BT512 – MICROBIAL BIOTECHNOLOGY

FINAL TERM IMPORTANT QUESTIONS

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Admin: **BS Biological Sciences** (One place to all your solutions)

1. What are the criteria for microorganism to include it in probiotic group?

1. Safety (GRAS Status)

A probiotic microorganism must be non-pathogenic, non-toxic, and safe for human consumption. It should not produce harmful metabolites or exhibit hemolytic activity. The strain must lack transferable antibiotic resistance genes and should be genetically stable to prevent mutation into virulent forms.

2. Ability to Survive Gastrointestinal Conditions

The microorganism must tolerate acidic gastric pH (2–3) and resist bile salts present in the small intestine. It should also withstand digestive enzymes such as pepsin and pancreatin. Survival through the GIT ensures that an adequate number of viable cells reach the colon.

3. Adhesion and Colonization Ability

Probiotic strains must adhere to intestinal epithelial cells and mucus layers. Adhesion allows temporary colonization and prolonged interaction with the host. Surface proteins and exopolysaccharides often facilitate this attachment and competitive exclusion of pathogens.

4. Antimicrobial Activity

A suitable probiotic should inhibit pathogenic microorganisms by producing bacteriocins, hydrogen peroxide, or organic acids such as lactic acid. These metabolites lower intestinal pH and suppress pathogen growth. Competitive exclusion for nutrients and binding sites also contributes to antimicrobial action.

5. Immunomodulatory Effect

The strain should stimulate the host immune system by enhancing IgA secretion and activating macrophages and dendritic cells. It may regulate cytokine production and balance pro-inflammatory and anti-inflammatory responses. This helps in maintaining gut immune homeostasis.

6. Technological Suitability

The microorganism must remain viable during industrial processing such as freeze-drying, spray drying, or encapsulation. It should maintain high cell counts during storage and have good shelf stability. Resistance to oxygen and temperature fluctuations is also important for commercial production.

7. Documented Health Benefits

The probiotic strain must demonstrate clinically proven health benefits in controlled studies. The beneficial effect should be strain-specific and dose-dependent. Effective concentration is generally between 10^6–10^9 CFU per day.

8. Metabolic Activity in Host

A probiotic should produce beneficial metabolites such as short-chain fatty acids (SCFAs), vitamins (e.g., B-complex), or enzymes like lactase. These metabolic activities improve nutrient absorption and gut health. Functional metabolic contribution is essential for its therapeutic value.

2. What is biodiesel and how it is produced?

Biodiesel

Biodiesel is a renewable, biodegradable biofuel composed of fatty acid methyl esters (FAME) produced from biological sources such as vegetable oils, animal fats, waste cooking oil, or microbial lipids. In microbial biotechnology, biodiesel production is linked to oleaginous microorganisms such as algae, yeast, and certain bacteria that accumulate high intracellular lipids.

Chemically, biodiesel is produced through transesterification, where triglycerides react with an alcohol (usually methanol or ethanol) in the presence of a catalyst to form fatty acid esters and glycerol as a by-product.

$$Triglyceride + 3Methanol- > 3FattyAcidMethylEsters + Glycerol$$

Production of Biodiesel (Microbial Biotechnology Approach)

1. ***Feedstock Selection***
   Lipid-rich sources such as microalgae (e.g., Chlorella, Spirulina), oleaginous yeast (e.g., Yarrowia lipolytica), and agricultural oils are selected. Microbial lipids are preferred because they do not compete with food crops.

2. ***Cultivation of Microorganisms***
   Microbes are grown in bioreactors under optimized conditions (nitrogen limitation enhances lipid accumulation). Under stress conditions, cells accumulate lipids up to 20–70% of dry cell weight.

3. ***Lipid Extraction***
   Biomass is harvested and lipids are extracted using solvents (hexane, chloroform-methanol) or mechanical disruption techniques.

4. ***Transesterification Process***
   Extracted triglycerides are reacted with methanol in presence of acid, alkali (NaOH/KOH), or lipase enzyme catalyst. Enzymatic transesterification using microbial lipases is an eco-friendly alternative.

5. ***Separation and Purification***
   Biodiesel (upper layer) is separated from glycerol (lower layer). The product is washed and purified to remove residual catalyst and alcohol.

