Introduction to Microbial Technology

- Traditional fermentation processes, such as those involved in the production of fermented dairy products and alcoholic beverages, have been performed for thousands of years.

-Pasteur also noted that certain organisms could spoil beer and wine, and that some fermentations were aerobic, whereas others were anaerobic.

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 advance-ments 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 Weiz- mann (1913-15) using the bacterium *Clostridium acetobutylicum*. In the early 1920s an industrial fermenta-tion 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 .

However, many of the greatest advances have followed the massive develop-ments in genetic engineering (recombinant DNA tech-nology) over the last 20 years. This technology has had, and will continue to have, a tremendous influence on traditional, established and novel fermentation process-es and products. It allows genes to be transferred from one organism to another and allows new approaches to strain improvement.

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 substrates 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.

Successful development of a fermentation process involves both upstream processing (USP) and downstream processing (DSP) stages (Fig. i)

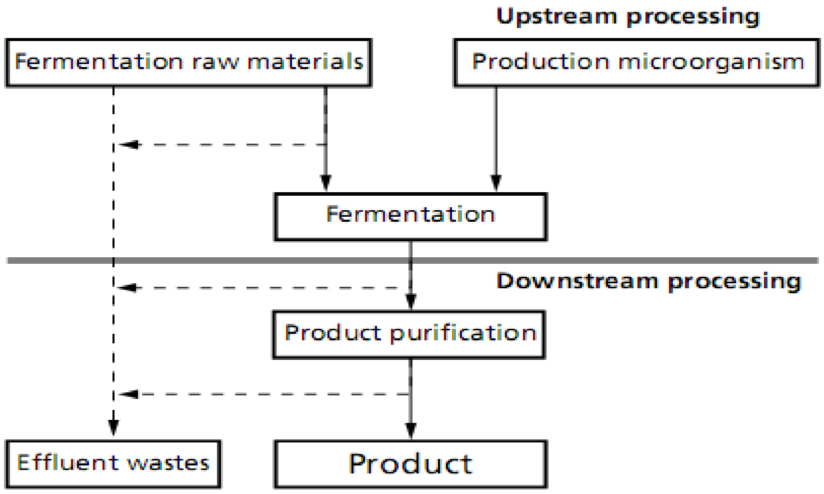


Fig. i Outline of a fermentation process.

The USP is associated with all factors and processes leading to and including the fermentation, and consists of three main areas.

1. The producer microorganism. Key factors relating to this aspect are: the strategy for initially obtaining a suitable industrial microorganism, strain improvement to enhance productivity and yield, maintenance of strain purity, preparation of a reliable inoculum and the continuing development of selected strains to improve the economic efficiency of the process. For example, the production of stable mutant strains that vastly overproduce the target compound is often essential.

Some microbial products are primary metabolites, produced during active growth (the trophophase), which include amino acids, organic acids, vitamins and industrial solvents such as alcohols and acetone .

However, many of the most important industrial products are secondary metabolites, which are not essential for growth, e.g. alkaloids and antibiotics. These compounds are produced in the stationary phase of a batch culture, after microbial biomass production has peaked (the idiophase)

2. The fermentation medium.

The selection of suitable cost-effective carbon and energy sources, and other essential nutrients, along with overall media optimization are vital aspects of process development to ensure maximization of yield and profit. In many instances, the basis of industrial media are waste products from other industrial processes, notably sugar processing wastes, lignocellulosic wastes, cheese whey and corn steep liquor.

3. The fermentation.

Industrial microorganisms are normally cultivated under rigorously controlled condi-tions developed to optimize the growth of the organism or production of a target microbial product. The synthesis of microbial metabolites is usually tightly regulated by the microbial cell. Consequently, in order to obtain high yields, the environmental conditions that trigger regulatory mechanisms, particularly repression and feedback inhibition, must be avoided.

Conventional DSP includes all unit processes that follow fermentation .

