) mutant selection, (ii) recombination, and (iii) recombinant DNA technology.