MICROBES AND HUMAN WELFARE

Microorganisms are essential components of the biological world and play a significant role in maintaining ecological balance and improving human welfare. Although certain microorganisms are responsible for causing diseases in humans, animals and plants, a large number of them are beneficial and have enormous applications in medicine, agriculture, food production, biotechnology and environmental management. The ability of microorganisms to grow rapidly, utilise different substrates and produce a wide variety of metabolic products makes them valuable biological resources.

In modern biotechnology, microorganisms are extensively employed for the commercial production of antibiotics, enzymes, organic acids, vitamins, vaccines, fermented foods and other industrial products. They are also used as biological control agents to manage agricultural pests and plant pathogens, thereby reducing dependence on chemical pesticides. Furthermore, microorganisms play a fundamental role in sewage treatment by decomposing organic pollutants, reducing biochemical oxygen demand and helping remove excess nutrients from wastewater. Thus, microbial technology contributes significantly to public health, agricultural productivity, industrial development and environmental sustainability.

1. MICROBIAL PRODUCTS

1.1 Introduction to Microbial Products

Microbial products are useful substances synthesised by microorganisms during their growth and metabolic activities. These products are obtained from bacteria, fungi, yeasts and actinomycetes and are widely utilised in pharmaceutical, food-processing, agricultural and chemical industries. Microorganisms are particularly suitable for industrial production because they have short generation times, can be cultivated on inexpensive substrates and can be genetically modified to improve their productivity.

Microbial products are broadly classified into two major categories based on their relationship with microbial growth: primary metabolites and secondary metabolites. Primary metabolites are directly associated with normal cellular growth and reproduction, whereas secondary metabolites are generally produced during specific stages of microbial development and are not directly required for basic cellular growth. Apart from these metabolites, microorganisms also produce industrially important enzymes, vitamins, recombinant proteins and other bioactive compounds.

1.2 Primary Metabolites

Primary metabolites are organic compounds produced by microorganisms during their active growth phase, particularly during the exponential or logarithmic phase. These substances are directly involved in essential cellular processes such as energy production, protein synthesis, nucleic acid synthesis and other metabolic activities. Since primary metabolites are closely associated with microbial growth, their production generally increases when microorganisms actively multiply under favourable environmental and nutritional conditions.

Several primary metabolites have considerable commercial importance. The most important examples include alcohols, organic acids, amino acids and certain vitamins.

A. Production of Alcohol

Alcohol production is one of the oldest and most important industrial applications of microorganisms. Ethanol is commercially produced through the fermentation of carbohydrates by yeast, particularly *Saccharomyces cerevisiae*. During this process, yeast utilises glucose under anaerobic conditions and converts it into ethanol and carbon dioxide. The process is known as alcoholic fermentation.

The biochemical reaction can be represented as:

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

During fermentation, glucose is first converted into pyruvate through glycolysis. Pyruvate is subsequently decarboxylated to form acetaldehyde, which is reduced to ethanol by the enzyme alcohol dehydrogenase. The regeneration of NAD^+ allows glycolysis to continue in the absence of oxygen.

Ethanol has several industrial applications. It is used in the production of alcoholic beverages, pharmaceutical preparations, perfumes, cosmetics and industrial solvents. It is also blended with petrol to produce gasohol, which serves as an alternative or supplementary transportation fuel. Therefore, microbial ethanol production has considerable significance in the development of renewable energy resources.

B. Production of Organic Acids

Organic acids are important primary metabolites produced by different microorganisms through fermentation. They are extensively used in food processing, pharmaceutical industries, chemical manufacturing and other industrial applications. The commercial production of organic acids depends on the ability of microorganisms to utilise carbohydrates and convert them into specific acidic compounds.

Citric acid is one of the most important industrially produced organic acids. It is commercially manufactured using the fungus *Aspergillus niger*. Under suitable conditions, particularly in a carbohydrate-rich medium, this fungus accumulates citric acid in the fermentation medium. Citric acid is widely used in the food industry as an acidulant, flavouring agent and preservative. It is also used in pharmaceutical preparations and cosmetic products.

Lactic acid is another important organic acid produced by lactic acid bacteria such as *Lactobacillus* and *Lactiplantibacillus*. During lactic acid fermentation, these bacteria convert carbohydrates into lactic acid. It is used in the preparation of curd, yoghurt, cheese and other fermented food products. Industrially produced lactic acid is also used in the manufacture of biodegradable plastics such as polylactic acid (PLA).

Acetic acid is produced biologically through the oxidation of ethanol by acetic acid bacteria, particularly *Acetobacter aceti*. The process occurs under aerobic conditions and is important in vinegar production. Gluconic acid is another commercially valuable organic acid produced by microorganisms such as *Aspergillus niger* and *Gluconobacter oxydans*. It is used in food, pharmaceutical and chemical industries.

C. Production of Amino Acids

Amino acids are essential components of proteins and play important roles in cellular metabolism, growth and development. Several microorganisms are employed for the

commercial production of amino acids because they can synthesise and accumulate large quantities of specific amino acids under controlled fermentation conditions.

Glutamic acid is one of the most commercially important amino acids produced by microorganisms. It is mainly manufactured using *Corynebacterium glutamicum*. Glutamic acid is used in the production of monosodium glutamate (MSG), which is widely employed as a flavour enhancer in food products. The bacterium is also used for the industrial production of lysine, an essential amino acid used in animal feed and nutritional supplements.

Other amino acids, including tryptophan and threonine, are produced using selected strains of genetically improved bacteria such as *Escherichia coli* and *Corynebacterium glutamicum*. Microbial amino acid production is important in the food and pharmaceutical industries, as well as in animal nutrition.

D. Production of Vitamins

Certain microorganisms have the ability to synthesise vitamins that are essential for normal growth, metabolism and physiological functioning in humans and animals. Microbial fermentation provides an efficient method for the large-scale production of several vitamins.

Vitamin B12, also known as cobalamin, is produced commercially by microorganisms such as *Propionibacterium freudenreichii* and selected strains of *Pseudomonas denitrificans*. It is essential for red blood cell formation, DNA synthesis and normal nervous system functioning.

Riboflavin or vitamin B2 is produced industrially using microorganisms such as *Ashbya gossypii* and certain strains of *Bacillus subtilis*. Riboflavin is an important component of the coenzymes FAD and FMN, which participate in cellular oxidation-reduction reactions. Microbial vitamin production is therefore important in the preparation of nutritional supplements, fortified foods and pharmaceutical products.