They involve:

1. Cell harvesting; cell disruption; product purification from cell extracts or the growth medium; and finishing steps .

2. Overall, DSP must employ rapid and efficient methods for the purification of the product, while maintaining it in a stable form. This is especially important where products are unstable in the impure form or subject to undesirable modifications if not purified rapidly. For some products, especially enzymes, retention of their biological activity is vital. Finally, there must be safe and inexpensive disposal of all waste products generated during the process.

3. 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 .

-Fermentation products can be broadly divided into two categories:

High volume, low value products or

Low volume; high value products.

Examples of the first category include most food and beverage fermentation products, whereas many fine chemicals and pharmaceuticals are in the latter category.

Food, beverages, food additives and supplements

- Fermented dairy products, for example, result from the activities of lactic acid bacteria in milk, which modify flavor 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, *S. 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 .
- A further aerobic fermentation involves citric acid production by the filamentous fungus, *A. niger*, which has become a major industrial fermentation product, as it has numerous food and nonfood applications .
- Also, most of the amino acids and vitamins used as supplements in human food and animal feed are produced most economically by microorganisms, particularly if high yielding over-producing 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 cultivated on low-cost carbon sources.

2. Health-care 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, penicillin and cephalosporin's, along with amino glycosides (e.g. streptomycin) and the tetracycline .

- Other important pharmaceutical products derived from microbial fermentation and/or biotransformation is 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.

3. Microbial enzymes

- Microbial enzymes, particularly extracellular hydrolytic enzymes, have numerous roles as process aids or in the production of a wide range of specific food and nonfood products. Proteases, for instance, are extensively used as additives to washing powders, in the removal of protein hazes from beer and as microbial rennet's for the production of cheese .

- All of these examples involve the use of 'bulk' enzymes. Smaller quantities of highly purified 'fine' enzymes are used for numerous specialized purposes.

- Immobilization of enzymes or whole cells, by their attachment to inert polymeric supports, allows easier recovery and reuse of the biocatalyst, and some enzymes are much more stable in this form. Also, the product does not become contaminated with the enzyme. Applications of immobilized biological catalysts include the production of amino acids, organic acids and sugar syrups.

4. 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 .

Fossil fuels, especially oil, are likely to become exhausted within the next 50-100 years, resulting in the need to develop alternative sources of energy. Biological fuel generation may make an increasing contribution, particularly in the conversion of renewable plant biomass to liquid and gaseous fuels. This plant biomass can be in the form of cultivated energy crops, natural vegetation, and agricultural, industrial and domestic organic wastes. Currently, methane and ethanol are the main products, although other potential fuels can be generated using microorganisms, including hydrogen, ethane, propane and butanol.

Environmental roles of microorganisms

Microorganisms are particularly important in wastewater treatment, which utilizes the metabolic activities of diverse mixed microbial populations capable of degrading any compound that may be presented to them .

The two main objectives are:

1. To destroy all pathogenic microbes present in the sewage, particularly the causal organisms of the waterborne diseases cholera, dysentery and typhoid .

2. The second objective is to break down the organic matter in wastewater to mostly methane and carbon dioxide, thereby producing a final effluent (outflow) that can be safely discharged into the environment. Microbial activities can also be employed in the degradation of manmade xenobiotic compounds within waste streams and in the bioremediation of environments contaminated by these materials.

Microbial cell structure and function

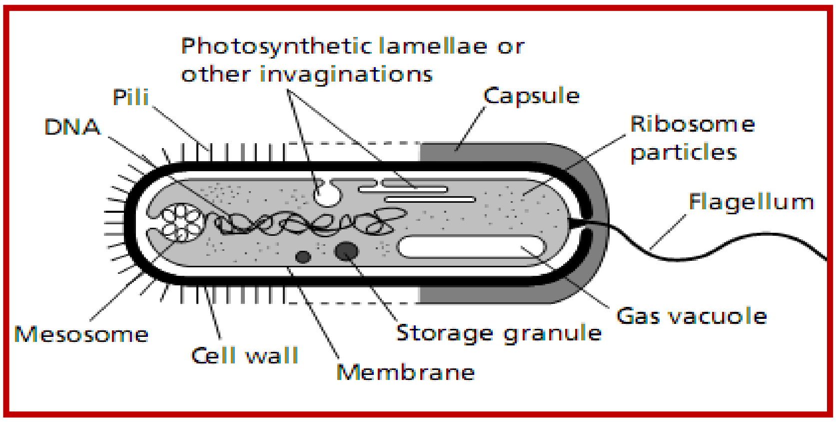
Cells are divided into two categories :

