UNIT-4

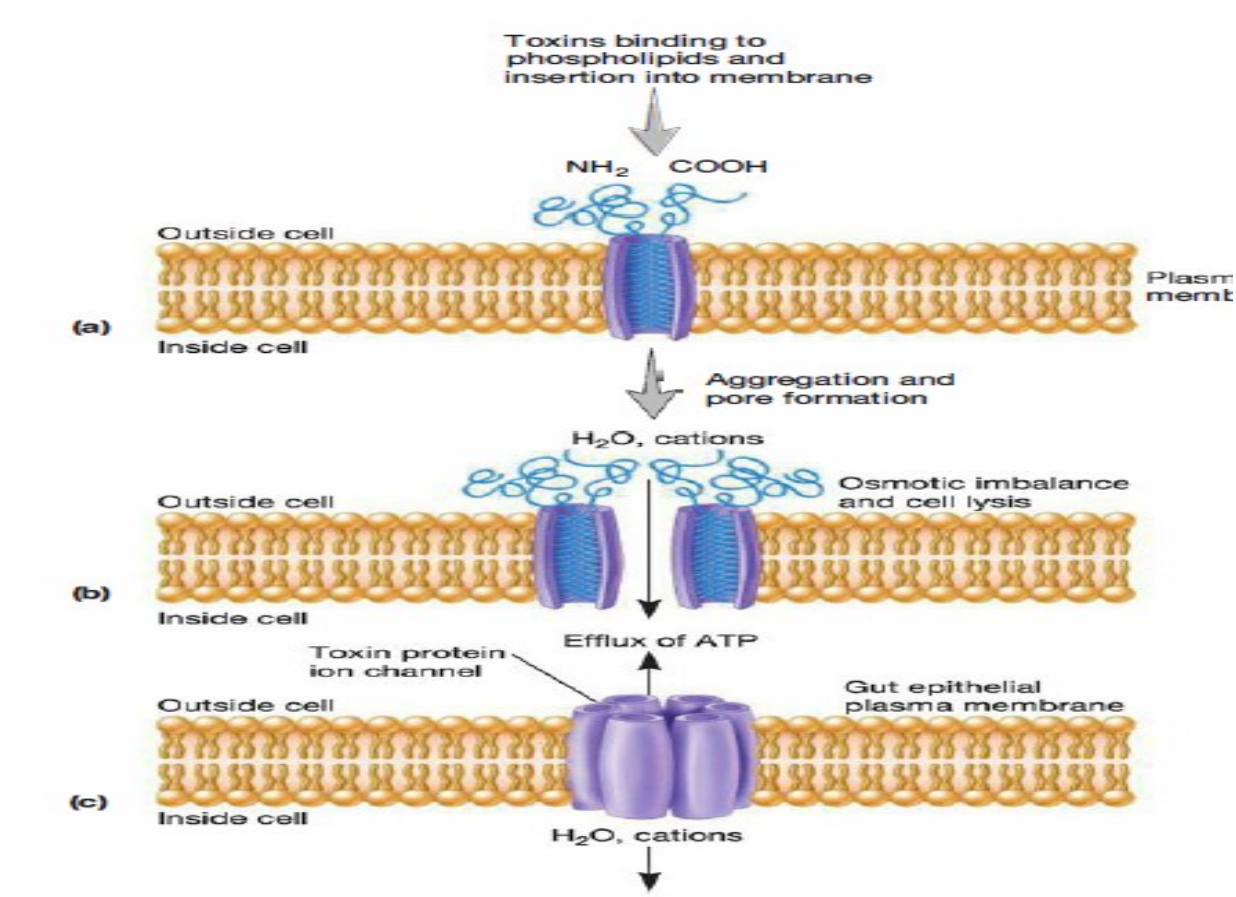
Economic importance of microorganisms i) Agricultural Microbiology (Biofertilizer, biopesticides), ii) Industrial Microbiology (in fermentation and Pharmaceuticals), iii) Medical Microbiology (air borne – Influenza; Water borne – Cholera; Food borne – Boutulism; Brief idea about epidemiology, causal organism and control).

Agricultural Microbiology (Biopesticides, Biofertilizer):

Biopesticides:

Microbial application to agriculture involves the use of bacteria, fungi, and viruses as bioinsecticides and biopesticides. These are defined as biological agents; that is, microbes or their components that can be used to kill susceptible insects. Bacterial agents include a variety of *Bacillus* species; however, *Bacillus thuringiensis* is most widely used.