1.3 Secondary Metabolites

Secondary metabolites are organic compounds produced by microorganisms that are not directly required for their normal growth, development or reproduction. Unlike primary metabolites, which are generally associated with the exponential growth phase, secondary metabolites are frequently produced during the late growth phase or stationary phase. Their production is often influenced by nutrient limitation, environmental conditions and specific regulatory mechanisms.

Secondary metabolites include antibiotics, pigments, toxins, immunosuppressive compounds and other biologically active substances. Many of these compounds have important pharmaceutical and agricultural applications. Antibiotics are among the most significant examples of microbial secondary metabolites.

A. Antibiotics

Antibiotics are antimicrobial substances produced naturally by certain microorganisms or manufactured through microbial and chemical processes. They inhibit the growth of susceptible microorganisms or destroy them. Antibiotics produced by bacteria, fungi and actinomycetes have revolutionised the treatment of bacterial infections and have become essential components of modern medicine.

Penicillin was discovered by Alexander Fleming in 1928 when he observed that a mould belonging to the genus *Penicillium* inhibited the growth of nearby bacteria. The antibiotic was subsequently developed for therapeutic use through the work of Howard Florey, Ernst Boris Chain and their collaborators. Industrial penicillin production is associated with high-yielding strains of *Penicillium rubens*, historically referred to as *Penicillium chrysogenum*. Penicillin acts by inhibiting bacterial cell wall synthesis. It interferes with the formation of peptidoglycan cross-links, resulting in weakened cell walls and bacterial cell death.

Streptomycin is an antibiotic produced by *Streptomyces griseus*, a soil-dwelling actinomycete. It belongs to the aminoglycoside group of antibiotics and acts by binding to the 30S subunit of bacterial ribosomes. This interferes with protein synthesis and causes errors in the translation of genetic information. Streptomycin has historically been important in the treatment of tuberculosis and several other bacterial infections.

Tetracycline is an antibiotic originally obtained from species of *Streptomyces*. It acts by inhibiting bacterial protein synthesis through binding to the 30S ribosomal subunit. It is a broad-spectrum antibiotic that acts against several Gram-positive and Gram-negative bacteria.

Erythromycin is produced by *Saccharopolyspora erythraea*, formerly known as *Streptomyces erythreus*. It belongs to the macrolide group of antibiotics and inhibits bacterial protein synthesis by binding to the 50S ribosomal subunit.

Chloramphenicol is produced naturally by *Streptomyces venezuelae*. It inhibits protein synthesis by interfering with peptidyl transferase activity in the 50S ribosomal subunit. Its clinical use is limited because of the possibility of serious adverse effects.

Cephalosporins are a group of beta-lactam antibiotics originally derived from the fungus *Acremonium chrysogenum*, formerly known as *Cephalosporium acremonium*. They interfere with bacterial cell wall synthesis and are widely used in the treatment of various bacterial infections.

B. Production of Industrial Enzymes

Enzymes are biological catalysts that accelerate biochemical reactions without being consumed during the reactions. Microorganisms are important sources of industrial enzymes because they can be cultivated in large quantities and are capable of producing enzymes with different biochemical properties. Microbial enzymes are extensively used in the food, textile, leather, detergent, paper and pharmaceutical industries.

Amylases are enzymes that break down starch into smaller carbohydrates. They are produced by microorganisms such as *Bacillus subtilis* and *Aspergillus oryzae*. These enzymes are used in starch processing, baking, brewing and the production of glucose syrups.

Proteases are enzymes that hydrolyse proteins into smaller peptides and amino acids. They are produced by bacteria such as *Bacillus licheniformis* and *Bacillus subtilis*. Proteases are important ingredients in detergent formulations because they help remove protein-based stains. They are also used in leather processing, food industries and pharmaceutical preparations.

Lipases are enzymes responsible for the hydrolysis of fats and oils into fatty acids and glycerol. They are produced by microorganisms such as *Candida rugosa* and *Yarrowia lipolytica*. They

are used in detergent manufacturing, dairy processing, biodiesel production and the food industry.

Cellulases are enzymes that break down cellulose into simpler sugars. They are produced by fungi such as *Trichoderma reesei*. Cellulases are used in textile processing, paper manufacturing, animal feed preparation and biomass conversion.

Pectinases are enzymes that degrade pectin, a structural polysaccharide present in plant cell walls. They are produced by *Aspergillus niger* and are widely used in fruit juice extraction and clarification.

C. Production of Other Pharmaceutical Compounds

Apart from antibiotics, microorganisms produce several other substances of pharmaceutical importance.

Cyclosporin A is an immunosuppressive compound produced by the fungus *Tolypocladium inflatum*. It suppresses the activity of certain immune cells, particularly T lymphocytes, and is used to prevent the rejection of transplanted organs.

Lovastatin is a cholesterol-lowering compound produced by *Aspergillus terreus*. It inhibits the enzyme HMG-CoA reductase, which plays an important role in cholesterol biosynthesis. Lovastatin is therefore used in the management of elevated blood cholesterol levels.

Griseofulvin is an antifungal compound produced by *Penicillium griseofulvum*. It is used in the treatment of certain fungal infections of the skin, hair and nails. It acts by interfering with fungal cell division through its effects on microtubule function.

These examples demonstrate the importance of microorganisms as sources of therapeutically valuable compounds.

1.4 Microorganisms in Food Production

Microorganisms have been used in food preparation and preservation for thousands of years. The process of fermentation involves the metabolic activities of microorganisms that convert carbohydrates and other organic substances into simpler products such as organic acids, alcohol and carbon dioxide. Fermentation improves the flavour, texture, aroma, digestibility and shelf life of several food products. Different microorganisms are selected depending on the type of food being prepared and the desired fermentation process.

A. Curd and Yoghurt Production

Curd is produced through the fermentation of milk by lactic acid bacteria. The bacteria utilise lactose, the major carbohydrate present in milk, and convert it into lactic acid. The accumulation of lactic acid lowers the pH of milk, resulting in the coagulation of casein proteins and the formation of curd. Common microorganisms involved in yoghurt production include *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*. These bacteria contribute to the characteristic sour taste, texture and aroma of yoghurt. Fermented dairy products may also contain beneficial microorganisms that contribute to gut microbial balance.