1. Those of archaeon's and eubacteria are prokaryotic ,

2. Whereas the cells of fungi, protozoa, algae and other plants, and animals are eukaryotic.

Prokaryotic cells are normally less than 5 μm in diameter.

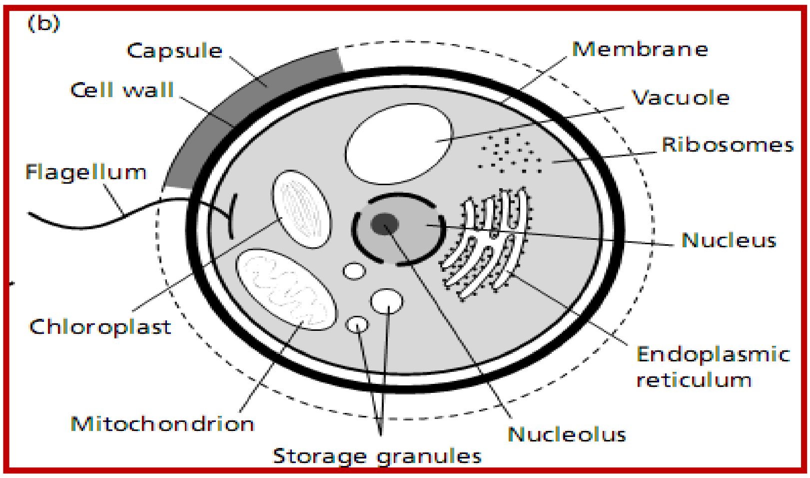
Prokaryotes rarely possess membrane- bound organelles and have little recognizable internal ultra-structure, apart from inclusion bodies (granules of organic or inorganic compounds), various vacuoles (Fig. 1.1a).



- Most prokaryotic cells contain a single chromosome composed of deoxyribonucleic acid (DNA), which is located in a region of the cell referred to as the nucleoid. The chromosome is usually circular, although in some prokaryotes it is linear. Prokaryotic ribosomes are 70S (Svedberg units), which refers to their rate of sedimentation on centrifugation and is a measure of their size, although density and shape of the particle can also influence this value .
- Almost all prokaryotes have cell walls or cell envelopes located outside the cytoplasmic membrane, which usually contain some peptidoglycan. Outside this wall they may have capsules or slime coats .
- Cell division in prokaryotes is normally by simple binary fission.

Eukaryotic cells are generally:

- Larger than those of prokaryotes and contain a range of membrane bound organelles, including mitochondria, lysosomes, Golgi bodies and an extensive endoplasmic reticulum (Fig. 1.1b). Photosynthetic cells also contain chloroplasts.
- The DNA of eukaryotic cells, in the form of several linear chromosomes, is characteristically complexes with histone proteins.
- Eukaryotic ribosomes, apart from those located within certain organelles, are 80S, somewhat larger than those of prokaryotes .
- If cell walls are present, they are composed of materials other than peptidoglycan, such as cellulose and related b-glucans, chitin or silica .
- Eukaryotic cells divide by a complex process of mitosis and usually have a sexual lifecycle, involving meiosis (reduction division).



Prokaryotes

Prokaryotes have been separated into two distinct groups on the basis of the study of phylogenetic (evolutionary) relationships. They are the archaea ('ancient' bacteria) and the eubacteria ('true' bacteria).

1. Archaea

These prokaryotes are quite different from eubacteria and have some features:

a. especially aspects of the transcription and translation machinery associated with protein synthesis, that are similar to eukaryotic cells.

b. Most archaeans live in extreme environments similar to those that early life forms are thought to have endured. Three basic physiological types are found, namely halophiles (adapted to high salt concentrations), methanogens (methane producers) and thermophiles (adapted to high temperatures), and some of these are also barophiles (adapted to high pressure).

