MICROBES IN HUMAN WELFARE

1.0 Microbes

- Microbes are the micro-organism which have a size of less than 0.1mm. These micro-organisms are omnipresent, they even exist on sites where no other life forms could possibly exist like high salinity (halophytes) deep inside the earth crust where temperature may be as high as 100^{O}C , highly acidic atmosphere and even under layers of snow.
- The term 'microbes' include bacteria fungi, viruses, mycoplasma, blue-green algae, nematodes and even protozoans.
- Microbes can be grown on artificial culture media where they form colonies. During their metabolisms, microbes has been in use by human beings, microbes can also be modified genetically to produce any type of chemicals. Therefore microbes are ideal for biotechnology.

2.0 Microbes in food processing and household products

1) Microorganism like Lactobacillus commonly called as Lactic Acid Bacteria (LAB) is used in preparation of curd from milk. Milk is boiled and cooled to temperature slightly less than 40^{O}C and small quantity of inoculum or curd is mixed. Inoculum or curd contain LAB like *Lactobacillus acidophilus*. These bacterium produce lactase which converts milk sugar lactose into lactic acid. It also partially digest milk protein casein. Lactic acid causes coagulation of protein to form curd. LAB improves nutritional quality of curd by increasing vitamin B_{12}. In our stomach, the LAB play very beneficial role in checking disease causing bacteria
2) Mixture of dosa and idli is allowed to undergo fermentation with the help of *Leuconostoc mesenteroides* and *Streptococcus faecalis* Coconut water is fermented to produce a refreshing drink called

toddy. It is a traditional drink in some part of southern India and is also made by fermenting sap from palms

3) Soybean preparations: (i) Tempeh is Indonesian food formed by fermenting, drying, salting and frying of soybean.
   (ii)Tofu are cheese-like products of soybean obtained after fermentation with mucor species
   (iii) Soy sauce is flavored salted brown sauce that is obtained from a mash of soybean, wheat and wheat bran fermented with the help of *Aspergillus oryzae*, *Lactobacillus. Saccharomyces rouxii* and *Torulopsis* species.
4) Cheese: Cheese is one of the oldest food items in which microbes are used. The large holes in "Swiss cheese" are due to production of a large amount of CO_2 by a bacterium named Propionibacterium Shermanii. The 'Roquefort cheese' is ripened by growing a specific fungus on them, which gives them a particular flavor. In order to prepare special cheese, such as blue Roquefort and soft camembert, the blue mold, Penicillium is commonly used.
5) Bread: Wheat flower is kneaded. A small quantity of Baker's yeast i.e. *Saccharomyces cerevisiae* is added to it. Yeast can be replaced by *Clostridium* species. The dough is allowed to ferment for a few hours. It leaves or rises. The puffed up appearance is due to production of CO_2 gas. Actually yeast produces three types enzyme – amylase, maltase and zymase complex. Amylase changes a small amount of starch into maltose, maltase changes maltose to glucose. Zymase complex acts on glucose. Zymase complex acts on glucose to form complex to form bubbles of ethyl alcohol and carbon dioxide.
6) Bacteria are used in the separation of fibres of flax, hemp and jute. For this purpose, the stems of plants are submerged in water, where the bacterial activity results in the rotting of softer parts. The tough bast fibres become loosened and easily separated from each other.

7) Sausages : They are fermented meats which possess particular taste due to microbes lactic acid bacterium *Pediococcus cerevisiae*. Lactic acid produced by the bacterium also preserves the sausage
8) Single cell protein (SCP) or probiotics: It is protein rich microbial biomass which can be used as food and feed e.g. *Methylophilus methylotrophus*, SCP has all the essential amino acids. Fat contents is low. Both autotrophs and heterotrophs are used as SCP. Amongst autotrophs, *Spirulina* has become an important food supplement which is used in various forms including tablets. It has 60% proteins, all minerals, vitamins and unsaturated fats. Amongst heterotrophs, yeast and mushrooms are being raised as SCP. There is an increasing use of low cost organic matter for raising SCP like Filamentous fungus *Fusarium graminearum* and common mushrooms. Live microbial food is called probiotic

3.0 Microbes in sewage treatment

- Large quantity of waste water is generated every day in cities and towns. This waste water is also called sewage. In sewage, most of the microorganisms are pathogenic. This huge quantity of sewage or urban waste water is discharged into natural water bodies like rivers and streams after treating in sewage treatment plant (STPs) so as to make it less polluting.
- The treatment of sewage is carried out in following two stages

(i) Primary treatment: It is a physical process of removal of large and small particles from sewage through sequential filtration and sedimentation. The sewage is first shredded and churned. It is then passed through many screens or skimmers to remove large pieces of organic matter. The sewage is now passed into a large primary settling tank having a gentle slope. Grit, sand and other heavy particles settle down. All solids that undergo sedimentation and screened organic matter constitution and screened organic

matter constitutes primary sludge. Primary sludge can be used for preparing compost or manure directly, or used in generation of biogas. It can also be burnt. The waste water after removing the primary sludge contain fine organic matter. It is passed for secondary treatment.