B. Bread Production

Bread production is an important commercial application of yeast fermentation. The yeast *Saccharomyces cerevisiae* is used as a leavening agent in bread-making. When yeast is added to dough containing fermentable sugars, it produces carbon dioxide and ethanol through alcoholic fermentation. The carbon dioxide becomes trapped within the gluten network of the dough, causing it to expand and rise. During baking, the alcohol evaporates, while the expanded structure becomes fixed, producing soft and porous bread.

C. Cheese Production

Cheese is prepared through the coagulation and fermentation of milk. Lactic acid bacteria, particularly species of *Lactococcus*, *Lactobacillus* and *Streptococcus*, convert lactose into lactic acid, which helps in milk coagulation. In certain varieties of cheese, specific fungi are also used during ripening. For example, *Penicillium roqueforti* is used in the production of blue cheese, whereas *Penicillium camemberti* is used in Camembert and Brie cheese. These microorganisms contribute to the characteristic flavour, texture and appearance of the cheese.

D. Idli and Dosa Fermentation

Idli and dosa are traditional fermented food products widely consumed in India. They are prepared using a mixture of rice and black gram (urad dal). During fermentation, naturally occurring lactic acid bacteria and yeasts multiply and produce organic acids and carbon dioxide. The production of carbon dioxide makes the batter soft and fluffy, while organic acids contribute to its characteristic taste. The fermentation process also improves the digestibility and nutritional properties of the batter.

E. Vinegar Production

Vinegar is produced through the biological oxidation of ethanol into acetic acid by acetic acid bacteria, particularly *Acetobacter aceti*. Unlike alcoholic fermentation, which occurs under anaerobic conditions, the conversion of ethanol into acetic acid requires oxygen. Vinegar is widely used as a food preservative, flavouring agent and condiment.

1.5 Large-Scale Production of Microbial Products

Commercial production of microbial products is generally carried out through a controlled fermentation process in large vessels known as fermenters or bioreactors. These vessels provide suitable environmental conditions for the growth of microorganisms and the production of desired metabolites. Industrial fermentation requires careful regulation of temperature, pH, oxygen supply, nutrient concentration and agitation to obtain maximum product yield.

The first step involves the selection of a suitable microbial strain capable of producing the required product in high quantities. The selected strain is maintained under controlled conditions and used for the preparation of an inoculum. A suitable nutrient medium containing carbon sources, nitrogen sources, minerals and growth factors is prepared and sterilised to prevent contamination. The sterilised medium is then inoculated with the selected microorganism.

During fermentation, the microorganisms multiply and produce the desired metabolite. The environmental conditions are continuously monitored and regulated to maintain optimum microbial activity. After completion of fermentation, the product is recovered from the fermentation broth or microbial cells, depending on whether it is an extracellular or

intracellular product. The recovered product undergoes downstream processing, which may include filtration, centrifugation, extraction, concentration and purification. Finally, the purified product is formulated, tested for quality and prepared for commercial use.

Thus, industrial fermentation provides an efficient method for the large-scale production of antibiotics, enzymes, vitamins, organic acids, amino acids and other commercially valuable microbial products.

2. MICROBIAL BIOCONTROL

2.1 Introduction to Microbial Biocontrol

Microbial biocontrol, also known as biological control using microorganisms, refers to the utilisation of beneficial microorganisms or their biologically active products to suppress the growth, reproduction and activities of harmful organisms. These harmful organisms may include plant pathogens, insect pests, weeds and other organisms responsible for agricultural losses. Microbial biocontrol is an important component of sustainable agriculture because it provides an alternative or supplementary approach to the excessive use of synthetic chemical pesticides.

Agricultural crops are frequently attacked by bacteria, fungi, viruses, nematodes and insect pests. Traditionally, chemical pesticides have been used to control these organisms. However, their excessive and indiscriminate use may cause environmental pollution, pesticide residues in food, destruction of beneficial organisms and the development of pesticide resistance in target pests. Microbial biocontrol offers a more environmentally compatible approach by utilising naturally occurring biological interactions.

Microbial biocontrol agents may act through several mechanisms, including antibiosis, competition, parasitism, predation, production of toxic substances and activation of natural defence mechanisms in plants. Depending on their mode of action, they can be used to control plant diseases, insect pests and soil-borne pathogens.

2.2 Types of Microbial Biocontrol Agents

Microbial biocontrol agents are broadly classified into bacterial, fungal and viral agents. Each group has distinct biological characteristics and mechanisms of action.

2.3 Bacterial Biocontrol Agents

Bacteria are among the most widely used microorganisms in biological control. Several soil- and plant-associated bacteria produce antimicrobial substances, enzymes and other metabolites that inhibit the growth of plant pathogens or directly kill insect pests. Some bacteria also colonise plant roots and stimulate the natural defence mechanisms of plants.

A. Bacillus thuringiensis (Bt)

Bacillus thuringiensis is a Gram-positive, rod-shaped, spore-forming bacterium commonly found in soil and other natural environments. It is one of the most important microbial insecticides used in agriculture and is particularly effective against the larval stages of several insect pests. The insecticidal activity of this bacterium is mainly associated with the production of crystalline protein inclusions during sporulation. These proteins are commonly called Cry proteins or delta-endotoxins.

The Cry proteins are produced as inactive protoxins and become active after ingestion by susceptible insect larvae. When the larvae feed on plant material contaminated with Bt spores and crystals, the crystals enter the insect digestive system. The alkaline conditions of the insect midgut dissolve the crystalline proteins, and digestive proteases convert the protoxins into active toxins. These activated toxins bind to specific receptors present on the epithelial cells of the insect midgut.

The binding of the activated toxin to the receptors results in the formation of pores in the intestinal cell membranes. This damages the epithelial cells, disrupts normal ion and water balance and causes the breakdown of the midgut lining. Consequently, the insect stops feeding, becomes paralysed or severely debilitated and eventually dies. The damaged gut may also allow secondary infections to develop.

Bt is particularly important in controlling caterpillar pests belonging to the order Lepidoptera. Different strains and toxin types are also effective against certain beetles and mosquito larvae. Commercial Bt formulations are used in agriculture as biological insecticides. Genes encoding specific Bt toxins have also been introduced into genetically modified crops, such as Bt cotton, to provide protection against susceptible insect pests.

