

BT604 – Industrial Biotechnology

Current + Past + Additional Questions

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We Grow Together

Fall-2022

Current + Past Questions

ATP bioluminometry? 3 Marks

The amount of ATP in a sample can be quantified using ATP bioluminometry. This technique utilizes an enzyme-substrate complex, luciferase- luciferin obtained from the firefly, photinus pyralis, which generates a photon of light for each molecule of ATP. When an aliquot of luciferase- luciferin is added to ATP extracted from a sample of cell suspension, the light generated can be detected in a bioluminometer. The resulting signal is amplified and then expressed as a digital or analogue data output. ATP bioluminometry is most suitable for direct measurement of samples that are not coloured, as quenching of light may be a problem. However, as the method is very sensitive, samples may require considerable dilution, which often overcomes any colour quenching problem.

Enlist Minor element? 5 Marks

- Phosphorus is generally provided as inorganic phosphate ions, often as a pH buffer. This element is essential for the synthesis of nucleic acids, intermediates of carbohydrate metabolism and compounds involved in energy transduction. E.G. Adenosine

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triphosphate (ATP) and nicotinamide adenine dinucleotide Phosphate (NADP).

- **Sulphur** is required for the production of the sulphur containing amino acids Cystine, cysteine and methionine, and some vitamins. It is often supplied as an inorganic sulphate or sulphide salt at a concentration of 20-30mg/l. Other minor elements, mostly calcium, iron, potassium and magnesium, must be provided in relatively small but significant quantities, usually less than 10-20mg/l.
- **Iron** is essential for several oxidation- reduction enzymes, particularly cytochromes, and potassium is required by enzymes involved in protein synthesis and as counter-ion for the DNA phosphate backbone.
- **Magnesium** ions are involved in the stabilization of ribosomes, and some are necessary for maintaining cell wall and membrane integrity.
- **Sodium and potassium** are used in chemiosmotic energy pumps, but the former does not appear to have other specific roles, except in salt loving' halophilic microorganisms.

Why bacteria form a capsule? 3 Marks

Because an outer layer of polysaccharides covers the cells of many different bacterial species. Capsules protect bacteria from toxic compounds and desiccation and allow them to adhere to surfaces.

What is Chemotaxis? 3 Marks

Chemotaxis is the movement of a cell towards attractant and away from repellent chemicals.

What is a fermentation vessel?

Fermenters used in microbial fermentation represent a wide range of devices from a simple type of tube aeration system fermenter operating on the airlift system or the deep jet principle or devices with rotary stirrers in which the air is sucked in or distributed under pressure into the stirrer space.

Properties for fermenter vessel? 3 Marks

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- It must be strong enough to withstand the pressure of a large volume of an aqueous medium.
- It must not corrode and contribute toxic ions to the growing microorganism.
- Provision for rapid incorporation of sterile air into the medium.
- Carbon dioxide released during fermentation must be flushed out.
- Stirrer must be available to mix the medium and microorganisms to facilitate the availability of nutrients and oxygen.
- Intermittent addition of antifoaming agent.
- Provision for controlling temperature.
- Aseptic withdrawal of culture sample during fermentation and introduction of inoculum at the initiation of fermentation.
- Determination of the pH and its adjustment, if required.
- Some means of sterilization of medium and addition of antifoaming agent.
- Air filter.
- Drain in the bottom.
- Access to the inside of the fermenter to clean it.

Write the applications of batch fermentation? 3 Marks

- Charging of the fermenter with fresh medium
- Sterilization of the fermenter and medium.
- Inoculation of the fermenter.
- Production of microbial products
- Harvesting of biomass and spent fermentation broth
- Cleaning of the vessel.

Petite yeast strains? 3 Marks

Petite' yeast mutants lack mitochondria and are therefore incapable of respiration. They can grow only fermentatively and on solid media their colonies are very small, compared with wild-type colonies, hence their name.

What is Hydrolysis enzyme?

Hydrolytic enzymes are the enzymes that catalyse the hydrolysis of various bonds such as peptide, ester, glycosidic, ether, carbon-carbon

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bonds, carbon-halide bonds and P-N bonds. They utilise water to break the bonds. They break a larger molecule into smaller molecules.

Fungal hyphae? 3 Marks

Hyphae can grow rapidly in length, at rates of up to several micrometres per minute. There is generally little increase in girth, which maximizes the surface area for absorption of nutrients. Individual hyphae are 1-15mm in diameter depending upon the species. Their cell walls are composed of 80--90% polysaccharide, along with some lipid and protein constituents.

Define Symport and Antiport? 3 Marks

Proton gradients are involved (see chapter 3, electron transport. The nutrient entering the cell, such as a sugar, amino acid or organic acid molecule, is simultaneously transported with a proton, and is referred to as Symport.

Proton gradients may also be used to establish a sodium ion gradient across the membrane. Here sodium ions leave the cell in exchange for the entry of protons which is termed Antiport.

Write a note on E.coli? 5 Marks

E. Coli was discovered in 1885 by the German Bacteriologist Theodor Escherich. A major inhabitant of the colon of humans and the lower gut of other warm-blooded animals. Some can cause food- and water-borne diseases that produce diarrhoea and can be especially problematical for human infants and young animals. A particularly virulent strain is e. Coli 0157:H7. Organism's rapid growth rate, with a doubling time as low as 20min, has led to it becoming an important industrial microorganism. E. Coli has been used extensively as a model for the study of molecular biology and is often considered to be the ideal host in gene-cloning experiments. Extremely useful for the production of heterologous proteins, derived from other organisms. E. Coli is a gram-negative facultative anaerobe belonging to the family enterobacteriaceae members are often referred to as enterobacteria or enteric bacteria.

Examples of food preservation with their use? 3 Marks

Preservative	Effective concentration	Uses
Acetic acid	0.4% (w/v)	Used in baked goods
Benzoic acid (E210), benzoates and parabens*	0.1% (w/v)	Antimicrobial agents in margarine, cider, soft drinks, cosmetics, etc.
Lactic acid	0.01–0.05% (w/v)* 0.3% (w/v)	Antimicrobial agent in cheeses, buttermilk, yoghurt and pickled foods
Nisin (E234)	50–500 IU/g effective against Gram (+) bacteria; particularly useful against spore formers	Used in processed cheese, other dairy produce and canned products at acid pH
Propionic acid (E280) and propionates	0.3% (w/v)	Antifungal agent in breads, cake, cheeses, dried fruits
Sodium nitrite (E250)	0.02% (w/v) more effective at pH 5.0–5.5	Antibacterial agent in cured meats and fish. Retards growth of <i>Clostridium botulinum</i> . Problems due to formation of nitrosamines on cooking.
Sorbic acid (E200) and sorbates	0.2% (w/v)	Antifungal agent in cheeses, syrups, cakes, fruit juices, etc.
Sulphur dioxide (E220), sulphites	0.02–0.03% (w/v) active form is undissociated sulphurous acid	Antimicrobial agent in dried fruits, grapes, molasses and beverages. Inhibits fungi and bacteria. Used extensively in the past, now reductions in use due to induction of asthmatic attacks in sensitive individuals

Write a brief note on method of sterile filtration for removal of microbial cells? 5 Marks

Sterile filtration involves the physical removal of all cells from a liquid or gas. It is especially useful for sterilizing heat labile solutions of antibiotics and other drugs, amino acids, sugars, vitamins, animal cell culture media and some beverages. There are three main types of sterile filter:

- **1 depth filters** are thick fibrous or granular filters that remove microorganisms by physical screening, entrapment and adsorption.
- **2 membrane filters** are thin filters with defined pore sizes, usually of 0.2 or 0.45mm, through which the microorganisms cannot pass
- **3 high-efficiency particulate air (HEPA) filters** are used in laminar flow biological safety cabinets and containment rooms to sterilize the air circulating in the enclosure.