This bacterium is only weakly toxic to insects as a vegetative cell, but during sporulation, it produces an intracellular protein toxin crystal, the parasporal body, that acts as a microbial insecticide for specific insect groups. The parasporal crystal, after exposure to alkaline conditions in the insect hind-gut, breaks apart to release protoxin. After protoxin reacts with a protease enzyme, active toxin is generated. Six of the active toxin units integrate into the plasma membrane to form a hexagonal-shaped pore through the mid-gut cell. This leads to the loss of osmotic balance and ATP, and finally to cell lysis. *Bacillus thuringiensis* has become an important industrial microbe. The toxin is widely marketed as the insecticide Bt, and the toxin genes can be used to genetically modify plants.



Biofertilizer:

Biofertilizers are defined as preparations containing living cells or latent cells of efficient strains of microorganisms that help crop plants' uptake of nutrients by their interactions in the rhizosphere when applied through seed or soil. They accelerate certain microbial processes in the soil which augment the extent of availability of nutrients in a form easily assimilated by plants.

Very often microorganisms are not as efficient in natural surroundings as one would expect them to be and therefore artificially multiplied cultures of efficient selected microorganisms play a vital role in accelerating the microbial processes in soil.

Use of biofertilizers is one of the important components of integrated nutrient management, as they are cost effective and renewable source of plant nutrients to supplement the chemical

fertilizers for sustainable agriculture. Several microorganisms and their association with crop plants are being exploited in the production of biofertilizers. They can be grouped in different ways based on their nature and function.

S.No.	Groups	Examples
N2 fixing Biofertilizers		
1.	Free-living	*Azotobacter, Beijerinkia, Clostridium, Klebsiella, Anabaena, Nostoc,*
2.	Symbiotic	*Rhizobium, Frankia, Anabaena azollae*
3.	Associative Symbiotic	*Azospirillum*
Phosphate Solubilizing Biofertilizers		
1.	Bacteria	*Bacillus megaterium* var. *phosphaticum, Bacillus subtilis Bacillus circulans, Pseudomonas striata*
2.	Fungi	*Penicillium sp, Aspergillus awamori*
Phosphate Mobilizing Biofertilizers		
1.	Arbuscular mycorrhiza	*Glomus sp.,Gigaspora sp.,Acaulospora sp., Scutellospora sp. & Sclerocystis sp.*
2.	Ectomycorrhiza	*Laccaria sp., Pisolithus sp., Boletus sp., Amanita sp.*
3.	Ericoid mycorrhizae	*Pezizella ericae*
4.	Orchid mycorrhiza	*Rhizoctonia solani*
Biofertilizers for Micro nutrients		
1.	Silicate and Zinc solubilizers	*Bacillus* sp.
Plant Growth Promoting Rhizobacteria		
1.	Pseudomonas	*Pseudomonas fluorescens*

Industrial Microbiology (in fermentation and Pharmaceuticals)

Fermentation

Fermentation technology is defined as field that involves the use of microbial enzymes for production of compounds that have application within the energy production, material, pharmaceutical industries, chemical, and food industries. The human beings are using it from the ancient times for preservation and organoleptic properties of food. It is a well-established technology of the ancient time used for food preservation, production of bread, beer, vinegar, yogurt, cheese, and wine. From time to time, it has got refined and diversified. It is the biological process in which various microorganisms such as yeast, bacteria, and fungi are involved in the conversion of complex substrate into simple compounds which are useful to humans (enzymes production, metabolites, biomass, recombinant technology, and biotransformation product) on industrial scale. Organic acid and alcohol are the main products of fermentation. In this process, there is liberation of secondary metabolites like antibiotics, enzymes, and growth factors.

Types of Fermentation Processes:

Solid-State Fermentation-

Solid-state (or substrate) fermentation (SSF) are define as fermentation that place in solid supporting, non-specific, natural state, and low moisture content. In this process, substrates such as nutrient rich waste can be reused. Bran, bagasses, and paper pulp are the solid substrates used in SSF. Since the process is slow the fermentation of substrate takes long time. So, the discharge of the nutrients is in controlled manner. It requires less moisture content so it is the best fermentation technology used for fungi and microorganism. However, this process is not applicable for bacteria because this fermentation cannot be used for organism that requires high water condition.

Some commonly used substrates in SSF are rice straw, vegetable waste, wheat bran, fruit bagasse, synthetic media, and paper pulp.

Submerged Fermentation-

In SmF, microorganism required a controlled atmosphere for proficient manufacture of good quality end products; attain optimum productivity and high yield.

Batch, fed-batch, or continuous modes are used in industrial bioreactors for the production of different type of microorganism in broad range [8].

For the manufacture of alcoholic beverages (whisky, beer, brandy, rum, and wine), preservatives or acidifiers (lactic acids, citric, and vinegar) are used in food industry and for flavor enhancers (monosodium glutamate) or sweeteners (aspartate) amino acid are used in submerged batch cultivation.

Liquid media, molasses, waste water, vegetable juices, and soluble sugar are common substrates used in SmF to extract bioactive compounds.