B. Pseudomonas fluorescens

Pseudomonas fluorescens is a Gram-negative, rod-shaped bacterium commonly found in soil, water and the rhizosphere, which is the region of soil surrounding plant roots. It is an important plant growth-promoting rhizobacterium and is widely studied for its ability to suppress several soil-borne plant pathogens.

The biocontrol activity of *Pseudomonas fluorescens* is mainly associated with the production of antimicrobial compounds, competition for nutrients and space, siderophore production and stimulation of plant defence mechanisms. Some strains produce antibiotics such as 2,4-diacetylphloroglucinol, pyoluteorin and phenazines, which inhibit the growth of pathogenic fungi and bacteria. Siderophores produced by the bacterium bind iron present in the soil, making it less available to competing pathogens.

The bacterium also colonises plant roots and establishes a protective microbial population around them. This reduces the opportunity for harmful microorganisms to establish infections. Certain strains stimulate induced systemic resistance in plants, thereby enhancing the plant's ability to defend itself against pathogen attack. Consequently, *Pseudomonas fluorescens* is used in the biological management of diseases such as root rot, damping-off and wilt caused by different soil-borne pathogens.

C. Bacillus subtilis

Bacillus subtilis is a Gram-positive, rod-shaped, endospore-forming bacterium commonly found in soil and associated with plant roots. It is an important biological control agent because of its ability to produce several antimicrobial compounds and extracellular enzymes. The bacterium also survives under unfavourable environmental conditions through the formation of resistant endospores, making it suitable for commercial microbial formulations.

The biocontrol activity of *Bacillus subtilis* is mainly associated with the production of cyclic lipopeptides such as iturin, surfactin and fengycin. These compounds can disrupt the cell membranes of susceptible fungal pathogens and inhibit their growth. The bacterium also

produces enzymes that degrade certain components of microbial cell walls. In addition, it competes with pathogens for nutrients and colonisation sites around plant roots.

Certain strains of *Bacillus subtilis* stimulate plant growth by producing growth-promoting substances and activating plant defence responses. It is therefore used in the biological management of several fungal and bacterial plant diseases.

2.4 Fungal Biocontrol Agents

Fungi are important biological control agents because several species are capable of parasitising plant pathogens or infecting insect pests. Certain fungi produce antimicrobial substances, extracellular enzymes and specialised structures that enable them to suppress harmful organisms. Fungal biocontrol agents are particularly useful in the management of soil-borne diseases and insect pests.

A. Trichoderma

Trichoderma is a genus of free-living, filamentous fungi commonly found in soil, decaying organic matter and the rhizosphere of plants. Species such as *Trichoderma harzianum*, *Trichoderma viride* and *Trichoderma asperellum* are widely used as biological control agents against different fungal plant pathogens.

The most important characteristic of *Trichoderma* is its ability to suppress pathogenic fungi through several biological mechanisms. One of its major mechanisms is mycoparasitism, in which the fungus directly attacks other fungi. During this process, *Trichoderma* recognises and attaches to the hyphae of the pathogenic fungus. Its hyphae may coil around the pathogen's hyphae and penetrate them through the combined action of mechanical pressure and hydrolytic enzymes. This results in the degradation of the pathogen's cell wall and disruption of its cellular structure.

Another important mechanism is antibiosis, in which *Trichoderma* produces antimicrobial substances that inhibit the growth of pathogenic fungi. It also produces extracellular enzymes such as chitinases, β-glucanases and proteases. These enzymes degrade the major structural components of fungal cell walls, thereby weakening or destroying the pathogen.

Competition is another mechanism through which *Trichoderma* suppresses pathogens. It grows rapidly and efficiently utilises available nutrients and colonisation sites, reducing the resources available to pathogenic organisms. Some strains also produce siderophores and other compounds that restrict nutrient availability to competing microorganisms.

Apart from direct pathogen suppression, *Trichoderma* can stimulate plant growth and activate plant defence mechanisms. This process is known as induced systemic resistance. Therefore, *Trichoderma* formulations are widely used for managing soil-borne diseases such as root rot, collar rot, damping-off and wilt.

B. Beauveria bassiana

Beauveria bassiana is an entomopathogenic fungus, meaning that it naturally infects and kills insects. It is commonly found in soil and has considerable importance as a biological insecticide. It can infect a wide range of insect hosts, including whiteflies, aphids, beetles and certain other agricultural pests.

Unlike many microbial pathogens that enter insects through ingestion, *Beauveria bassiana* generally infects insects through their external body covering, known as the cuticle. The fungal conidia come into contact with the insect's body and attach to the cuticular surface. Under favourable environmental conditions, the conidia germinate and produce germ tubes. The fungus then penetrates the insect cuticle through mechanical pressure and the secretion of enzymes such as proteases, chitinases and lipases.

After entering the insect's body cavity or haemocoel, the fungus multiplies and spreads through the internal tissues. It may produce biologically active metabolites that contribute to insect mortality. The insect eventually dies due to tissue damage, physiological disruption and fungal infection. Under favourable conditions, fungal hyphae emerge through the insect's cuticle and produce new conidia, which can infect other insects.

The disease caused by *Beauveria bassiana* is commonly called white muscardine disease because infected insects often become covered with white fungal growth. Commercial formulations of this fungus are used in biological pest management and integrated pest management programmes.

C. Metarhizium anisopliae

Metarhizium anisopliae is an entomopathogenic fungus commonly found in soil. It is used as a biological control agent against several insect pests, including termites, locusts, grasshoppers and certain beetles. Like *Beauveria bassiana*, it infects insects primarily through the external cuticle.

The conidia of *Metarhizium* attach to the insect body, germinate and penetrate the cuticle through mechanical action and enzyme secretion. Once inside the insect, the fungus multiplies and damages internal tissues, eventually causing death. After the death of the insect, the fungus may grow externally and produce green-coloured conidia. The disease caused by this fungus is therefore commonly known as green muscardine disease.

D. Entomopathogenic Fungi and Their Importance

Entomopathogenic fungi are important because they can infect insects without requiring the insect to consume the fungal spores. Their ability to penetrate the insect cuticle makes them useful against several sucking and chewing insect pests. However, their effectiveness depends on environmental conditions, particularly temperature and humidity. High humidity generally favours spore germination and fungal infection.