What are the key factors relating to improve the economic efficiency of fermentation? 3 Marks

- ✓ The strategy for initially obtaining a suitable industrial microorganism
- ✓ Strain improvement to enhance productivity and yield
- ✓ Maintenance of strain
- ✓ Purity, preparation of a reliable inoculum and the continuing
- ✓ Development of selected strains
- ✓ Some microbial products are primary metabolites,
- ✓ Produced during active growth (the Trophophase),
- ✓ Include amino acids, organic acids, vitamins
- ✓ Industrial solvents such as alcohols and acetone

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

Difference between prototroph and auxotroph? 3 Marks

Prototroph

Microorganisms that are able to grow on this minimal medium are referred to as prototroph.

Auxotroph

Those that are unable to grow without additional organic substances, such as amino acids or vitamins, are called auxotrophs.

Trace elements and their source? 3 Marks

Trace elements include cobalt, copper, manganese, molybdenum, nickel, selenium and zinc. These elements are usually required at concentrations of 0.1-1mg/L, or less for a number of specific enzymes. Their normal concentrations in water supplies, or as contaminants in other media ingredients, often fulfil this requirement. Overall nutrient demands vary for different microorganisms. Many bacteria can grow on media merely containing carbon and energy sources and basic mineral elements.

Microorganisms that are able to grow on this minimal medium are referred to as prototrophs. However, other microorganisms must be given specific compounds in a part or fully constructed form. Those that are unable to grow without additional organic substances, such as amino acids or vitamins, are called auxotrophs. Culture media for many organotrophic microorganisms often require certain vitamin and growth factor supplements, especially B vitamins (thiamine, B1 riboflavin, B2; pyridoxine, B6; cobalamin, B12; biotin; nicotinic acid; and pantothenic acid). Few microorganisms require fat-soluble vitamins (A, D, E and K); and vitamin C. Although often improving growth, is not a true microbial growth factor.

Microbial growth kinetics? 5 Marks

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Microbial growth can be defined as an orderly increase in cellular components resulting in cell enlargement and eventually leading to cell division. This definition is not strictly accurate as it implies that a consequence of growth is always an increase in cell numbers. However, under certain conditions growth can occur without cell division. In this situation the cell numbers remain constant, but the concentration of biomass continues to increase. This is also true for coenocytic organisms, such as some fungi, that are not divided into separate cells. Their growth results only in increased size. The growth model that will be examined is bacterial binary fission in homogeneous suspension cultures, where cell division produces identical daughter cells. Each time a cell divides is called a generation and the time taken for the cell to divide is referred to as the generation time. The generation time or doubling time (t_d) is the time required for a microbial population to double. Theoretically, after one generation, both the microbial cell population and biomass concentration have doubled. At any one time there are cells at different stages of their cell cycle. This is termed asynchronous growth. Microbial fermentations in liquid media can be carried out under different operating conditions, i.e. Batch growth, fed- batch growth or continuous growth. Batch growth involves a closed system where all nutrients are present at the start of the fermentation within a fixed volume. The only further additions may be acids or bases for pH control, or gases (e.g. Aeration, if required). In fed-batch systems fresh medium or medium components are fed continuously, intermittently or are added as a single supplement and the volume of the batch increases with time.

Environmental effects on microorganisms? 5 Marks

Growth and development of microorganisms are greatly affected by the chemical and physical conditions of their environment. Nevertheless, microorganisms have evolved to occupy niches throughout the range of environments on earth, some of which are very hostile. Microorganisms from some of these extreme environments have useful properties that can be exploited.

What are the Environmental roles of microorganisms?

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Microorganisms are particularly important in wastewater treatment, which utilizes the metabolic activities of microbial populations capable of degradation.

The **two main objectives** are.

- To destroy all pathogenic microbes present in the sewage, particularly the causal organisms of the water-borne diseases cholera, dysentery and typhoid.
- The second objective is to break down the organic matter in wastewater to mostly methane and carbon dioxide, thereby producing a final effluent (outflow) that can be safely discharged into the environment.

Microbial activities can also be employed in the degradation of man-made xenobiotic compounds. Environmental biological control is a further area where microorganisms are employed in an effort to reduce our reliance on synthetic chemical pesticides. Bacteria, fungi, protozoa and viruses are cultivated to produce biomass or cell products for the control of fungal, insect and nematode pests of agricultural crops, along with some vectors of human and animal diseases.

Describe the 12 subgroups of Eubacteria?

Eubacteria

The eubacteria are a very diverse group that may be divided into 12 subgroups.

1. The proteobacteria is a major kingdom of gram negative bacteria that is divided into five groups, a, b, g, d and e. They include purple photosynthetic bacteria.
2. The gram-positive eubacteria are composed of two major subdivisions:
 - The low guanine (G) + cytosine (C) group
 - The high G+ C group.
3. Cyanobacteria and relatives, which are oxygenic phototrophs, e.g. Anabaena, nostoc and spirulina.
4. Chlamydia, a group of obligate intracellular parasites.

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5. Planctomyces and pirella, bacteria lacking peptidoglycan; some with membrane-bound nucleoid.
6. Bacteroides and flavobacteria, a subgroup that contains a mixture of physiological types.
7. Green sulphur bacteria, such as chlorobium, an anaerobic phototroph.
8. Spirochetes and relatives which are gram-negative coiled bacteria.
9. Deinococci, radioresistant micrococci and relatives, E.G. Deinococcus Radiodurans and thermus aquaticus.
10. Green non-sulphur bacteria and anaerobic Phototrophs.
11. Thermotoga and thermosulfobacteria, thermophiles From hot springs and marine sediments.
12. Aquiflex, a group of obligate chemolithotrophic And autotrophic hyperthermophiles.

Temperature on growth? 3 Marks

All organisms have an optimum temperature for growth a typical microorganism, referred to as stenothermal, can grow over a temperature range of approximately 30°C. Eurythermal organisms grow over even wider ranges. As for temperature optima, mesophiles have optimum growth temperatures in the range 20- 45°C and a minimum around 15-20°C. Psychrophiles usually have their optimum temperatures below 15°C and these organisms are often killed by exposure to room temperature. They are able to function at low temperature because their membranes contain a high proportion of unsaturated fatty acids. Organisms with optima above 50°C are termed thermophiles. Several algal, protozoal and fungal species have temperature maxima up to 55°C. However, it is only certain prokaryotes that are truly thermophilic, as no eukaryotic microbes grow at such high temperatures. Some extreme (hyper-) thermophiles can grow at 100°C or higher. For example, pyrolobus fumarii has an optimum of 106°C and continues growing up to 113°C. All of these are archaea's, which are mainly anaerobic sulphate reducers or have other metabolism with lower requirements for thermolabile cofactors such as NADH and NADPH.