Batch Cultivation:

Batch culture is a closed system which works under aseptic condition. In these cultivations, inoculums, nutrients, and medium are mixed in the bioreactor in which the volume of the culture broth remains constant.

Fed-Batch Cultivation:

In fed batch cultivation, one or more nutrients are added aseptically, it is a semi-open system and the culture is supplemented step-by-step into the bioreactor at the same time the volume of the liquid culture in the bioreactor increase within this time.

The increase in productivity, enhanced yield by controlled sequential addition of nutrients, ability to achieve higher cell densities, and prolonged product synthesis are the main advantages of fed-batch over batch cultures.

Fundamental difference between SmF and SSF

Submerged fermentation	Solid-state (substrate) fermentation
Water cultivation medium (~95%).	Water cultivation medium is low (40–80%).
Liquid–gas are the two phase of the system.	Solid–liquid–gas are three phase of system.
Homogeneous.	Heterogeneous.
Low nutrient content, water soluble.	High nutrient content, water insoluble.
Oxygen transfer: gas–liquid.	Oxygen transfer: liquid–solid and gas–liquid.
Microorganism growth: liquid medium.	Microorganism growth: medium surface.
Only oxygen is transfer, process is not limited.	Oxygen, heat, and nutrient transfer is limited.
Product: soluble in the liquid phase.	Product: high concentration.

Pharmaceuticals

Industrial microbiology has profoundly changed our lives and lifespans. Microbial products include antibiotics and other medicines, food additives, enzymes with industrial applications, and Biofuels etc.

i. Production of Antibiotics:

More than 65% of all antibiotics are produced by microorganisms. Currently most antibiotics are produced by members of the genus *Streptomyces* and by filamentous fungi. Antibiotics are secreted by these microbes and either used in the native state or chemically modified to produce semi-synthetic derivatives. Such derivatives generally are manufactured to address the problem of antibiotic resistance or the desire for a broader spectrum drug.

A number of antibacterial (Bacitracin, Pelicillin) and antifungal (Griseofulvin, Ketoconazole) and antiviral (Acyclovir, Amantadine) antibiotics.

ii. Production of Vitamins:

Different kinds of vitamins are produced from bacteria like riboflavin from *Clostrodium butylicum*, vitamin B-12 from *Bacillus megatherium* and vitamin K and B-complex from *Escherichia coli* etc.

iii. Genetic Engineering:

Bacteria have short reproduction cycles. Some of them can divide in minutest to produce new daughter cells. This factor of bacteria is used in biotechnology to produce biological compounds. Products like insulin, vitamin B-12 etc. are supplied in large scale in continuous basis due to their manufacture using genetically modified bacteria cells.

iv. Production of Vaccines:

Bacteria are used to produce vaccines by either separating their antigens or sometimes dead form or else even live one with lack of pathogenic character. Example: TB-vaccine is one where dead bacteria of TB are administered to build up resistances to tuberculosis in humans. Once administered, these bacteria cannot cause diseases, but the body will be able to produce antibodies to kill any infections of *Mycobacterium.*

Medical Microbiology (Air borne- Influenza; water borne- Cholera; Food borne- Boutulism)

Air borne- Influenza:

Influenza is caused by an RNA virus of the orthomyxovirus group. Influenza virus is a single-stranded, negative sense, helical RNA genome surrounded by an envelope made up of protein, a lipid bilayer, and external glycoproteins. There are three different types of influenza viruses: influenza A, influenza B, and influenza C. Here we consider only influenza A, because it is the most important human pathogen.

Influenza Epidemiology:

Human influenza virus is transmitted from person to person through the air, primarily in droplets expelled during coughing and sneezing. The virus infects the mucous membranes of the upper respiratory tract and occasionally invades the lungs.

Symptoms include a low-grade fever lasting 3–7 days, chills, fatigue, headache, and general aching. Recovery is usually spontaneous and rapid.

Influenza Prevention and Treatment:

Influenza epidemics can be controlled by immunization. However, the selection of appropriate strains for vaccines is complicated by the large number of existing strains and the ability of existing strains to undergo antigenic drift and antigenic shift.

Influenza A may also be controlled by use of antiviral drugs. The adamantanes, amantadine and rimantadine, are synthetic amines that inhibit viral replication. The neuraminidase inhibitors oseltamivir (Tamiflu) and zanamivir (Relenza) block release of newly replicated virions of influenza A and B and H5N1 avian virus. These drugs are used to treat ongoing influenza and shorten the course and severity of infection. They are most effective when given very early in the course of the infection. The adamantanes and oseltamivir also prevent the onset and spread of influenza.

Treatment of influenza symptoms with aspirin, especially in children, is not recommended. Aspirin treatment of influenza has been linked to development of Reye's syndrome, a rare but occasionally fatal complication involving the central nervous system.