2. Eubacteria

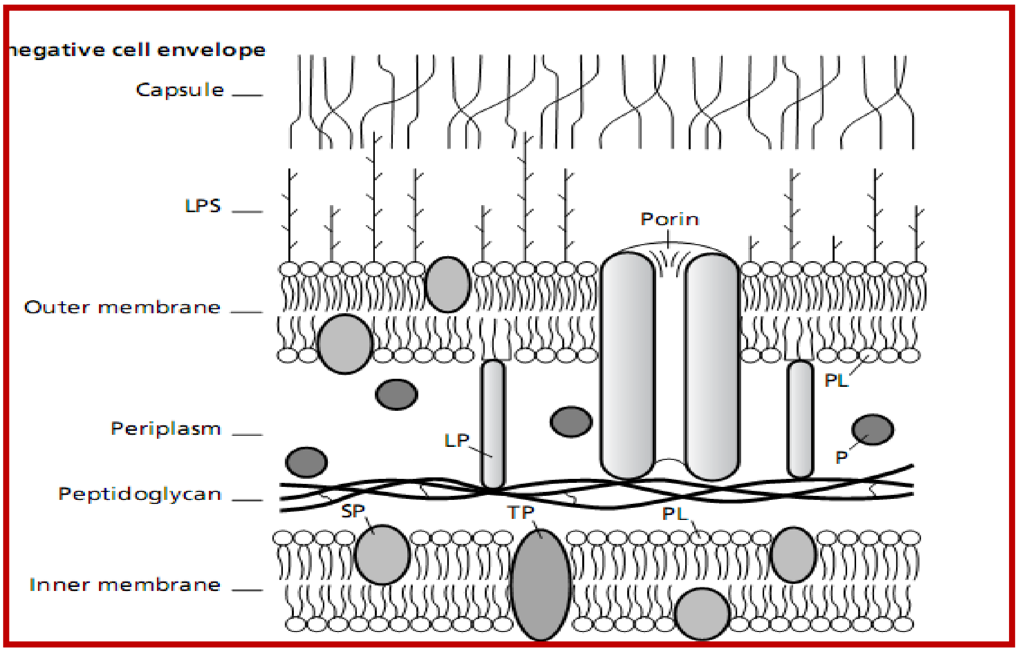
The eubacteria are a very diverse group that may be divided into 12 subgroups. However, almost all industrial bacteria are contained within just two of them, the proteobacteria (gram-negative bacteria) and the Gram-positive eubacteria.

Outer membrane of gram-negative bacteria

The outer coverings of Gram-negative bacterial cells are often referred to as envelopes, rather than walls, as they are more complex than the cell walls of Gram-positive bacteria (Fig. 1.2a). They are essentially composed of two layers that protect the cell and provide rigidity. The outermost layer is called the outer membrane, which is approximately 7-8 nm thick, containing:

1. Lipopolysaccharide: This structure does not impede the movement of small molecules, charged or uncharged, and is more permeable than the cell/cytoplasmic membrane, but is a barrier to hydrophobic molecules and proteins. It contains proteins, composed of three subunits that form narrow channels of about 1-2 nm diameters through which small molecules can pass. Lipopolysaccharide components of the envelope are effective in protecting the cell from detergents and other antimicrobial agents. The most common outer membrane protein is lipoprotein, which extends through the outer membrane and links to the underlying peptidoglycan.

2. Some strains also possess capsules located outside the outer membrane, which are composed of polysaccharides. Their production is influenced by the chemical and physical conditions within the local environment. These exopolysaccharides may provide a barrier to certain molecules, help protect against desiccation, or aid attachment of pathogenic strains to host cell surfaces.



Peptidoglycan and the periplasmic space

- Within the outer membrane of Gram-negative bacteria, and covalently attached to it through lipoprotein, is a thin layer of peptidoglycan some 2-3 nm thick. It constitutes only 5-10% of the cell envelope and is composed of one to three layers, compared with the 20-25 layers of peptidoglycan in the walls of many Gram-positive bacteria .