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Fed-Batch and Continuous fermentation? 3 Marks

Fed-batch fermentation is a modification to batch fermentation in which nutrients are systematically added.

Continuous fermentation is a continuous process where nutrients are continually added to the bioreactor and the culture broth (containing cells and metabolites) is removed at the same time.

How penicillin inhibit the growth of microbes? 3 Marks

Penicillin kills bacteria through binding of the beta-lactam ring to DD-trans peptidase, inhibiting its cross-linking activity and preventing new cell wall formation. Without a cell wall, a bacterial cell is vulnerable to outside water and molecular pressures, which causes the cell to quickly die.

How temperature effect the microbes? 5 Marks

The higher the temperature, the more easily microorganisms can grow up to a certain point. Very high and low temperatures both obstruct the enzyme processes microorganisms depend on to survive. Microbes multiply rapidly between 40 and 140 degrees. Microbes will not multiply but may start to die between 140 and 165 degrees. Microbes will die at temperatures above 212 degrees.

Temperature range of bacteria? 5 Marks

These bacteria can grow at temperatures between 5°C and 60°C, which is known as the temperature danger zone. The fastest rate of growth is at around 37°C, the temperature of the human body. The temperature range over which a microorganism grows reflects the temperatures at which its enzymes function. Within this range, three cardinal temperatures can be identified:

- Minimum growth temperature
- Optimum growth temperature
- Maximum growth temperature

What are antibiotics? And effects on different microbes? 5 Marks

Antibiotics

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A drug used to treat infections caused by bacteria and other microorganisms.

OR

An antibiotic is a type of antimicrobial substance active against bacteria. It is the most important type of antibacterial agent for fighting bacterial infections, and antibiotic medications are widely used in the treatment and prevention of such infections. They may either kill or inhibit the growth of bacteria. Antibiotics kill bacteria, not archaea, fungi, or protists. It has been shown that antibiotics can increase mutation, recombination, gene transfer and prophage induction, all of which have inheritable consequences. To be evolutionarily relevant, these changes need to be fixed, and fixation is achieved only if bacteria are under selection.

Antibiotics' effects on different microbes

Antibiotics can either have a narrow or broad spectrum of activity. Narrow-spectrum antibiotics are more specific and are only active against certain groups or strains of bacteria. Broad-spectrum antibiotics instead inhibit a wider range of bacteria.

What is the outer strain of cell membrane? 5 Marks

Surrounding the cell wall is the outer membrane, a hallmark of Gram-negative species. The outer membrane is an atypical biological membrane: while it is a lipid bilayer, it is asymmetrical, with phospholipids in the inner. Some strains also possess capsules located outside the outer membrane, which are composed of polysaccharides. They may provide a barrier to certain molecules, help protect against desiccation, or aid attachment of pathogenic strains to host cell surfaces. The plasma membrane also called the cell membrane, is the membrane found in all cells that separates the interior of the cell from the outside environment. In bacterial and plant cells, a cell wall is attached to the plasma membrane on its outside surface.

Outer membrane of gram negative bacteria? 5 Marks

The cell envelope of gram-negative bacteria consists of two membranes, the inner and the outer membrane, that are separated by the periplasm.

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The outer membrane consists of phospholipids, lipopolysaccharides, integral membrane proteins, and lipoproteins. The Gram-negative cell wall is composed of an outer membrane, a peptidoglycan layer, and a periplasm. In Gram-negative Bacteria the cell wall is composed of a single layer of peptidoglycan surrounded by a membranous structure called the outer membrane. The gram-negative bacteria do not retain crystal violet but can retain a counterstain, commonly safranin, which is added after the crystal violet. Safranin is responsible for the red or pink colour seen with gram-negative bacteria. The Gram-negative cell wall is thinner (10 nm thick) and less compact than that of Gram-positive bacteria but remains strong, tough, and elastic to give them shape and protect them against extreme environmental conditions. The outer membrane of Gram-negative bacteria invariably contains a unique component, lipopolysaccharide (LPS) in addition to proteins and phospholipids.

Methods to detect microbial growth? 5 Marks

Identification of microorganisms provides the name of the organism to genus or species level which can help in determining whether it is a safety or spoilage concern or is likely to be heat resistant, for example.

- **DNA sequencing** to identify bacteria, moulds and yeasts.
- **Ribo-printer analysis** for bacterial identification and characterisation.
- **Repeat based polymerase chain reaction** for assessing the similarity of microorganisms.
- **Rapid** pathogen confirmation by PCR.

What is imperfect fungi? 2 Marks

Deuteromycetes are called imperfect fungi because only their vegetative and asexual phases are known. Imperfect fungi are fungi that do not fit into the commonly established taxonomic classifications of fungi that are based on biological species concepts or morphological characteristics of sexual structures because their sexual form of reproduction has never been observed.

What is Fungi? 2 Marks

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Fungi are eukaryotic organisms that include microorganisms such as yeasts, moulds and mushrooms. These organisms are classified under kingdom fungi. The organisms found in Kingdom fungi contain a cell wall and are omnipresent. They are classified as heterotrophs among the living organisms.

How penicillin work? 5 Marks

Penicillin kills bacteria through binding of the beta-lactam ring to DD-trans peptidase, inhibiting its cross-linking activity and preventing new cell wall formation. Without a cell wall, a bacterial cell is vulnerable to outside water and molecular pressures, which causes the cell to quickly die.

Define microbial growth? 3 Marks

Microbial growth refers to an increase in number of cells rather than an increase in cell size. Many microbes (including *Escherichia coli*, *Salmonella enterica*, and *Listeria monocytogenes*) are unicellular, meaning they are made of only one cell.

What is the building block in industrial biotechnology? 3 Marks

To achieve these, seven major areas of research and technology were identified cooperatively by the stakeholders:

1. Novel enzymes and micro-organisms
2. Microbial genomics and bio-informatics
3. Metabolic engineering and modelling
4. Bio-catalytic process design
5. Fermentation science and engineering
6. Innovative downstream processing
7. Biocatalyst function and optimisation

Brief note on yeast? 5 Marks

The term 'yeast' refers to a unicellular form rather than to a phylogenetic classification. It can be used to describe a unicellular phase of the life-cycles of fungi that may be predominantly filamentous, but exhibit yeast-mould dimorphism. This term is used more commonly as a generic name for fungi that have only a unicellular phase. These true yeasts and many

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others of industrial importance are strains of *S. Cerevisiae*, a member of the ascomycetes yeasts are heterotrophic. They are found in a wide range of natural habitats, being particularly common aerial plant organs, especially flowers, and plant debris in the surface layers of soil. Many yeasts are facultative anaerobes, able to grow in the absence of oxygen. Strains of *S. Cerevisiae* are the most well-known and commercially important yeasts, and have long been employed to produce alcoholic beverages and leaven bread. They are now also used in the production of several other fermentation products particularly fuel ethanol, single cell protein, enzymes and heterologous proteins, e.g. Human insulin. Very few yeasts are animal pathogens. *Cryptococcus neoformans* causes a relatively rare form of meningitis, but the best known is *Candida albicans*. It may commonly infect the skin, and mucosal membranes of the mouth, vagina and alimentary tract. *C. Albicans* can become a serious opportunistic pathogen, particularly in individuals whose immune response is weakened. Such infections are difficult to treat, due to the similarity between the host's and the pathogen's metabolism.