3. What are main objectives of transgenic plants?

Main Objectives of Transgenic Plants

1. ***Insect Resistance***
   Introduction of genes such as *Bt toxin gene* from *Bacillus thuringiensis* enables plants to produce insecticidal proteins. This reduces crop loss and minimizes the use of chemical pesticides.
2. ***Herbicide Resistance***
   Transgenic plants are engineered to tolerate specific herbicides (e.g., glyphosate). This allows selective weed control without damaging the crop.
3. ***Disease Resistance***
   Genes conferring resistance against viral, bacterial, and fungal pathogens are inserted to enhance plant immunity. Example: coat protein genes for viral resistance.
4. ***Abiotic Stress Tolerance***
   Genetic modification improves tolerance to drought, salinity, heat, and cold. Stress-responsive genes help plants survive adverse environmental conditions.
5. ***Improved Nutritional Quality (Biofortification)***
   Transgenic plants are engineered to enhance vitamins, minerals, and essential amino acids. Example: Vitamin A-enriched crops to combat malnutrition.
6. ***Enhanced Yield and Productivity***
   Genes regulating growth hormones and metabolic pathways are modified to increase biomass and grain yield.
7. ***Delayed Ripening and Extended Shelf Life***
   Suppression of ethylene production or cell wall–degrading enzymes delays fruit ripening. This reduces post-harvest losses and improves storage life.
8. ***Production of Recombinant Proteins (Molecular Farming)***
   Plants are engineered to produce vaccines, antibodies, and therapeutic proteins. This provides a cost-effective and scalable production system.
9. ***Industrial Product Formation***
   Modification of metabolic pathways enables production of biodegradable plastics, biofuels, and industrial enzymes in plants.

4. What is second generation biofuel?

Second Generation Biofuel

Second generation biofuels are advanced biofuels produced from non-food lignocellulosic biomass such as agricultural residues (wheat straw, rice husk), forest waste, wood chips, and dedicated energy crops (switchgrass). Unlike first-generation biofuels, they do not compete directly with food crops and are considered more sustainable. These biofuels are mainly derived from cellulose, hemicellulose, and lignin components of plant cell walls. Microbial biotechnology plays a key role in converting complex polysaccharides into fermentable sugars using cellulases and hemicellulases, followed by microbial fermentation to produce bioethanol, biobutanol, or biohydrogen.

5. What is metabolomics?

Metabolomics is the comprehensive study of all small molecular weight metabolites (metabolome) present within a cell, tissue, or organism under specific conditions. It reflects the functional state of cellular metabolism and provides a direct link between genotype and phenotype. In microbial biotechnology, metabolomics is used to analyze metabolic pathways, optimize fermentation processes, improve strain performance, and enhance production of antibiotics, enzymes, biofuels, and other industrial metabolites using techniques such as GC–MS, LC–MS, and NMR.

6. Define bio-pesticides and bio-fertilizers.

Bio-pesticides are formulations containing living microorganisms or their metabolites that control pests, pathogens, or weeds through mechanisms such as parasitism, predation, toxin production, or competition, offering an eco-friendly alternative to chemical pesticides. Examples include *Bacillus thuringiensis* (insecticidal bacteria) and *Trichoderma* spp. (fungal pathogen control).

Bio-fertilizers are preparations containing live microorganisms that enhance soil fertility by fixing atmospheric nitrogen, solubilizing phosphorus, or producing growth-promoting substances, thus improving plant nutrient uptake and growth. Examples include *Rhizobium* (nitrogen fixation), *Azotobacter* (free-living nitrogen fixer), and phosphate-solubilizing bacteria.

7. Write two examples of bio-fertilizers.

Rhizobium – Symbiotic nitrogen-fixing bacteria associated with leguminous plants, converting atmospheric nitrogen into a form usable by plants.

Azotobacter – Free-living nitrogen-fixing bacteria that enhance soil nitrogen content and promote growth of non-leguminous crops.

8. Describe genome sequencing of viruses.

Genome Sequencing of Viruses

Viral genome sequencing is the process of determining the complete nucleotide sequence of a virus's genetic material, which can be DNA or RNA. It involves several steps:

1. ***Viral Isolation and Purification*** – Virus particles are isolated from infected hosts or cell cultures and purified to remove host nucleic acids.
2. ***Nucleic Acid Extraction*** – Viral DNA or RNA is extracted using chemical or enzymatic methods. RNA viruses are first converted to complementary DNA (cDNA) using reverse transcriptase.
3. ***Library Preparation*** – The extracted nucleic acids are fragmented and adapters are added to create a sequencing library compatible with high-throughput sequencing platforms.
4. ***Sequencing*** – Techniques like Next-Generation Sequencing (NGS), Illumina sequencing, or Nanopore sequencing are used to read the nucleotide sequence.
5. ***Data Analysis*** – Bioinformatics tools assemble sequence reads into a complete genome, annotate genes, and identify mutations, variants, or phylogenetic relationships.