Water borne- Cholera

Cholera is a severe diarrheal disease that is now largely restricted to the developing world. Cholera is an example of a major waterborne disease that can be controlled by application of appropriate public health measures for water treatment. It is an indicator of socio-economic problems and is a global threat to public health. Worldwide, approximately 3–5 million cholera cases and 100,000–120,000 deaths due to cholera occur annually. Cholera is transmitted by drinking water or eating food.

Biology and Epidemiology:

Cholera is typically caused by ingestion of contaminated water containing *Vibrio cholerae*, a gram-negative, curved rod–shaped Proteobacterium.

Pathogenesis:

The ingestion of 10^8–10^9 cholera vibrios is generally required to cause disease. The ingested *Vibrio cholerae* cells attach to epithelial cells in the small intestine where they grow and release cholera toxin, a potent enterotoxin. Cholera enterotoxin causes severe diarrhea that can result in dehydration and death unless the patient is given fluid and electrolyte therapy. The enterotoxin causes fluid losses of up to 20 liters per day.

Diagnosis of Cholera

Cholera is diagnosed by the presence of the gram-negative, comma-shaped V. cholerae bacilli in the “rice water” stools.

Treatment of Cholera

Cholera treatment is simple, effective, and in expensive. Intravenous or oral liquid and electrolyte replacement therapy [20 g of glucose, 4.2 g of sodium chloride (NaCl), 4.0 g of sodium bicarbonate (NaHCO3), and 1.8 g of potassium chloride (KCl) dissolved in 1 liter of water] is the most effective means of cholera treatment. Oral treatment is preferred because no special equipment or sterile precautions are necessary.

Streptomycin or tetracycline may shorten the course of infection and the shedding of viable cells, but antibiotics are of little benefit without simultaneous fluid and electrolyte replacement.

Food borne- Boutulism:

Food poisoning, also called **food intoxication**, is disease that results from ingestion of foods containing preformed microbial toxins. The microorganisms that produced the toxins do not have to grow in the host and are often not alive at the time the contaminated food is consumed; the ingestion and action of a bioactive toxin causes the illness. Some of these toxins, notably the exotoxin of *Clostridium botulinum* and the super-antigen toxins of *Staphylococcus* and *Streptococcus*.

Food infection is ingestion of food containing sufficient numbers of viable pathogens to cause infection and disease in the host.

Botulism:

Botulism is a severe, often fatal, food poisoning caused by the consumption of food containing the exotoxin produced by *Clostridium botulinum*. This bacterium normally inhabits soil or water, but its endospores may contaminate raw foods. If the foods are properly processed so that the *Clostridium botulinum* endospores are removed or killed, no problem arises; however, if viable endospores remain in the food, they may germinate and produce botulinum toxin. Ingesting even a small amount of this neurotoxin can be dangerous.

Botulinum toxin is a neurotoxin that causes flaccid paralysis, usually affecting the autonomic nerves that control body functions such as respiration and heartbeat. The toxins are destroyed by heat (80^0C for 10 minutes), thoroughly cooked food, even though contaminated with toxin, and are totally harmless. Most cases of food borne botulism are caused by eating processed foods contaminated with *C. botulinum* endospores. Typically, such foods are consumed without cooking after processing.

Diagnosis, Treatment, and Prevention:

Botulism is diagnosed when botulinum toxin is found in patient serum or when toxin or live *C. botulinum* is found in food the patient has ingested. Laboratory findings are coupled with clinical observations, including neurological signs of localized paralysis (impaired vision and speech) beginning 18–24 hours after ingestion of contaminated food.

Treatment is by administration of botulinum antitoxin if the diagnosis is early, and mechanical ventilation for flaccid respiratory paralysis. Antitoxin administration is not recommended. Respiratory failure causes occasional deaths.

Prevention of botulism requires careful control of canning and preservation methods. Susceptible foods should be heated to destroy endospores; boiling for 20 minutes to destroys the toxin.

Brief idea about epidemiology:

Epidemiology is the study of the determinants, distribution, and frequency of disease (who gets the disease and why) i.e. the study of the distribution and determinants of health-related states or events in specified populations, and the application of this study to control of health problems.

Uses of Epidemiology:

i. To study the cause (or etiology) of disease(s), or conditions, disorders, disabilities, etc.

ii. To determine the primary agent responsible or ascertain causative factors.

iii. To determine the characteristics of the agent or causative factors.

iv. To determine the mode of transmission.

v. To determine contributing factors.

vi. To identify and determine geographic patterns.

Types of Epidemiology:

Descriptive epidemiology: examining the distribution of disease in a population, and observing the basic features of its distribution.

Analytic epidemiology: investigating a hypothesis about the cause of disease by studying how exposures relate to disease.