Enlist the two distinct groups of phylogenetic prokaryotes? 3 Marks

On the basis of the study of phylogenetic (evolutionary) prokaryotes have been separated into two distinct groups:

- Archaea (ancient' bacteria)
- The eubacteria ('true' bacteria)

Enlist the features of prokaryotes that are quite different from eubacteria?

These prokaryotes are quite different from eubacteria and have some features

- Especially aspects of the transcription.
- Translation machinery associated with protein synthesis, that are similar to eukaryotic cells.
- Archaeans have relatively small genomes containing microbial cell structure and function less than half the DNA of eubacteria. For example: The genome of *Methanococcus jannaschii* has been

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sequenced and found to contain 1760 genes composed of 1700 kilobase pairs (kbp).

Facultative anaerobic need Carbon dioxide for growth. From where Carbon dioxide come? 3 Marks

Facultative anaerobes function with or without oxygen, but grow more efficiently when oxygen is available. Suitable procedures include:

- The use of media containing reducing agents, such as thioglycollate, which chemically combine with oxygen.
- Physical removal of oxygen from a growth chamber, by pumping out the air with a vacuum pump, and then flushing the vessel with nitrogen gas carbon dioxide.

Write the five main groups of microorganisms on the basis of their requirements for oxygen? 5 Marks

Microorganisms are classified into five main groups on the basis of their requirements for oxygen

1. **Obligate aerobes** grow only in the presence of oxygen, as it is required as the terminal electron acceptor for electron transport in aerobic respiration.
2. **Facultative anaerobes** function with or without oxygen, but grow more efficiently when oxygen is available.
3. **Micro-aerophiles** require some oxygen for the biosynthesis of certain compounds, but cannot grow at normal atmospheric oxygen concentrations of 21% (v/v). They must have lower oxygen levels of 2- 10% (v/v).
4. **Aero-tolerant anaerobes** essentially ignore oxygen and grow equally well in its presence or absence.
5. **Obligate anaerobes** cannot tolerate oxygen; exposure to it results in their death.

Effect of Radiations on microbial growth? 5 Marks

Visible and ultraviolet (UV) light are parts of the electromagnetic spectrum, which extends from strong radiation (g-rays) to very weak radiation (heat and radio waves). Even visible light, particularly the more

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energetic violet and blue regions, can be damaging. It may generate singlet oxygen, a very powerful oxidizing A which can cause damage to cellular components and may kill some microorganisms. UV light, which is most harmful at 260nm, causes specific damage to DNA notably the formation of thymine dimers, ionizing radiation, such as g-rays emitted from the excited nucleus of ^{60}Co generates free radicals, OH^- and H^- , and hydrated electrons. Resulting damage includes breakage of hydrogen bonds in functional molecules, oxidation of many chemical groups and breakage of DNA strands. Many air-borne bacteria are afforded some protection by pigments that absorb radiation, and in other microorganisms resistance involves effective repair mechanisms, especially for damaged DNA. Some microorganisms, including *E. Coli*, are also less susceptible to radiation damage in anaerobic environments than when respiring aerobically, and may be partially protected by reducing agents and sulfhydryl compounds.

Note on bacillus subtilis? 5 Marks

The genus *Bacillus* consists of a large number of diverse, rod-shaped, chemoheterotrophic gram positive bacteria, 0.5-2.5mm wide and 1.2-10mm long. Some species are strictly aerobic, others are facultative anaerobes or microaerophilic, but all are catalase positive. *Bacillus* species also produce oval or cylindrical endospores. Endospores are resistant to adverse environmental conditions and provide a selective advantage for survival and dissemination. *B. Anthracis*, the cause of anthrax. *B. Subtilis* is a common soil microorganism that is often recovered from water. Air and decomposing plant residues. *B. Subtilis* has been used for the production of industrial enzymes, particularly amylases and proteases. *B. Subtilis* has also proved very useful for the manufacture of fine chemicals especially nucleosides, vitamins and amino acids, and some strains are used in crop protection against fungal pathogens. The main features of *B. Subtilis* that distinguish it from *E. Coli* are the cell wall structure and the ability to produce spores. They are 20-50nm thick and simply composed of 20-25 layers of peptidoglycan, associated with some lipid, protein and teichoic acid. Flagella may be present and flagellated forms are capable of chemotaxis. Vegetative cells continue to grow and

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12 chapter 1 spore coat cortex DNA exosporium cytoplasm with ribosomes cytoplasmic membrane. The structure of a typical bacterial endospore. Divide by binary fission until nutrients become limiting unequal cell division produces a smaller forespore cell and a larger mother cell by the formation of an asymmetric septum near one pole of the cell.

What is Spread plating? 3 Marks

In spread plating, samples, usually 0.1 ml, are spread on the surface of a suitable agar-based nutrient medium using a sterile spreading device, e.g. A bent glass rod. The plates are then inverted and "" incubated caudated at the optimum growth temperature. All resultant colonies should be well separated and easily counted. This also enables the isolation of pure cultures where required

What is pour plates? 3 Marks

Pour plates result in the development of microbial colonies throughout the agar. Those organisms with lower oxygen tolerance grow within the agar. Colonies of aerobic organisms often have variable sizes, as those nearer the surface have a better oxygen supply. Consequently, it is often harder to see similarity in colonial morphology between colonies on the surface and those within the agar.

Floc formation? 3 Marks

Flocs are aggregates of cells, not chains of unseparated buds. There are two main theories of floc formation.

1. The first concerns calcium bridging, where a calcium ion links two cell via negatively charged cell wall components. Support for this theory comes from the observation that flocs are dispersed by the chelating agent ethylenediamine Tetraacetic acid (EDTA).
2. Secondly, the lectin hypothesis, involving protein-carbohydrate binding. Proposes that a surface protein of one cell links to a mannose residue on an adjacent cell. This hypothesis is supported by the fact that sugars, particularly mannose, inhibit floc formation.

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What are the biotechnological products resulting from recombinant DNA technology? 5 Marks

Products which are produced by recombinant DNA technology produced by biotechnology :

- ✓ Antibiotics
- ✓ Vaccines
- ✓ GMO's
- ✓ Transgenic plants
- ✓ Beverages

Characteristics of E.coli? 5 Marks

- E.coli is a straight, rod shape (bacillus) bacterium.
- The size of E.coli is about $1-3 \mu\text{m} \times 0.4-0.7 \mu\text{m}$ (micrometre).
- E.coli is arranged singly or in pairs.
- E.coli is a motile bacterium. Some strains of E.coli are non-motile.
- E.coli is a flagellated bacterium with a peritrichous flagella arrangement.
- E.coli is a gram negative bacterium.
- E.coli is a non sporing bacterium.
- Capsules are present in some strains of E. Coli which can easily be demonstrated using Indian ink preparation and appear as a clear halo on a dark background.