9. Proteins do function with other proteins. Explain with an example.

Proteins often perform their functions by interacting with other proteins to form complexes or signaling pathways. These protein–protein interactions are essential for structural support, enzymatic activity, transport, and regulation of cellular processes.

Example: In microbial biotechnology, the ATP synthase complex in bacteria consists of multiple protein subunits (F_0 and F_1 components) that work together to synthesize ATP. F_0 forms a proton channel across the membrane, while F_1 contains catalytic subunits that convert ADP and inorganic phosphate into ATP. Alone, none of the subunits can produce ATP efficiently; only the assembled multi-protein complex performs the function.

Another example is two-component regulatory systems in bacteria, where a sensor kinase protein phosphorylates a response regulator protein to control gene expression in response to environmental signals.

10. What is microarray?

Microarray is a high-throughput technology used to simultaneously measure the expression levels of thousands of genes or detect specific DNA/RNA sequences. It consists of a solid surface (glass, silicon, or nylon) onto which DNA probes or oligonucleotides are immobilized in a grid-like pattern. In microbial biotechnology, microarrays are used to study gene expression profiles under different environmental conditions, identify pathogen-specific genes, monitor metabolic pathways, and screen for mutations or antibiotic resistance genes. Target nucleic acids from the sample are fluorescently labeled and hybridized to the probes, and fluorescence intensity at each spot indicates the presence and abundance of the corresponding gene.

11. Explain polyesters.

Polyesters are polymers formed by the condensation reaction between diacids (or their derivatives) and diols, resulting in ester bonds (–COO–) linking repeating monomer units. They can be synthetic, like polyethylene terephthalate (PET) used in plastics and textiles, or biodegradable, like polyhydroxyalkanoates (PHAs) produced by microorganisms. In microbial biotechnology, microbial polyesters (PHAs) are accumulated as intracellular energy storage granules by bacteria such as *Cupriavidus necator* and *Bacillus* spp. These biopolyesters are biodegradable, biocompatible, and used in medical applications, packaging, and bio-plastics, providing a sustainable alternative to petrochemical plastics.

12. Write benefits of composing.

Benefits of Composting

1. ***Soil Fertility Improvement*** – Compost adds organic matter, nutrients (N, P, K), and beneficial microbes to the soil, enhancing its structure, aeration, and water-holding capacity.
2. ***Waste Management*** – Converts kitchen, agricultural, and garden waste into useful organic fertilizer, reducing landfill load and environmental pollution.
3. ***Reduction of Chemical Fertilizer Use*** – Provides natural nutrients, lowering dependency on synthetic fertilizers and reducing chemical runoff into water bodies.
4. ***Microbial Diversity Enhancement*** – Encourages growth of beneficial microorganisms that suppress soil-borne pathogens and promote plant growth.
5. ***Environmental Benefits*** – Reduces methane emission from organic waste in landfills and helps in carbon sequestration, contributing to climate change mitigation.
6. ***Cost-Effective and Sustainable*** – Produces free or low-cost organic fertilizer, supporting sustainable agriculture and circular economy practices.

13. Discuss structure of Xanthan gum.

Structure of Xanthan Gum

Xanthan gum is a microbial polysaccharide produced by *Xanthomonas campestris*. Its structure consists of a cellulose-like backbone of β-(1→4)-linked D-glucose units, similar to cellulose, with trisaccharide side chains attached to alternate glucose residues. Each side chain contains one D-mannose–D-glucuronic acid–D-mannose unit, where the terminal mannose can be acetylated or pyruvylated.

This branched structure gives xanthan gum high viscosity, pseudoplasticity, and stability in solution. The presence of charged groups (carboxyl of glucuronic acid and pyruvate residues) allows it to form thick, shear-thinning solutions, making it widely used in food, pharmaceuticals, and industrial applications as a stabilizer, thickener, and emulsifier.