2.5 Viral Biocontrol Agents

Viruses are also used as biological control agents, particularly against agricultural insect pests. Among them, baculoviruses are the most important group because of their natural pathogenicity towards several insect larvae. Baculoviruses are members of the family Baculoviridae and are generally highly host-specific, making them suitable for environmentally selective pest management.

Nucleopolyhedrovirus (NPV)

Nucleopolyhedrovirus, commonly abbreviated as NPV, is an insect-specific virus that infects the larval stages of several insects, particularly caterpillars. The virus produces characteristic proteinaceous structures called occlusion bodies, which protect the virus particles in the

external environment. The occlusion bodies contain infectious virions that are released after entering the digestive system of susceptible insect larvae.

When an insect larva consumes food contaminated with NPV occlusion bodies, the alkaline environment of its midgut dissolves the protective protein matrix and releases the infectious virus particles. These particles infect the epithelial cells of the midgut and subsequently spread to other tissues. The virus multiplies within the host cells, causing cellular damage and disruption of normal physiological activities. As the infection progresses, the insect becomes inactive, stops feeding and eventually dies.

After the death of the infected insect, the body may disintegrate and release large quantities of occlusion bodies into the surrounding environment. These occlusion bodies can contaminate plant surfaces and infect other susceptible larvae.

Important examples include *Helicoverpa armigera* nucleopolyhedrovirus (HaNPV), which is used against the gram pod borer and other susceptible caterpillar pests, and *Spodoptera litura* nucleopolyhedrovirus (SlNPV), which is used against the tobacco cutworm.

NPV formulations are particularly valuable in integrated pest management because of their high specificity towards target insect pests and their comparatively low impact on humans, livestock and many beneficial organisms.

2.6 Mechanisms of Microbial Biocontrol

Microorganisms suppress harmful organisms through different biological mechanisms. These mechanisms may operate individually or together, depending on the microorganism and the target pathogen or pest.

A. Antibiosis

Antibiosis refers to the suppression of one microorganism by another through the production of antimicrobial substances. Certain bacteria and fungi produce antibiotics, toxins or other inhibitory compounds that interfere with the growth, metabolism and reproduction of competing microorganisms. For example, *Bacillus subtilis* produces iturin and fengycin, which inhibit several fungal pathogens. Similarly, *Pseudomonas fluorescens* produces antimicrobial compounds that suppress certain soil-borne pathogens.

B. Competition

Competition occurs when beneficial microorganisms and harmful pathogens require the same nutrients, space or environmental resources. Beneficial microorganisms that colonise plant roots rapidly may prevent pathogens from establishing themselves. For example, *Pseudomonas fluorescens* competes with soil-borne pathogens for iron by producing siderophores. Similarly, *Trichoderma* competes effectively for nutrients and space in the rhizosphere.

C. Mycoparasitism

Mycoparasitism refers to a biological interaction in which one fungus directly parasitises another fungus. The biocontrol fungus attaches to the pathogen, grows around its hyphae and may penetrate its cell wall. It subsequently releases hydrolytic enzymes that degrade the pathogen's cellular structures. *Trichoderma* is a well-known example of a mycoparasitic fungus used in agricultural disease management.

D. Pathogenesis

Pathogenesis refers to the ability of a microorganism to infect and cause disease in another organism. Entomopathogenic fungi such as *Beauveria bassiana* and *Metarhizium anisopliae* infect insects and cause their death. Similarly, NPV infects susceptible insect larvae and destroys their tissues through viral multiplication.

E. Induced Systemic Resistance

Some beneficial microorganisms stimulate the natural defence mechanisms of plants. This process is known as induced systemic resistance (ISR). When beneficial bacteria or fungi colonise plant roots, they may activate signalling pathways involving jasmonic acid and ethylene, preparing the plant to respond more effectively to subsequent pathogen attacks. *Pseudomonas* and *Trichoderma* species are well-known examples of microorganisms capable of inducing plant defence responses.

2.7 Applications of Microbial Biocontrol in Agriculture

Microbial biocontrol has several important applications in modern agriculture. It is used for the management of insect pests, fungal diseases, bacterial diseases and certain soil-borne pathogens. Bt formulations are commonly used to control susceptible caterpillar larvae, while NPV formulations are used against specific agricultural pests such as *Helicoverpa armigera* and *Spodoptera litura*.

Fungal agents such as *Trichoderma* are used for the management of root rot, damping-off and other soil-borne fungal diseases. *Beauveria bassiana* and *Metarhizium anisopliae* are used against several insect pests. Beneficial bacteria such as *Pseudomonas fluorescens* and *Bacillus subtilis* are used to suppress plant pathogens and improve plant health.

Microbial biocontrol agents may be applied to seeds, soil, roots or plant surfaces depending on their target organism and formulation. Seed treatment with suitable microbial formulations helps establish beneficial microbial populations around developing roots, while soil application supports the suppression of soil-borne pathogens.

2.8 Advantages and Limitations of Microbial Biocontrol

Microbial biocontrol has several advantages over conventional chemical pest control. It generally reduces chemical pesticide use and helps minimise environmental contamination and pesticide residues. Many microbial agents exhibit a high degree of host specificity and have comparatively less harmful effects on beneficial organisms. They can also be incorporated into integrated pest management programmes and contribute to sustainable agricultural practices.

However, microbial biocontrol also has certain limitations. The effectiveness of microbial agents may vary with environmental conditions such as temperature, moisture, sunlight and soil pH. Some microorganisms act more slowly than chemical pesticides, and their survival may be affected by ultraviolet radiation and unsuitable storage conditions. Certain agents have a narrow host range and therefore cannot control all types of pests. Proper selection, formulation, storage and application are essential for obtaining satisfactory results.

3. MICROBIAL SEWAGE WATER TREATMENT

3.1 Introduction

Sewage refers to wastewater generated from domestic, commercial, institutional and industrial activities. It generally contains human excreta, food residues, detergents, oils, suspended particles, dissolved organic substances, inorganic salts and a large number of microorganisms. Depending on its source, sewage may also contain toxic chemicals, heavy metals and disease-causing microorganisms.

Untreated sewage is one of the major sources of water pollution. When it is directly discharged into rivers, lakes, ponds and other water bodies, the organic matter present in it is decomposed by microorganisms. This process consumes dissolved oxygen from the water and may cause oxygen depletion. As a result, aquatic organisms, particularly fish and other oxygen-dependent organisms, may experience stress or die.