Explain applications of bioinformatics in industrial biotechnology? 5 Marks

- Genomics
- Comparative Genomics
- Proteomics
- Drug Discovery
- Cancer Research and Analysis
- Phylogenetic Studies
- Forensic Science
- Bio-Defence

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What are the net overall products of the Entner-Doudoroff pathway? 3 Marks

The pathway can generate two pyruvate molecules, one ATP, one NADH and one NADPH.

Certain chemicals may be added to different food substances to prevent the growth of microorganisms. These are known as food preservatives. Give three examples of food preservatives with their uses. 5 Marks

Preservative	Effective concentration	Uses
Acetic acid	0.4% (w/v)	Used in baked goods
Benzoic acid (E210), benzoates and parabens*	0.1% (w/v) 0.01–0.05% (w/v)*	Antimicrobial agents in margarine, cider, soft drinks, cosmetics, etc.
Lactic acid	0.3% (w/v)	Antimicrobial agent in cheeses, buttermilk, yoghurt and pickled foods

A student prepares a nutrient medium for bacterial growth. She takes glucose, water and mineral salts to formulate the medium but observes that no growth occurs. Which component needed for bacterial growth is missing from her medium? Give examples of compounds that can be added to the medium. 5 Marks

Any medium for the cultivation of bacteria must provide certain basic nutritional requirements, which include

- A carbon source that may also serve as an energy source
- Water
- A nitrogen source
- A phosphate source
- Various mineral nutrients, such as iron and magnesium.

Three kingdom of Archaea? 5 Marks

The archaea may be divided into three kingdoms:

1. Euryarchaeota are primarily methanogens such as methanobacterium and methanosarcina, and the extreme halophiles halobacterium and halococcus.
2. Crenarchaeota are mostly extreme barophiles and thermophiles that include pyrodictium, pyrolobus, sulfolobus and thermoproteus.

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3. 3 korarchaeota are hyperthermophiles which as yet, have not been isolated as pure cultures.

Role of bioinformatics in industrial biotechnology? 5 Marks

Bioinformatics is the use of computing technologies to manage and analyse biological data. The huge amounts of genomic data produced through DNA sequencing can often be very confusing and difficult to analyse using bioinformatics techniques, we can map the positions of genes, elicit their functions and infer evolutionary relationships.

Explain application of bioinformatics in industrial biotechnology? 5 Marks

There exists a number of applications of bioinformatics for accelerating research in the area of industrial biotechnology that include automatic genome sequencing, gene identification, prediction of gene function, prediction of protein structure, phylogeny, drug designing and development, identification of organisms, vaccine designing, understanding the gene and genome complexity, understanding protein structure, functionality and folding.

Three disadvantages of batch fermentation? 3 Marks

- Charging of the fermenter with fresh medium
- Sterilization of the fermenter and medium.
- Inoculation of the fermenter.
- Production of microbial products
- Harvesting of biomass and spent fermentation broth
- Cleaning of the vessel.

Disease caused by caladium? Marks

Common diseases of caladium are caused by *Fusarium solani*, *Pythium* spp., *Rhizoctonia solani*, *Sclerotium rolfsii*, *Pectobacterium carotovorum*, *Xanthomonas axonopodis*, Dasheen mosaic virus, and *Meloidogyne* spp.

Microorganisms growth on basis of type of temperature? 5 Marks

The results show that microbes have a higher growth rate at 20, 25, and 30°C than at 3°C. In general, an increase in temperature will increase enzyme activity. But if temperatures get too high, enzyme

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activity will reduce, and the protein (the enzyme) will denature.

Microorganisms are classified into three primary groups based on their preferred range of temperature:

- Psychrophiles (cold-loving microbes)
- Mesophiles (moderate-temperature-loving microbes)
- Thermophiles (heat-loving microbes).

Enlist five points of challenges face by biotechnology industry? 5 Marks

Despite the boom in the biotech industry, there are some grave challenges faced by this domain.

- High levels of risk
- GM food adoption
- Societal concerns
- Environmental harm
- Production/laboratory safety
- Bioterrorism
- Loss of patents, bringing competition from biosimilar
- High R&D costs
- Low R&D productivity
- A strong requirement of implementing M&A to
- Strengthen the pipeline

What are sterile filtration? 3 Marks

Sterile filtration involves the physical removal of all cells from a liquid or gas. It is especially useful for sterilizing heat labile solutions of antibiotics and other drugs, amino acids, sugars, vitamins, animal cell culture media and some beverages.

Name and description of three sterile filters? 3 Marks

There are three main types of sterile filter:

- **1 depth filters** are thick fibrous or granular filters that remove microorganisms by physical screening, entrapment and adsorption.
- **2 membrane filters** are thin filters with defined pore sizes, usually of 0.2 or 0.45mm, through which the microorganisms cannot pass

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- **3 high-efficiency particulate air (HEPA) filters** are used in laminar flow biological safety cabinets and containment rooms to sterilize the air circulating in the enclosure.

Define Plasmid. Where it is present? 2 Marks

Plasmids, relatively small circular extrachromosomal DNA molecules. It is present in Bacterial cell in the cytoplasmic matrix.

Define Water activity? 2 Marks

Actual water E to a microorganism is indicated by the term water activity (aw). This is the ratio of the vapour pressure of water in the solution surrounding the microorganism, p_{soln} , to the vapour pressure of pure water.

Write down the form of plant biomass used in fermentation? 3 Marks

Plant biomass can be in the form of:

- Cultivated energy crops
- Natural vegetation
- Agricultural wastes
- Industrial wastes
- Domestic organic wastes

How bacitracin inhibit the growth of bacteria? 3 Marks

It acts by inhibiting the release of peptidoglycan subunits from the lipid-carrier molecule that transports them across the cytoplasmic membrane.

Enlist the minor element and write down the role of each? 5 Marks

Phosphorus is generally provided as inorganic phosphate ions, often as a pH buffer. This element is essential for the synthesis of nucleic acids, intermediates of carbohydrate metabolism and compounds involved in energy transduction sulphur is required for the production of the sulphur containing amino acids cystine, cysteine and methionine, and some vitamins. Iron is essential for several oxidation-reduction enzymes, particularly cytochromes, and potassium is required by enzymes involved in protein synthesis and as counter-ion for the DNA phosphate backbone. Magnesium ions are involved in the stabilization of ribosomes, and some

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are necessary for maintaining cell wall and membrane integrity. Sodium and potassium are used in chemiosmotic energy pumps, but the former does not appear to have other specific roles, except in salt “loving” halophilic microorganisms.

Differentiate between Primary and secondary metabolite? 5 Marks

It is Produced during active growth (the some microbial products are organic acids, vitamins. Industrial solvents such as trophophase). It is included amino acids, organic acids, vitamins. Industrial solvents such as alcohols and acetone. Many of the most important industrial products are secondary metabolites, which are not essential for growth e.g. Alkaloids and antibiotics.

What is Batch and Fed-batch fermentation? 3 Marks

Batch growth involves a closed system where all nutrients are present at the start of the fermentation within a fixed volume. The only further additions may be acids or bases for pH control, or gases (e.g. Aeration, if required). In fed-batch systems fresh medium or medium components are fed continuously, intermittently or are added as a single supplement and the volume of the batch increases with time.