14. What are types of bio-fertilizers?

Types of Bio-fertilizers

1. ***Nitrogen-Fixing Bio-fertilizers*** – Microorganisms that convert atmospheric nitrogen into ammonia, making it available to plants. **Examples**: *Rhizobium* (legumes), *Azotobacter* (non-legumes), *Azospirillum* (cereals).
2. ***Phosphate-Solubilizing Bio-fertilizers*** – Microbes that solubilize insoluble phosphorus compounds in soil, enhancing phosphorus availability. **Examples**: *Bacillus megaterium*, *Pseudomonas striata*.
3. ***Potassium-Solubilizing Bio-fertilizers*** – Microorganisms that solubilize potassium from minerals for plant uptake. **Examples**: *Frateuria aurantia*, *Bacillus mucilaginosus*.
4. ***Cyanobacterial Bio-fertilizers*** – Photosynthetic cyanobacteria that fix atmospheric nitrogen in paddy fields. **Examples**: *Anabaena*, *Nostoc*.
5. ***Mycorrhizal Bio-fertilizers*** – Symbiotic fungi that enhance nutrient (P, Zn) and water uptake by plants. **Examples**: *Glomus* spp., *Gigaspora* spp.
6. ***Vermicompost/Organic Bio-fertilizers*** – Microbially enriched organic matter that improves soil fertility and microbial activity.

15. How biofuels are used to charge electronics?

Biofuels for Charging Electronics

Biofuels can be converted into electricity to power or charge electronic devices through microbial fuel cells (MFCs) or bio-batteries. In an MFC, electrogenic microorganisms (e.g., *Shewanella*, *Geobacter*) oxidize organic substrates from biofuels such as glucose, ethanol, or biodiesel-derived compounds. During this metabolic process, electrons are transferred to an anode, flow through an external circuit, and generate electric current, which can be used to charge batteries or directly power small electronics.

16. Can we produce a protein array?

Yes, a protein array can be produced. A protein array is a high-throughput platform in which multiple proteins are immobilized on a solid surface (glass, nitrocellulose, or microchip) in a defined pattern to simultaneously analyze protein expression, interactions, or activity. There are two main types:

1. ***Analytical Protein Arrays*** – Detect and quantify proteins or antibodies in complex samples. **Example**: antibody arrays for biomarker screening.

2. ***Functional Protein Arrays*** – Contain active proteins to study protein–protein, protein–DNA, or protein–ligand interactions.

17. What is antimicrobial pesticides?

Antimicrobial Pesticides are bio-based or chemical agents that inhibit the growth of or kill microorganisms such as bacteria, fungi, or viruses that cause plant diseases. They are used in agriculture to protect crops from microbial pathogens without harming beneficial organisms. Examples include:

- ***Bacterial pesticides:*** *Bacillus thuringiensis* toxins targeting insect vectors that carry pathogens.
- ***Fungal pesticides:*** *Trichoderma* spp. that suppress plant pathogenic fungi.
- ***Natural antimicrobial compounds:*** Neem extracts, chitosan, or microbial metabolites like bacteriocins.

18. What are methods used to add bio-fertilizers to the crop?

Methods of Applying Bio-fertilizers to Crops

1. ***Seed Treatment (Seed Inoculation)*** – Seeds are coated with a bio-fertilizer slurry containing live microorganisms before sowing. Example: *Rhizobium* on legume seeds.
2. ***Soil Application (Soil Inoculation)*** – Bio-fertilizers are mixed directly into the soil or placed in the planting furrows to enhance microbial colonization around roots.
3. ***Root or Seedling Dipping*** – Roots of seedlings are dipped in a microbial suspension before transplanting, ensuring immediate contact with beneficial microbes.
4. ***Foliar Spray*** – Microbial suspensions are sprayed on leaves to promote nutrient uptake or provide plant growth–promoting effects, though less common than soil or seed methods.
5. ***Combination with Organic Manure*** – Bio-fertilizers are mixed with compost or farmyard manure to improve microbial survival and nutrient availability.

19. Differentiate between proteomics and transcriptomics.

Proteomics vs. Transcriptomics

Proteomics is the large-scale study of the complete set of proteins (proteome) expressed by a cell, tissue, or organism, focusing on protein structure, function, interactions, and post-translational modifications. It provides a direct view of the functional molecules driving cellular processes. Transcriptomics, on the other hand, analyzes the entire set of RNA transcripts (transcriptome) produced under specific conditions, reflecting gene expression patterns but not necessarily protein abundance or activity. While transcriptomics identifies which genes are being transcribed, proteomics reveals which proteins are present, their modifications, and interactions, making it more closely linked to phenotype and cellular function.