- Nevertheless, it is a very important structural component. When the peptiglycan layer is incomplete, bacterial cells may swell and ultimately burst.

The peptidoglycan extends down into the underlying periplasmic space, which is approximately 12-15 nm wide. This region is not empty; it contains a range of proteins, binding proteins, chemoreceptors and various enzymes. Binding proteins initiate transport of specific substances into the cell by taking them to their membrane-bound carriers. The chemoreceptors are involved in chemotaxis, which is the movement of a cell towards attractant and away from repellant chemicals .

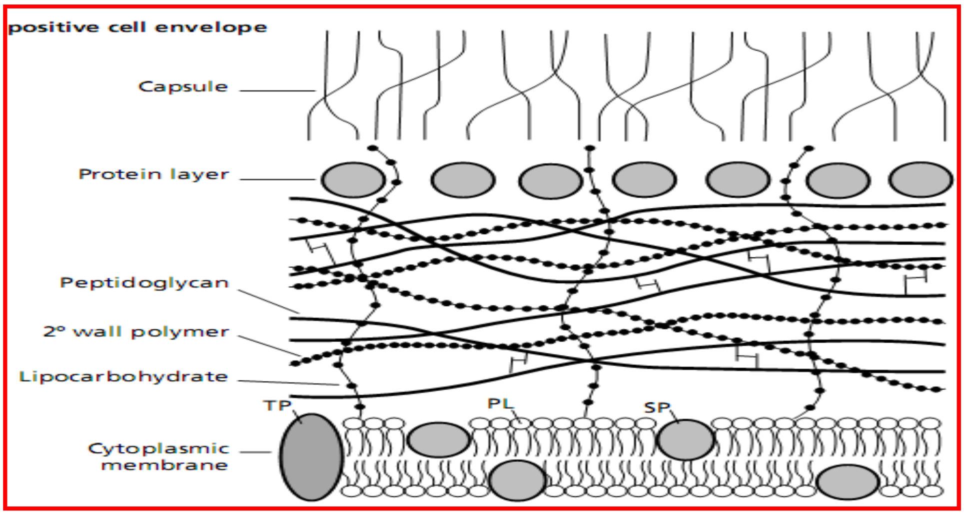
BACILLUS SUBTILIS, A GRAM-POSITIVE BACTERIUM

- These cells are usually somewhat larger than E. coli, at 0.5-2.5 mm wide and 1.2-10 mm long. Some species are strictly aerobic; others are facultative anaerobes or microaerophilic .
- Bacillus species also produce oval or cylindrical endospores that are resistant to adverse environmental conditions and provide a selective advantage for survival and dissemination (Fig. 1.3).

- Several members of the genus have important industrial roles, particularly as sources of enzymes, antibiotics (bacitracin, gramicidin and polymyxin) and insecticides.

- B. subtilis is a common soil microorganism that is often recovered from water, air and decomposing plant residues. The range of extracellular enzymes produced by this microorganism enables it to degrade a variety of natural substrates and contributes to nutrient cycling .

- B. subtilis has also proved very useful for the manufacture of fine chemicals, especially nucleosides, vitamins and amino acids, and some strains are used in crop protection against fungal pathogens.



- The main features of *B. subtilis* that distinguish it from E. coli are the cell wall structure and the ability to produce spores .

- *B. subtilis* cell walls are typical of Gram-positive bacteria, being much less complex than those of Gram-negative bacteria such as E. coli. They are 20-50 nm thick and simply composed of 20-25 layers of peptidoglycan, associated with some lipid, protein and teichoic acid (Fig. 1.2b). Teichoic acid is a distinctive anionic polymer of glycerol phosphate, ribitol phosphate and other sugar phosphates. It is covalently linked to the acid units of the peptidoglycan or to lipids of the underlying cell membrane. This component is not found in Gram-negative bacteria .

- Outside the cell wall, *B. subtilis* produces a polypeptide capsule .