Write down the impact of industrial biotechnology on environment? 3 Marks

The Environment benefits from the industrial biotechnology because:

- Very little wastes are released in the environment
- Excessive materials are used in the further processing
- Significant reduction in carbon dioxide emission
- Less energy consumption

Name of various form of fuel which comprises hydrocarbon? 2 Marks

Alcohol, Acetone, Methane, Ethane.

How acidophiles and Alkalophiles maintain their pH? 3 Marks

True acidophiles have PH optima between 1 and 5.5 , and often have mechanisms for the exclusion of protons in order to maintain internal pH at a higher level.

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Alkalophiles, such as species of bacillus and micrococcus, have pH optima between 8.5 and 11.5 but take up protons to maintain their internal pH at a lower value.

Silent feature of fermentation material? 3 Marks

The fermenter material should have the following characteristics:

- Usually, stainless steel
- Can be repeatedly sterilized
- Will not react adversely with the microorganisms
- Will not react with the target products

Three kingdom of Archaea? 5 Marks

The archaea may be divided into three kingdoms.

1. Euryarchaeota are primarily methanogens, such as Methanobacterium and methanosarcina, and the Extreme halophiles halobacterium and halococcus.
2. Crenarchaeota are mostly extreme barophiles and thermophiles that include pyrodictium, pyrolobus, Sulfolobus and thermoproteus.
3. Korarchaeota are hyperthermophiles which, as yet. Have not been isolated as pure cultures.

Location of Tricarboxylic acid in eukaryotes and prokaryotes? 2 Marks

Tricarboxylic acid cycle enzymes are located within the mitochondrial matrix in eukaryotes whereas in prokaryotes they are cytoplasmic.

Define Autotrophs, Phototrophs and Organotrophs? 3 Marks

Autotrophs utilize CO₂ as they source of carbon.

Phototrophs use energy derived from light.

Organotrophs oxidize performed organic molecules, such as sugars, to obtain electrons or hydrogen.

Write a note on Metabolic engineering? 5 Marks

Metabolic engineering is the practice of optimizing genetic and regulatory processes within cells to increase the cells' production of a certain substance, some of the common strategies used for metabolic engineering are:

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1. Over-expressing the gene encoding the rate-limiting enzyme of the biosynthetic pathway.
2. Blocking the competing metabolic pathways
3. Heterologous gene expression
4. Enzyme engineering

Metabolic engineering typically uses recombinant DNA technology to develop micro-organisms giving improved product yields or even having totally new pathways. Such designer organisms can be seen as cell factories and form the cornerstone of industrial biotechnology.

Goals:

1. Mathematical modelling of microbial metabolism, directed towards both steady state and dynamic models.
2. Design of new pathways and synthetic microorganisms focusing on the synthesis of molecules and products new to nature.

Amino acid Catabolism microorganisms? 5 Marks

THE TRICARBOXYLIC ACID CYCLE

In many bacteria, yeasts, filamentous fungi, algae and protozoa, further catabolism of pyruvate under aerobic conditions involves its direction into the tricarboxylic acid (TCA) cycle. TCA cycle enzymes are located within the mitochondrial matrix in eukaryotes, whereas in prokaryotes they are cytoplasmic. The step immediately before the cycle involves the oxidative decarboxylation of pyruvate to form the C₂ unit acetyl coenzyme A (acetyl CoA), catalysed by the multi-enzyme complex, pyruvate dehydrogenase.

What are the limitations containing dry weight to control microbial growth culture? 2 Marks

Further limitations are the time needed to obtain the results and the relatively large volume of sample required to obtain sufficient biomass for accurate weighing, as an individual bacterium weighs only about 10⁻¹² g.

What is nitrogen fixing bacteria? Give example. 3 Marks

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In addition, certain specialized nitrogen-fixing bacteria, notably azotobacter and rhizobium. Species can utilize molecular nitrogen.

What are Hydrolytic enzymes? Give example. 3 Marks

Hydrolytic enzymes, notably alkaline phosphatase, nucleases and are secreted into the periplasm from the cytoplasm and are retained close to the cell as they cannot normally pass through the outer membrane. Breakdown of large impermeable nutrients into smaller molecules that can be transported across the cell membrane into the cell. For example, Penicillinase.

Define acidophiles, Alkalophiles and neutrophiles? 3 Marks

- **Acidophiles** or acidophilic organisms are those that thrive under highly acidic conditions. These organisms can be found in different branches of the tree of life, including Archaea, Bacteria, and Eukarya. True acidophiles have PH optima between 1 and 5.5 , and often have mechanisms for the exclusion of protons in order to maintain internal pH at a higher level.
- **Alkaliphiles** are a class of extremophilic microbes capable of survival in alkaline environments, growing optimally around a pH of 10. Alkalophiles are high-pH growing bacteria which is adapted to survive and reproduce in an alkaline environment. Alkalophiles, such as species of bacillus and micrococcus, have pH optima between 8.5 and 11.5 but take up protons to maintain their internal pH at a lower value.
- A **neutrophile** is a neutrophilic organism that thrives in a neutral pH environment. Escherichia coli is a neutrophilic organism. The majority of organisms are neutrophiles, growing between PH 5 and 9, as most natural environment falls in this range.

Explain the effect of temperature on the growth of microorganisms? 5 Marks

All organisms have an optimum temperature for growth. A typical microorganism, referred to as stenothermal, can grow over a temperature range of approximately 30sec, Eurythermal organisms grow over even wider ranges. As for temperature optima, mesophiles have

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optimum growth temperatures in the range 20-4500°C and a minimum around 15-2000°C.

Psychrophiles usually have their optimum temperatures below 15°C and these organisms are often killed by exposure to room temperature. They are able to function at low temperature because their membranes contain a high proportion of unsaturated fatty acids.

Several algal, protozoal and fungal species have temperature maxima up to 55°C. Some extreme (hyper-) thermophiles can grow at 100°C or higher. For example, *pyrolobus fumarii* has an optimum of 106°C and continues growing up to 113°C.

Microbial spores are particularly heat resistant. These dormant structures have thick spore coats and low water activity. Bacterial endospores contain high levels of calcium dipicolinate which may constitute up to 15% of their dry weight.

Define Group translocation? 3 Marks

Some compounds may be modified during uptake sugars for example can be phosphorylated using phosphoenolpyruvate (PEP) as the phosphate donor. This is referred to as group translocation which is performed by many prokaryotic.

Why E. Coli is important in industry microorganism? 2 Marks

Organism's rapid growth rate with a doubling time as low as 20 min, has led to E. Coli becoming an important industrial microorganism.

Define broad and narrow spectrum antibodies? 2 Marks

Antibiotics effective against a wide range of gram-positive and gram negative bacteria are said to be broad spectrum.

If they are predominantly effective against gram-positive or gram-negative bacteria, they are narrow spectrum.

Closed loop fermentation? 3 Marks

The development of a closed loop fermentation cycle (where the "bio-waste" of one process can be recycled as input for another process), e.g. sugar beet pulp as an untapped biomass feeds stock for future use.