20. What are nitrogen bio-fertilizers?

Nitrogen Bio-fertilizers are preparations containing living microorganisms that fix atmospheric nitrogen (N_2) into ammonia or other bioavailable forms for plants, reducing the need for chemical nitrogen fertilizers. They enhance soil fertility and promote plant growth by supplying nitrogen naturally. Examples include:

- ***Rhizobium*** – Symbiotic bacteria forming nodules on legume roots to fix nitrogen.
- ***Azotobacter*** – Free-living nitrogen-fixing bacteria for non-leguminous crops.
- ***Azospirillum*** – Associative nitrogen-fixing bacteria promoting cereals and grasses.
- ***Cyanobacteria*** – Photosynthetic microbes like *Anabaena* and *Nostoc* that fix nitrogen in paddy fields.

21. Define prebiotics.

Prebiotics are non-digestible food ingredients, usually oligosaccharides or dietary fibers, that selectively stimulate the growth or activity of beneficial microorganisms in the gut, such as *Lactobacillus* and *Bifidobacterium*. They enhance host health by improving gut microbiota composition, promoting nutrient absorption, and supporting immune function without being metabolized by the host.

22. Define types of fermentation.

Types of Fermentation

1. ***Alcoholic Fermentation*** – Anaerobic conversion of sugars into ethanol and carbon dioxide by microorganisms such as *Saccharomyces cerevisiae*. Used in brewing, winemaking, and bioethanol production.
2. ***Lactic Acid Fermentation*** – Anaerobic metabolism of glucose to lactic acid by lactic acid bacteria (*Lactobacillus*, *Streptococcus*). Used in yogurt, cheese, and probiotic foods.
3. ***Acetic Acid Fermentation*** – Oxidation of ethanol to acetic acid by acetic acid bacteria (*Acetobacter*), used in vinegar production.
4. ***Butyric Acid Fermentation*** – Anaerobic conversion of carbohydrates into butyric acid, CO_2, and H_2 by *Clostridium* species, applied in biofuel and industrial chemical production.
5. ***Mixed Acid Fermentation*** – Formation of multiple organic acids (lactic, acetic, formic, succinic) and gases by enteric bacteria like *Escherichia coli*.
6. ***Propionic Acid Fermentation*** – Conversion of lactate to propionic acid and CO_2 by *Propionibacterium*, used in cheese ripening and industrial propionate production.

23. Explain economic development in biotechnology.

Economic Development in Biotechnology

Biotechnology contributes to economic development by creating high-value products, generating employment, and promoting sustainable industries. Microbial biotechnology enables production of biofuels, enzymes, antibiotics, vaccines, and bioplastics, reducing dependence on imports and petrochemicals. Agricultural biotechnology increases crop yield and reduces losses through bio-fertilizers, bio-pesticides, and transgenic crops, enhancing food security and farm incomes. Industrial-scale microbial fermentation and bioprocessing open new markets for pharmaceuticals, nutraceuticals, and specialty chemicals, attracting investments and supporting biotech startups. Additionally, innovations in molecular diagnostics, personalized medicine, and microbial metabolomics stimulate the health sector and research-driven economy. Overall, biotechnology integrates science and commerce to drive technological growth, environmental sustainability, and global competitiveness.

24. Write a note of whole genome sequencing.

Whole Genome Sequencing (WGS)

Whole genome sequencing is the process of determining the complete DNA sequence of an organism's genome at a single time, including coding and non-coding regions. In microbial biotechnology, WGS is used to analyze bacteria, viruses, and fungi for strain identification, evolutionary studies, pathogenicity assessment, and metabolic potential. The process involves isolation of genomic DNA**,** library preparation with fragmentation and adapter ligation, high-throughput sequencing using platforms like Illumina, PacBio, or Nanopore, and bioinformatic assembly and annotation of the sequence reads. WGS allows detection of mutations, antibiotic resistance genes, virulence factors, and horizontal gene transfer events, supporting microbial strain improvement, epidemiological tracking, and development of vaccines or therapeutics.