Sewage may also contain pathogens responsible for diseases such as cholera, typhoid, dysentery and hepatitis. Therefore, proper sewage treatment is essential for protecting public health and maintaining aquatic ecosystem quality.

Microorganisms play a central role in sewage treatment because of their ability to decompose complex organic substances and convert them into simpler and less harmful products. Bacteria, fungi, protozoa and archaea participate in different stages of the treatment process. The biological treatment of sewage mainly involves aerobic and anaerobic microbial activities.

3.2 Objectives of Sewage Treatment

The primary objective of sewage treatment is to remove pollutants and improve wastewater quality before it is discharged into the environment or reused. Treatment processes are designed to remove large suspended materials, reduce biodegradable organic matter, remove excess nutrients and decrease the number of pathogenic microorganisms.

One of the most important objectives is the reduction of biochemical oxygen demand (BOD). BOD indicates the amount of dissolved oxygen required by aerobic microorganisms to decompose biodegradable organic matter present in a water sample under specified conditions. It is commonly measured over five days at 20°C and is therefore expressed as BOD_5. High BOD indicates a high concentration of biodegradable organic pollutants, whereas a reduction in BOD indicates that the organic load has been reduced through treatment.

Another important objective is the reduction of chemical oxygen demand (COD), which measures the oxygen equivalent required to chemically oxidise substances present in water. Sewage treatment also aims to remove suspended solids, nitrogen, phosphorus and disease-causing microorganisms.

3.3 Major Stages of Sewage Treatment

Conventional sewage treatment is generally divided into preliminary treatment, primary treatment, secondary treatment and tertiary or advanced treatment. Preliminary and primary treatments mainly involve physical separation processes, whereas secondary treatment relies heavily on microbial activities. Tertiary treatment provides additional purification to meet specific discharge or reuse standards.

A. Preliminary Treatment

Preliminary treatment is the first stage of sewage treatment. Its main purpose is to remove large and heavy materials that may damage pumps, pipes and other treatment equipment. It involves screening and grit removal.

During screening, sewage passes through metal bars or screens that retain large floating materials such as plastics, paper, rags, sticks and other debris. The collected materials are removed and disposed of appropriately.

After screening, the sewage enters a grit chamber, where the flow velocity is controlled to allow heavy inorganic particles such as sand, gravel and small stones to settle. Removal of these materials prevents abrasion and accumulation of sediments in subsequent treatment units.

Preliminary treatment generally does not involve significant microbial decomposition. Instead, it prepares the sewage for the subsequent stages of physical and biological treatment.

B. Primary Treatment

Primary treatment is mainly a physical process involving the removal of suspended solids through sedimentation. The sewage obtained after preliminary treatment is transferred into large sedimentation tanks, where the flow is slowed down to allow heavier suspended particles to settle under gravity.

The settled material accumulates at the bottom of the tank and is known as primary sludge. It contains organic and inorganic solids that have been separated from the wastewater. Oils, grease and other floating substances form a layer on the surface and are removed by skimming equipment.

The partially clarified wastewater, called primary effluent, is then transferred to the secondary treatment unit. Primary treatment significantly reduces suspended solids and a portion of the organic load, but it cannot remove most dissolved biodegradable organic substances. Therefore, secondary biological treatment is necessary.

C. Secondary Treatment or Biological Treatment

Secondary treatment is the most important microbial stage of conventional sewage treatment. It involves the utilisation of microorganisms to decompose dissolved and finely suspended organic matter present in the primary effluent. Under suitable environmental conditions, bacteria and other microorganisms utilise organic substances as sources of carbon and energy and convert them into carbon dioxide, water and new microbial biomass.

Secondary treatment is generally carried out through aerobic biological processes such as the activated sludge process and trickling filter method. These methods depend on the metabolic activities of microbial communities growing either as suspended flocs or attached biofilms.

During aerobic treatment, oxygen is supplied to the wastewater to support the growth and activity of aerobic and facultative microorganisms. The microbial population utilises biodegradable organic substances, thereby reducing the organic pollution load and BOD of the wastewater.

3.4 Activated Sludge Process

The activated sludge process is one of the most widely used biological methods for secondary sewage treatment. It involves the activity of a mixed population of aerobic and facultative microorganisms that decompose organic pollutants present in wastewater. The process is called the activated sludge process because the microbial biomass formed during treatment remains biologically active and can be recycled to maintain a high concentration of microorganisms in the aeration tank.

Working Mechanism of the Activated Sludge Process

1. Entry of Primary Effluent into the Aeration Tank

The partially treated sewage obtained from the primary sedimentation tank is transferred into a large aeration tank. In this tank, air or oxygen is continuously supplied through mechanical aerators or diffusers. Continuous aeration maintains sufficient dissolved oxygen for the growth and metabolic activities of aerobic microorganisms. It also helps keep microbial flocs suspended in the wastewater.

2. Formation of Microbial Flocs

The aeration tank contains a mixed population of bacteria, fungi, protozoa and other microorganisms. These microorganisms grow and aggregate into small, irregular masses known as microbial flocs. Flocs consist mainly of bacterial cells embedded in extracellular polymeric substances (EPS), which help the microorganisms adhere to one another.

Bacteria are the principal organisms responsible for the decomposition of organic matter. Certain bacteria, such as *Zoogloea* species, are particularly important in floc formation because of their ability to produce extracellular polymeric substances. Protozoa and other microorganisms are also present and contribute to the biological stability and clarification of the wastewater.

3. Decomposition of Organic Matter

The microorganisms present in the flocs utilise organic substances present in sewage as sources of carbon and energy. Complex organic compounds are enzymatically broken down into simpler substances and subsequently metabolised through cellular respiration. Under aerobic conditions, organic matter is converted mainly into carbon dioxide, water and new microbial biomass.

The continuous decomposition of organic pollutants results in a considerable reduction in BOD. The microbial population also undergoes multiplication, resulting in an increase in the quantity of activated sludge.

4. Secondary Sedimentation

After sufficient aeration and biological treatment, the mixture of wastewater and microbial flocs, known as mixed liquor, is transferred to a secondary sedimentation tank or secondary clarifier. In this tank, the flow is slowed down, allowing the microbial flocs to settle at the bottom under gravity.