Silent features of Eukaryotic cells? 3 Marks

- The DNA of eukaryotic cells in the form of several linear chromosomes is characteristically complexed with histone proteins and is housed in a double membrane bound nucleus.
- Eukaryotic ribosomes, apart from those located within certain organelles, are 80 s.
- Eukaryotic cells divide by a complex process of mitosis and usually have a sexual lifecycle involving meiosis (reduction division).

Additional Questions

Silent features of Prokaryotic cells?

- Prokaryotic cells are normally less than 5 mm in diameter.
- Prokaryotes rarely possess membrane bound organelles.
- Most prokaryotic cells contain a single chromosome composed of deoxyribonucleic acid (DNA), which is located in a region of the cell referred to as the **nucleoid**.
- Prokaryotic ribosomes are 70 s.
- Cell division in prokaryotes is normally by simple binary fission

Brief note on outer member?

The outer coverings of gram-negative bacterial cells are often referred to as envelopes which is composed of two layers. Layers protect the cell and provide rigidity. The outermost layer is called the outer membrane. They are 7-8nm thick and contain lipopolysaccharide and mucopeptide. This structure does not impede the movement of small molecules, charged or uncharged more permeable than the cell/cytoplasmic membrane. A barrier to hydrophobic molecules and proteins contains porin proteins, composed of three subunits, that form narrow channels of about 1-2nm diameter through which small molecules can pass. Non-specific porins allow the passage of molecules up to 600-700Da. Specific porins have binding sites for one or more substances of up to 5000Da.

Brief note on flagella?

Most common outer membrane protein is Braun's lipoprotein. Flagella of motile strains propel the cell through aqueous media. Each flagellum is several micrometres long, composed of the protein flagellin. In addition, fibrils (fimbriae or pili), may be attached to the outer membrane which are short hair-like projections, 5-7nm in diameter and 400nm long. They enable E. Coli to attach to surfaces, such as intestinal epithelium. Some strains also possess capsules located outside the outer membrane, which are composed of polysaccharides. They may provide a barrier to certain molecules, help protect against desiccation, or aid attachment of pathogenic strains to host cell surfaces

What is phycomycetes?

Phycomycetes are the lower fungi. These are subdivided into:

- Mastigomycotina (zoosporic, motile spores)
- Zygomycotina (zygosporic)

They are the simplest fungi. Their vegetative hyphae are aseptate. Industrially important members of the zygomycotina include mucor, rhizomucor and rhizopus species which are used in some traditional food fermentations, and whole cell and enzyme bioconversions.

What is Sexual or Asexual reproduction of phycomycetes?

Sexual Reproduction

In sexual reproduction mycelia of opposite mating types each form gametangia containing several haploid nuclei plasmogamy of + and – gametangia, and pairing of haploid nuclei, results in the formation of a dikaryotic zygosporangium. This structure is metabolically inactive and resistant to desiccation. When conditions become favourable, karyogamy occurs between paired nuclei and the resulting diploid nuclei undergo meiosis to produce haploid spores.

Asexual Reproduction

Asexual reproduction results in the formation of saclike sporangia at the tips of upright hyphae or sporangiophores. Hundreds of haploid spores

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are produced by mitosis within the sporangium, which are then wind dispersed.

What is ascomycetes or ascomycotina (Sac Fungi)?

This is the largest class of fungi and includes the yeasts that are utilized in many industrial fermentation processes neurospora species, claviceps species, and important edible fungi, including morchella species (morels) and tuber species (truffles) are industrially important. Their hyphae are septate

What is Sexual or Asexual reproduction of ascomycetes or ascomycotina (Sac Fungi)?

Asexual Reproduction:

In asexual reproduction, the tips of specialized hyphae (conidiophores) form chains of haploid conidiospores that are usually wind dispersed

Sexual Reproduction:

Sexual spores or ascospores are contained within a sac-like ascus, which may be enclosed within a fruiting body or ascocarp. The yeasts, such as saccharomyces cerevisiae, produce the equivalent of an ascus during sexual reproduction and most bud during asexual reproduction in a manner similar to the formation of conidiospores.

What is White biotechnology?

White biotechnology also known as industrial biotechnology applies to industrial processes. Designing of an organism to use to produce a useful chemical. Use of enzymes as industrial catalysts to produce valuable chemicals or destroy hazardous/ polluting chemicals. It is started in California in 1970s.

Write the importance of White biotechnology?

- White biotechnology tends to consume less in resources than traditional processes used to produce industrial, goods.
- The modern use and application of biotechnology for the sustainable processing and production of chemicals.
- The use of biological methods to optimize industrial processes..

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- Research of new enzymes (proteins that remove oily and protein-based stains)
- Applied by the manufacturers of laundry detergents.

Which things is produces by the used of biotechnology?

Biotechnology is used to produces many things like:

- Pulp & paper
- Cotton
- Leather
- Biogas from organic wastes

Write two modern uses of white biotechnology?

Modern uses of white biotechnology includes:

1. Phytoremediation by using transgenic plants
2. Biofuel production by using plants

Write the overall economy benefits of biotechnology?

Biotechnology is also benefited for the overall economy:

- Due to less energy consumption
- More efficient processes
- Fermentation and enzymatic processes are commonly used in the fine chemicals sector, to produce for example vitamins, pharmaceutical intermediates and flavours.
- They are also making their first inroads into larger volume segments such as polymers, bulk chemicals and bio-fuels, and many other industrial sectors.
- Regarding all these environmental and economical impacts the industrial biotechnology is most favourable for the sustainability of the society.

What are the important products of industrial biotechnology?

The following are the important products of industrial biotechnology:

- Recovery of metals

Desulphuration of the coal by *Thiobacillus* spp.

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- **Metabolic production**

Antibiotics, enzymes, alcohols, organic acids, vitamins

- **Biocatalysts**

Yeast, bacteria & enzymes

- **Biopolymers**

Polyactic acid and Poly 3-hydroxy butyrate

Write the future visions 2025 of biotechnological processes?

Fermentation processes are used to produce a number of important chemicals at very-high volumes, including vitamin C. Looking to the future, biotechnology will allow the production of new polymers with improved functionality, not possible by conventional processes. The development and use of industrial biotechnology is essential to the future competitiveness. By the year 2025, Biotechnological processes will be used to produce chemicals and materials which are hard or impossible to produce conventionally, or to make existing products in a more efficient way.

What is the strategic research agenda of industrial biotechnology?

Modern white biotechnology is a relatively new discipline, with major areas of knowledge still to be explored. It is by nature a multidisciplinary area, comprising biology, microbiology, biochemistry, molecular biotechnology, chemistry, engineering etc. The industrial biotechnology section of the sustainable chemistry technology platform (suschem) has developed a strategic research agenda, for the development of the white biotechnology. The suschem technology platform will also cooperate on industrial biotechnology issues with other technology platforms such as: the bio-fuels, plants for the future, innovative and sustainable use of forest resources, food for life, textile and clothing and future manufacturing technologies.

Write the seven major areas of research and technology?

To achieve these, seven major areas of research and technology were identified cooperatively by the stakeholders:

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8. Novel enzymes and micro-organisms
9. Microbial genomics and bio-informatics
10. Metabolic engineering and modelling
11. Bio-catalytic process design
12. Fermentation science and engineering
13. Innovative downstream processing
14. Biocatalyst function and optimisation

Which horizontal issues need to be addressed during the implementation of program?