25. Write role of microbes in polysaccharides.

- Microbes synthesize exopolysaccharides (EPS) such as xanthan gum (*Xanthomonas campestris*), dextran (*Leuconostoc* spp.), and curdlan (*Agrobacterium* spp.) used as thickeners, stabilizers, and gelling agents.
- They produce intracellular storage polysaccharides like glycogen and microbial starch as carbon and energy reserves.
- Microbial enzymes (amylases, cellulases, pectinases) degrade complex polysaccharides for biofuel production, bioremediation, and industrial applications.
- Microbial polysaccharides contribute to biofilm formation, enhancing microbial survival and adhesion.
- They have industrial, pharmaceutical, and food applications, including viscosity modification, drug delivery, and immunomodulatory roles.

26. Explain role of biotechnology in agriculture.

Role of Biotechnology in Agriculture

- ***Crop Improvement*** – Genetic engineering produces transgenic crops with enhanced yield, pest resistance, herbicide tolerance, and stress tolerance (drought, salinity, heat).
- ***Bio-fertilizers*** – Microbial inoculants like *Rhizobium*, *Azotobacter*, and cyanobacteria fix atmospheric nitrogen, solubilize phosphorus, and promote nutrient uptake, reducing chemical fertilizer use.
- ***Bio-pesticides*** – Microbial products such as *Bacillus thuringiensis* and *Trichoderma* spp. control insect pests and plant pathogens sustainably.
- ***Disease Diagnostics*** – Molecular tools (PCR, ELISA, DNA microarrays) allow early detection of plant pathogens, minimizing crop losses.
- ***Post-Harvest Technology*** – Biotechnological interventions extend shelf life of fruits and vegetables using controlled ripening, edible coatings, and microbial treatments.
- ***Plant Tissue Culture*** – Micropropagation produces disease-free, high-quality planting material rapidly and maintains genetic uniformity.
- ***Soil Health and Waste Management*** – Microbes degrade organic waste into compost, enhancing soil fertility and sustainability.
- ***Biofortification*** – Genetic modification enhances nutritional content of crops (vitamins, minerals, essential amino acids), improving human health.

27. What are types of prebiotics?

Types of Prebiotics

- ***Fructo-oligosaccharides (FOS)*** – Short-chain fructose polymers that selectively stimulate *Bifidobacterium* and *Lactobacillus* growth.
- ***Galacto-oligosaccharides (GOS)*** – Derived from lactose, promote beneficial gut bacteria and improve calcium absorption.
- ***Inulin*** – Naturally occurring polysaccharide from plants (chicory, garlic) that enhances gut microbiota and improves bowel health.
- ***Lactulose*** – Synthetic disaccharide that resists digestion in the upper GI tract and supports beneficial bacteria in the colon.
- ***Xylo-oligosaccharides (XOS)*** – Derived from xylan in plant cell walls; selectively stimulate *Bifidobacteria* and improve intestinal health.
- ***Other oligosaccharides*** – Arabinooligosaccharides, pectin-derived oligosaccharides, and resistant starches acting as selective microbial substrates.

28. What is prokaryotic genome?

Prokaryotic Genome

A prokaryotic genome is the complete set of genetic material present in a prokaryotic cell, including chromosomal DNA and extrachromosomal elements. The chromosomal DNA is typically a single, circular double-stranded DNA molecule containing most essential genes for growth, metabolism, and reproduction. Many prokaryotes also carry plasmids, small circular DNA molecules that replicate independently and often encode beneficial traits such as antibiotic resistance, virulence factors, or metabolic enzymes. Prokaryotic genomes are compact, lack introns, and have high gene density, allowing rapid adaptation and expression of metabolic pathways, which is exploited in microbial biotechnology for recombinant protein production, metabolic engineering, and synthetic biology.

29. How prebiotics are produced?

Production of Prebiotics

Prebiotics are produced mainly through enzymatic or microbial processes that generate non-digestible oligosaccharides from natural carbohydrates:

- ***Fructo-oligosaccharides (FOS)*** – Produced by enzymatic hydrolysis of inulin from plants (chicory, Jerusalem artichoke) using inulinase or by transfructosylation with fructosyltransferase from microbes like *Aspergillus niger*.
- ***Galacto-oligosaccharides (GOS)*** – Synthesized from lactose using β-galactosidase enzymes from *Kluyveromyces lactis* or *Bacillus* spp., forming oligosaccharides with prebiotic activity.
- ***Xylo-oligosaccharides (XOS)*** – Produced by enzymatic hydrolysis of xylan-rich plant materials (corn cobs, wheat straw) using xylanases from bacteria or fungi.
- ***Inulin*** – Extracted directly from inulin-rich plants and purified; can also be partially hydrolyzed to obtain short-chain FOS.