The clear liquid above the settled sludge is called secondary effluent. It contains considerably less biodegradable organic matter than the original sewage and is transferred for further treatment or disinfection.

5. Recycling of Activated Sludge

A portion of the settled microbial sludge is pumped back into the aeration tank. This recycled material is known as return activated sludge (RAS). It contains a large population of active microorganisms that help maintain the required microbial concentration in the aeration tank.

The remaining sludge, known as waste activated sludge (WAS), is removed from the system and transferred for further treatment, generally through sludge digestion and dewatering.

The activated sludge process is highly effective in reducing organic pollution and is widely used in municipal sewage treatment plants.

Microorganisms Involved in the Activated Sludge Process

The activated sludge system contains a complex microbial community in which different organisms perform different functions.

Bacteria: Bacteria are the dominant microorganisms responsible for the decomposition of organic matter. Heterotrophic bacteria utilise dissolved organic substances and convert them into microbial biomass and simpler metabolic products. Genera such as *Pseudomonas*, *Bacillus* and *Zoogloea* are commonly associated with wastewater treatment systems.

Protozoa: Protozoa such as *Vorticella*, *Paramecium*, *Amoeba* and other ciliates feed on free-floating bacteria and fine organic particles. Their feeding activity helps maintain microbial balance and improves the clarity of treated wastewater. The presence and abundance of different protozoan groups can also provide information about the condition and performance of an activated sludge system.

Fungi: Certain fungi participate in the decomposition of complex organic substances, particularly under conditions where the wastewater has a low pH or contains substances that are difficult for bacteria to degrade. However, excessive fungal growth may interfere with normal sludge settling.

Nitrifying bacteria: Specialised microorganisms participate in the oxidation of ammonia into nitrite and nitrate. They are important for nitrogen removal from wastewater.

3.5 Trickling Filter Method

The trickling filter is another important biological method of secondary sewage treatment. Unlike the activated sludge process, where microorganisms remain suspended in the wastewater, the trickling filter method involves the growth of microorganisms attached to a solid supporting material. This type of biological treatment is known as an attached-growth or biofilm process.

A trickling filter generally consists of a tank containing a bed of stones, gravel, plastic modules or other suitable filter media. The surface of these materials provides a large area for the attachment and growth of microorganisms. The microorganisms develop into a biological layer known as a biofilm.

Primary-treated sewage is distributed over the filter bed using rotating distributors or other distribution systems. As the wastewater trickles down through the filter media, organic substances come into contact with the microbial biofilm. Bacteria, fungi, protozoa and other

microorganisms present in the biofilm utilise the organic matter as nutrients and decompose it through their metabolic activities.

Air enters the spaces between the filter media and supplies oxygen required for aerobic microbial activity. As microorganisms grow, the biofilm becomes thicker. Portions of the biofilm may eventually detach from the filter surface and flow along with the treated wastewater. This process is called sloughing. The wastewater is subsequently passed through a secondary clarifier, where the detached microbial biomass is separated from the treated effluent.

The trickling filter method is effective in reducing the organic load of sewage and is commonly used in municipal wastewater treatment systems.

3.6 Anaerobic Treatment of Sewage Sludge

The sludge produced during primary and secondary sewage treatment contains a considerable quantity of organic matter and microbial biomass. If it is not properly treated, it may cause unpleasant odours, attract insects and create environmental and public health problems. Therefore, sewage sludge is generally subjected to further biological treatment.

Anaerobic digestion is an important method used for stabilising sewage sludge. It is a biological process in which microorganisms decompose organic matter in the absence of molecular oxygen. During this process, complex organic substances are converted into simpler compounds, ultimately producing biogas containing mainly methane and carbon dioxide.

Anaerobic digestion is carried out in closed vessels known as anaerobic digesters. These digesters provide oxygen-free conditions that support the growth of anaerobic bacteria and methanogenic archaea. The process generally occurs through four major stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis.

A. Hydrolysis

Hydrolysis is the first stage of anaerobic digestion. During this stage, complex organic substances present in sewage sludge, such as carbohydrates, proteins and lipids, are broken down into simpler soluble compounds.

Carbohydrates are converted into simple sugars, proteins into amino acids and lipids into fatty acids and glycerol. These reactions are catalysed by extracellular enzymes produced by hydrolytic microorganisms. Hydrolysis is particularly important because many complex organic substances cannot be directly utilised by the microorganisms involved in the subsequent stages.

B. Acidogenesis

Acidogenesis is the second stage of anaerobic digestion. During this stage, the soluble products formed during hydrolysis are fermented by acidogenic bacteria into various simpler substances. These include volatile fatty acids, alcohols, hydrogen, carbon dioxide and other organic compounds.

Examples of acidogenic bacteria include different species of *Clostridium* and other fermentative anaerobes. The accumulation of organic acids may lower the pH of the digester if their production exceeds their consumption.

C. Acetogenesis

Acetogenesis is the third stage of anaerobic digestion. During this stage, the organic acids and alcohols produced during acidogenesis are converted into acetate, hydrogen and carbon dioxide by acetogenic microorganisms.

These products are important because acetate and hydrogen serve as major substrates for methanogenic archaea during the final stage of anaerobic digestion. The proper functioning of acetogenic microorganisms is therefore essential for maintaining the balance of the overall digestion process.

D. Methanogenesis

Methanogenesis is the final stage of anaerobic digestion. During this stage, methanogenic archaea convert acetate, hydrogen and carbon dioxide into methane and carbon dioxide. Methanogens are strictly anaerobic microorganisms and are highly sensitive to oxygen.

Methane may be produced through two major pathways. In the acetoclastic pathway, acetate is converted into methane and carbon dioxide. In the hydrogenotrophic pathway, carbon dioxide is reduced by hydrogen to produce methane.

Important methanogenic archaea include *Methanobacterium*, *Methanococcus* and *Methanosarcina*.

The biogas produced during anaerobic digestion contains mainly methane and carbon dioxide, along with small quantities of other gases such as hydrogen sulphide. Methane is combustible and can be used as a source of energy for heating, electricity generation and other purposes. The digested sludge may also be further processed for safe disposal or beneficial use, depending on its quality and regulatory requirements.