When the program is implemented, a number of key horizontal issues also need to be addressed, such as:

1. Technology transfer
2. Overcoming bottlenecks
3. Contribution to standards
4. Impact assessment
5. Issues of perception, awareness and education.

Which characteristics should have the new enzymes and the microorganisms?

For this purpose the new enzymes and the microorganisms are needed. These enzymes and the microorganisms should have the following characteristics:

- Cope with extreme environments like high temperature and
- High pressure
- Either in fresh water or marine environment

For the purpose of making new enzymes and the microorganisms which two techniques are used nowadays?

For this purpose two techniques are used nowadays

- **Metagenomics:**

Metagenomics allows the sequencing and genetic characterisation of the full range of species present in a sample from a particular environment at once.

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- **High throughput screening:**

Automated high throughput screening technologies can then help researchers find candidate genes which have both the desired function and are likely to be expressed when transferred to a target micro-organism.

Which characteristics should have the fermenter material?

The fermenter material should have the following characteristics:

- Usually stainless steel
- Can be repeatedly sterilized
- Will not react adversely with the microorganisms
- Will not react with the target products

What are the major influences on fermentation performance?

Major influences on fermentation performance are:

- The mode of fermenter operation (batch, Fed-batch or continuous systems)
- The method of its aeration and agitation
- Approach taken to process scale-up

Write the vital aspects of process development to ensure maximization of yield and profit?

Vital aspects of process development to ensure maximization of yield and profit are:

- Cost-effective carbon and energy sources
- Essential nutrients
- Media optimization
- In many instances, the basis of industrial media are waste products from other industrial processes, notably:
 - Sugar processing wastes
 - Lignocellulose wastes
 - Cheese whey and corn
 - Steep liquor

Write the two categories of fermentation products?

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Fermentation products can be broadly divided into two categories.

- **High volume, low value products**

Most food and beverage fermentation products

- **Low volume, high value products**

Many fine chemicals and pharmaceuticals

Define single-cell protein (SCP)?

Some microorganisms contain high levels of protein with good nutritional characteristics suitable for both human and animal consumption. This so-called single-cell protein (SCP) can be produced from a wide range of microorganisms cultivated on low-cost carbon sources.

Health-care products?

- Secondary metabolites synthesized by filamentous fungi and bacteria particularly the actinomycetes.
- Over 4000 antibiotics have now been isolated, but only about 50 are used regularly in antimicrobial chemotherapy.
- The most medically useful antibiotics are the β -lactams, penicillins cephalosporins, and the tetracyclines.

Enlist the forms of plant biomass?

Plant biomass can be in the form of:

- Cultivated energy crops
- Natural vegetation
- Agricultural wastes
- Industrial wastes
- Domestic organic wastes
- Methane and ethanol are the main products, although other potential fuels can be generated using microorganisms including hydrogen, ethane, propane and butanol.

How to control microbial growth by chemical agents?

Chemical antimicrobial agents can be used to kill microorganisms or inhibit their growth and may be of natural or synthetic origin. They

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include chemical preservatives, disinfectants, antiseptics, and drugs used in antimicrobial chemotherapy. Some phenolic compounds, for example, are used as disinfectants at high concentration, but at low concentrations they are suitable for use as antiseptics. Disinfectants are agents that kill microorganisms, but not necessarily their spores. They are not safe enough for direct application to living tissues and are used on inanimate objects such as tables, floors, utensils, etc. Chlorine, hypochlorites, chlorine compounds and phenols are extensively used as disinfectants. Disinfectants and antiseptics are distinguished on the basis of whether they are safe for application to mucous membranes. Often, safety depends on the concentration of the compound. Disinfectants at high concentration, but at low concentrations they are suitable for use as antiseptics.

How to control microbial growth by physical agents?

Heat is the most important and widely used means of sterilization and may be achieved through:

1. Incineration, where burning at 500°C physically destroys the organisms and is particularly useful for some solid wastes.
2. Moist heat, which is suitable for sterilizing most items, except heat-labile substances that would be denatured or destroyed. It is carried out using steam under pressure to achieve 121°C for 15 min, and is extensively used in fermentation processes for the sterilization of vessels, connecting pipe work and culture media.
3. Dry heat is less efficient than moist heat, requiring higher temperatures and much longer exposure times. It is performed in hot air ovens at 160°C for 2 h and can be used for glassware, metal objects and moisture-sensitive materials such as powders and oils.

Heat sterilization is commonly employed in canning and bottling, and ultra-high temperature treatments (UHT) are used in some sterile packaging procedures. Pasteurization is a milder heat treatment, used to reduce the number of microorganisms in products or foods that are heat-sensitive and unable to withstand prolonged exposure to high temperatures. These treatments involve refrigeration or freezing.

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Organisms are not usually killed, but the majority do not grow or grow very slowly at temperatures below 5°C. Perishable foods are stored at low temperatures to slow the rate of microbial growth and consequent spoilage. Often, it is psychrotrophs, rather than true psychrophiles, that are the cause of food spoilage in refrigerated foods. Low water activity this is used extensively to preserve foods, especially fruits, grains and some meat products. Methods involve removal of water from the product by heating or freeze-drying: alternatively, water activity may be reduced by the addition of solutes, usually salt or sugar.

How to control or inhibit the microbial growth?

The control or prevention of microbial growth is necessary in many practical situations, particularly in:

- Health care
- Food processing and preparation
- Preservation of materials

Control may be achieved using physical or chemical agents that either kill microorganisms or inhibit their further growth. Agents which kill cells are called '-cidal' agents, whereas '-static' agents inhibit the growth of cells without killing them. Sterilization procedures completely destroy or eliminate all viable organisms including spores, and may be performed using heat, radiation and chemicals or by the physical removal of cells.

Write down the factors that influence the effectiveness of any antimicrobial treatment?

The population decreases exponentially, by a constant fraction at constant intervals, and several factors influence the effectiveness of any antimicrobial treatment. These include:

Population size the larger the microbial population, the longer the time

Required to kill all the microorganisms present

Population composition different microorganisms vary in their sensitivity to a specific lethal agent, and its effectiveness may be influenced by their age, morphology and physiological condition.

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Period of exposure to the lethal agent the longer the exposure, the greater the number of organisms killed

Temperature normally a higher temperature increases the effectiveness of the agent.

Brief note on On-line estimation?

On-line monitoring of fermenters can provide real-time estimation of biomass and minimizes the requirement for repeated sampling and off-line analysis monitoring systems may involve optical density or capacitance based probes the microorganisms involved in most fermentation processes will either have a requirement for oxygen and/or will produce carbon dioxide. In such cases it is theoretically possible to establish a mathematical relationship between factors, such as:

- Carbon dioxide evolution
- Oxygen utilization
- The biomass concentration within the bioreactor.

Estimation of biomass concentrations and even product formation can be made by measuring these parameters on-line using carbon dioxide or oxygen detectors and biosensors attached to a computer. This can give an accurate estimate of the biomass concentration, provided that the mathematical algorithms developed are reliable