30. Write techniques of percolator leaching.

Techniques of Percolator Leaching

Percolator leaching is a hydrometallurgical method used to extract soluble metals from ores using a percolating solvent. The main techniques include:

1. ***Heap Leaching*** – Crushed ore is piled into heaps on an impermeable pad, and leaching solution (e.g., cyanide for gold) is percolated from the top. The metal-laden solution is collected at the base for recovery.
2. ***Column Leaching*** – Ore is placed in vertical columns, and the leaching solvent is passed through under controlled flow. It allows better control of retention time, temperature, and solution distribution.
3. ***Tank Leaching (Percolation in Tanks)*** – Finely ground ore is loaded into tanks, and the leaching solution is circulated or percolated through the ore. It provides faster extraction and higher recovery than heap leaching.
4. ***In-Situ Leaching (Solution Mining)*** – The leaching solution is injected directly into the ore body underground, dissolving metals without mining the ore physically. The metal-rich solution is pumped out for processing.

31. Why is it important to use cotton plug during fermentation?

Using a cotton plug during fermentation is important because it allows gas exchange while preventing contamination. Microbial fermentation often produces gases like CO_2, which need to escape to avoid pressure buildup. The cotton plug acts as a sterile barrier, blocking dust, airborne microbes, and other contaminants from entering the culture vessel, ensuring a pure and controlled microbial growth environment for efficient fermentation.

32. What is fermentation and what key microorganisms are involved in this process?

Fermentation

Fermentation is an anaerobic metabolic process in which microorganisms convert carbohydrates (like glucose, sucrose, or lactose) into simpler compounds such as organic acids, alcohols, gases, or other metabolites, releasing energy for microbial growth. It is widely used in food, beverage, pharmaceutical, and biofuel industries.

Key Microorganisms Involved:

- ***Yeasts*** – *Saccharomyces cerevisiae* (ethanol production in alcoholic fermentation).
- ***Lactic Acid Bacteria (LAB)*** – *Lactobacillus*, *Streptococcus*, *Leuconostoc* (lactic acid fermentation in yogurt, cheese, and fermented vegetables).
- ***Acetic Acid Bacteria*** – *Acetobacter*, *Gluconobacter* (conversion of ethanol to acetic acid in vinegar production).
- ***Clostridium Species*** – *Clostridium butyricum*, *Clostridium acetobutylicum* (butyric acid, acetone-butanol fermentation).
- ***Propionibacterium*** – *Propionibacterium freudenreichii* (propionic acid fermentation in Swiss cheese).

33. Write advantages of bio-pesticides.

Advantages of Bio-pesticides

- ***Environmentally Friendly*** – They are biodegradable and don't leave toxic residues in soil, water, crops.
- ***Target-Specific*** – Act against specific pests or pathogens without harming beneficial insects, animals, or humans.
- ***Reduced Chemical Usage*** – Decrease reliance on synthetic pesticides, low chemical pollution & cost.
- ***Resistance Management*** – Reduces development of pest resistance compared to repeated chemical pesticide use.
- ***Sustainable Agriculture*** – Promote integrated pest management (IPM) and long-term soil and crop health.
- ***Safety for Humans and Livestock*** – Lower toxicity reduces health hazards for farmers and consumers.
- ***Enhance Crop Productivity*** – By controlling pests naturally, they indirectly support higher yield and quality.

34. Write types of interaction of microbe and plants.

Types of Microbe–Plant Interactions

- ***Symbiotic Interaction*** – Both the plant and microbe benefit. Example: *Rhizobium* forms root nodules on legumes, fixing nitrogen for the plant while receiving carbohydrates.
- ***Commensalism*** – Microbe benefits without affecting the plant. **Example**: Epiphytic bacteria on leaf surfaces that utilize plant exudates without harming the host.
- ***Parasitism/Pathogenic Interaction*** – Microbe benefits at the expense of the plant, causing disease. **Example**: *Agrobacterium tumefaciens* causing crown gall disease.
- ***Mutualism*** – Similar to symbiosis, with reciprocal benefits enhancing plant growth or stress tolerance. **Example**: Mycorrhizal fungi improving nutrient and water uptake.
- ***Endophytic Association*** – Microbes live inside plant tissues without causing harm, often promoting growth or stress resistance. **Example**: *Bacillus* or *Pseudomonas* endophytes enhancing disease resistance.
- ***Rhizospheric Interaction*** – Microbes in the rhizosphere enhance nutrient availability, suppress pathogens, and promote root development. **Example**: Phosphate-solubilizing bacteria or nitrogen-fixing bacteria.