3.7 Tertiary Treatment of Sewage

Tertiary treatment, also known as advanced wastewater treatment, is the final stage of sewage treatment. It is carried out when additional purification is required beyond the level achieved through primary and secondary treatment. The main purpose is to remove residual suspended particles, excess nutrients, dissolved pollutants and pathogenic microorganisms.

The exact treatment methods depend on the quality of wastewater and the intended use of the treated water. Tertiary treatment is particularly important when wastewater is intended for agricultural irrigation, industrial reuse or discharge into environmentally sensitive water bodies.

A. Filtration

Filtration involves passing treated wastewater through suitable filtering materials to remove fine suspended particles that may remain after secondary treatment. Sand filters, multimedia filters and other filtration systems are commonly used. This process improves water clarity and helps prepare wastewater for subsequent disinfection.

B. Nutrient Removal

Wastewater may contain excess nitrogen and phosphorus derived from human excreta, detergents and other organic wastes. If these nutrients enter rivers and lakes in excessive

quantities, they can stimulate the rapid growth of algae and aquatic plants, resulting in eutrophication.

Biological nutrient removal involves specialised microorganisms that convert nitrogen into gaseous forms or accumulate phosphorus within their cells. Nitrogen removal is generally achieved through nitrification and denitrification, whereas phosphorus removal may involve specialised phosphorus-accumulating organisms.

C. Disinfection

Disinfection is an important final treatment process designed to reduce the number of disease-causing microorganisms present in wastewater. Common disinfection methods include chlorination, ultraviolet radiation and ozonation.

Chlorination involves the addition of chlorine or chlorine-based compounds to wastewater. These compounds damage microbial cellular components and inactivate many pathogenic microorganisms.

Ultraviolet radiation damages microbial genetic material, particularly DNA and RNA, thereby preventing microorganisms from reproducing. Ozonation involves the use of ozone, a strong oxidising agent that damages microbial cells and oxidises certain organic substances.

Disinfection is particularly important before treated wastewater is discharged into water bodies or reused for applications where human exposure is possible.

3.8 Biological Removal of Nitrogen from Wastewater

Nitrogen is an important component of domestic and industrial wastewater. It occurs in different forms, including organic nitrogen, ammonia, nitrite and nitrate. Excessive discharge of nitrogen into natural water bodies can contribute to eutrophication and deterioration of water quality. Microorganisms play an essential role in nitrogen removal through two major biological processes known as nitrification and denitrification.

A. Nitrification

Nitrification is an aerobic biological process in which ammonia or ammonium is oxidised into nitrite and subsequently nitrate by specialised microorganisms. This process occurs in the presence of oxygen and is carried out mainly by autotrophic nitrifying bacteria and archaea.

The process involves two major steps. During the first step, ammonia is oxidised into nitrite by ammonia-oxidising microorganisms such as *Nitrosomonas*. During the second step, nitrite is oxidised into nitrate by nitrite-oxidising microorganisms such as *Nitrospira*.

The overall process can be represented as:

$$NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$$

Nitrification reduces the concentration of ammonia in wastewater. However, nitrate may still remain in the treated effluent and can contribute to eutrophication. Therefore, nitrification is often followed by denitrification.

B. Denitrification

Denitrification is a biological process in which nitrate is reduced to gaseous nitrogen compounds under anoxic conditions, where oxygen is absent but nitrate is available as an alternative electron acceptor.

During this process, denitrifying bacteria use organic carbon compounds as electron donors and convert nitrate into nitrite, nitric oxide, nitrous oxide and finally nitrogen gas.

The process can be represented as:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

Examples of denitrifying bacteria include *Pseudomonas* and *Paracoccus*. The nitrogen gas produced is released into the atmosphere, thereby reducing the nitrogen content of wastewater.

Nitrification and denitrification together constitute an important biological nitrogen removal system used in modern sewage treatment plants.

3.9 Biological Removal of Phosphorus

Phosphorus is another important nutrient present in domestic and industrial wastewater. Excessive phosphorus discharge into lakes and ponds can stimulate algal blooms and lead to eutrophication. Biological phosphorus removal is achieved through the activity of specialised microorganisms known as phosphorus-accumulating organisms (PAOs).

These microorganisms have the ability to take up and store phosphorus inside their cells in the form of polyphosphate granules. During enhanced biological phosphorus removal, PAOs are exposed to alternating anaerobic and aerobic conditions.

Under anaerobic conditions, these organisms take up suitable organic compounds, particularly volatile fatty acids, and release phosphate into the surrounding water while utilising stored intracellular energy. Under subsequent aerobic conditions, they take up phosphate in quantities greater than those released and store it as intracellular polyphosphate.

The phosphorus is ultimately removed from the treatment system when excess microbial sludge containing polyphosphate is withdrawn. This process is known as enhanced biological phosphorus removal (EBPR).

3.10 Importance of Microorganisms in Sewage Treatment

Microorganisms are responsible for the biological degradation of organic pollutants and the transformation of several inorganic substances present in sewage. Heterotrophic bacteria utilise organic matter and reduce the BOD of wastewater. Nitrifying bacteria convert ammonia into nitrate, while denitrifying bacteria convert nitrate into nitrogen gas. Methanogenic archaea produce methane during anaerobic sludge digestion.

Protozoa help improve the quality of treated wastewater by consuming free-floating bacteria and fine organic particles. Certain fungi participate in the degradation of complex organic compounds, while specialised bacteria accumulate excess phosphorus within their cells.

Thus, the combined activities of different microbial groups make biological sewage treatment an efficient and essential process for wastewater management.

3.11 Advantages and Limitations of Microbial Sewage Treatment

Microbial sewage treatment offers several environmental and economic advantages. It reduces the organic pollution load of wastewater and decreases BOD, thereby helping protect dissolved oxygen levels in receiving water bodies. Biological treatment also contributes to nitrogen and phosphorus removal, reducing the possibility of eutrophication. Anaerobic digestion allows the recovery of methane-rich biogas from organic sludge, providing an additional source of renewable energy.

However, microbial sewage treatment requires proper environmental control. Temperature, pH, dissolved oxygen, nutrient availability and retention time influence microbial activity and treatment efficiency. Toxic chemicals and heavy metals may inhibit microbial growth. Improper aeration, excessive sludge production and poor operational management can also affect treatment performance. Therefore, regular monitoring and maintenance are necessary for the effective functioning of sewage treatment plants.

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