35. Write aspects of polysaccharides structure.

Aspects of Polysaccharide Structure

- ***Monomer Composition*** – Polysaccharides are composed of monosaccharide units such as glucose, galactose, mannose, or xylose.
- ***Glycosidic Linkages*** – Monomers are connected via α- or β-glycosidic bonds, determining linear or branched structure.
- ***Branching*** – Polysaccharides may be linear (cellulose) or branched (glycogen, amylopectin), affecting solubility and function.
- ***Molecular Weight*** – They are high-molecular-weight polymers, ranging from thousands to millions of daltons.
- ***Functional Groups*** – Presence of hydroxyl, carboxyl, acetyl, or pyruvate groups influences solubility, charge, and interaction with other molecules.
- ***Three-Dimensional Structure*** – Folding, helicity, or sheet-like arrangements determine physical properties like viscosity, gel formation, and mechanical strength.
- ***Homopolysaccharides vs. Heteropolysaccharides*** – Homopolysaccharides contain one type of monosaccharide (e.g., starch), while heteropolysaccharides contain two or more types (e.g., xanthan gum).

36. What is the role of microbial polysaccharides in nature?

Role of Microbial Polysaccharides in Nature

- ***Biofilm Formation*** – Exopolysaccharides (EPS) help microbes adhere to surfaces and form biofilms, protecting them from desiccation, toxins, and predation.
- ***Environmental Protection*** – EPS retain water, stabilize soil aggregates, and protect microbes against harsh conditions like pH changes, salinity, and temperature stress.
- ***Nutrient Storage*** – Intracellular polysaccharides like glycogen serve as carbon and energy reserves during nutrient scarcity.
- ***Symbiotic Interactions*** – Microbial polysaccharides facilitate symbiosis with plants (e.g., nodulation in legumes) and other organisms by mediating adhesion and signaling.
- ***Biogeochemical Cycling*** – Polysaccharide-degrading microbes recycle carbon and other elements, contributing to soil fertility and ecosystem sustainability.
- ***Defense Mechanism*** – Some polysaccharides act as protective capsules around pathogenic bacteria, preventing phagocytosis and enhancing survival.

37. How microbes help in oil recovery?

Microbial Enhanced Oil Recovery (MEOR)

Microbes assist in oil recovery by modifying reservoir conditions and mobilizing trapped oil through their metabolic activities:

- ***Biosurfactant Production*** – Microorganisms like *Bacillus*, *Pseudomonas*, and *Acinetobacter* produce biosurfactants that reduce oil–water interfacial tension, improving oil flow and displacement.
- ***Biogas Generation*** – Fermentative microbes produce CO_2, CH_4, and H_2 gases that increase reservoir pressure, pushing oil toward production wells.
- ***Polymer Production*** – Microbial exopolysaccharides increase the viscosity of injection water, improving sweep efficiency in the reservoir.
- ***Selective Plugging*** – Some microbes grow in high-permeability zones, blocking them and redirecting water to extract oil from low-permeability areas.
- ***Biodegradation of Heavy Oils*** – Hydrocarbon-degrading microbes partially break down viscous crude oil into lighter fractions, facilitating easier extraction.

38. Differentiate between metagenomics and metabolomics.

Metagenomics vs. Metabolomics

Metagenomics is the study of the collective genetic material (DNA) of microbial communities in a specific environment, providing information about microbial diversity, potential metabolic capabilities, and gene functions without the need for culturing. It reveals which microorganisms are present and what genes they carry. Metabolomics, in contrast, analyzes the complete set of small-molecule metabolites produced by a cell, tissue, or microbial community under specific conditions, reflecting the actual biochemical activities and physiological state. While metagenomics predicts potential functions based on gene content, metabolomics provides a direct snapshot of cellular metabolism and functional output, making it closely linked to phenotype and real-time metabolic processes.

LMS Handling

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