(ii) Secondary treatment: It involves biological process of microbial degradation of organic matter. There are three main methods –oxidation tanks trickling filter method and activated sludge method. In activated sludge method the effluent from primary settling tank is passed into an aeration tank. It is agitated mechanically. Air is pumped into the effluent. Part of the previous activated sludge is inoculated into it. It contains a large population of aerobic heterotrophic microbes including bacteria, fungi and protozoans. The microbes form flocs and floccules with help of slime. The BOD of the effluent falls to 10-15% of the raw sewage. Biochemical oxygen demand (BOD) refers to the amount of oxygen that would be consumed, if all the organic matter in one liter of water is oxidized by bacteria. Thus, indirectly BOD is a measure of the organic matter present in the water. The greater the BOD of waste water more is its polluting potential. After decrease in BOD, it is taken to secondary settling tank where the flocs undergo sedimentation. The sediment is called activated sludge. The supernatant is allowed to pass into rivers and streams. Activated sludge is taken to anaerobic sludge digesters along with the primary sludge. Here, anaerobic microbes act upon organic matter to first produce monomer and then organic acids. Methanogens then convert the latter into a mixture of gases like methane, hydrogen sulphide and carbon dioxide. The gaseous mixture is called biogas. It is inflammable and is used as source of energy. The

spent sludge is used as a manure, land fill or can be burnt. Pathogens present in the original sewage, get killed during anaerobic digestion.

4.0 Microbes in biofuels

- Biofuels are fuels of biological origin, which are used for the production of heat and other forms of energy. The energy derived from the biofuels is called bioenergy. Fuel wood and biogas are the sources of bioenergy. Biologically generated hydrogen, methane, ethanol, butanol and diesel are referred as bio-hydrogen, bio-methane, bioethanol and biodiesel respectively
- The advantages of biofuels are
  1. These are renewable energy resources
  2. They release relatively low greenhouse gases including carbon dioxide emission than fossil fuels
  3. The raw materials used in biofuel production are often wastes, including municipal waste. Therefore, it helps in pollution control

4.1 Biogas or Gobar gas

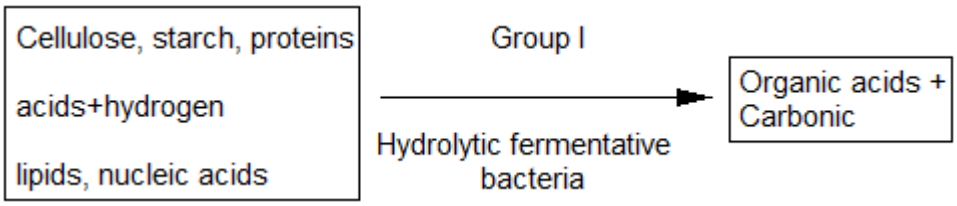
- Biogas is a methane rich fuel gas produced by the anaerobic breakdown or digestion of biomass with the help of methanogenic bacteria. It is mainly produced from the animal waste. The main ingredient of biogas is methane (CH_4, 50-70%). Other components are carbon dioxide (CO_2, 25-35%), hydrogen (H_2 1-5%), nitrogen (N_2, 2-7%) and hydrogen sulphide traces. Besides generation of biogas, biogas plants also produce manure which is equivalent to manure produced by conventional method of dumping biomass in open.
- The technology for biogas plants was developed in India by IART (Indian Agriculture Research Institute) and KVIC (Khadi and village industries commission)
- A family sized biogas plants has a large (10-15 feet deep) concrete or brick lined air tight cylindrical tank called digester. It has a

charge pit for passage of slurry into digester, a floating gas holder of metal with an outlet for gas and a pit for removal of sludge or manure

- Various microorganisms are involved in production of biogas are as follows

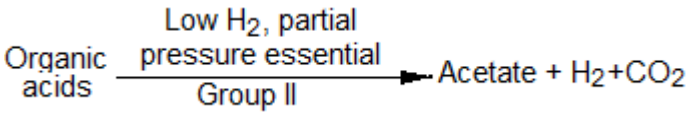
1. Hydrolytic and fermentative bacteria

   These include both obligate and facultative anaerobes, which can hydrolyse and ferment the organic materials and produce organic acid, carbon dioxide and hydrogen



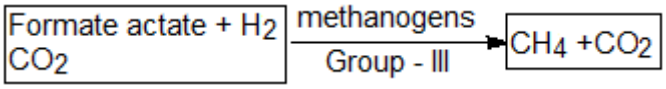
2. Syntrophic hydrogen producing bacteria:

   This group includes obligate hydrogen producing or obligate proton producing bacteria, which breakdown, organic acids having more than two carbon atoms in their chain to produce acetate, carbon dioxide and hydrogen

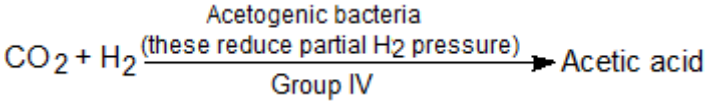


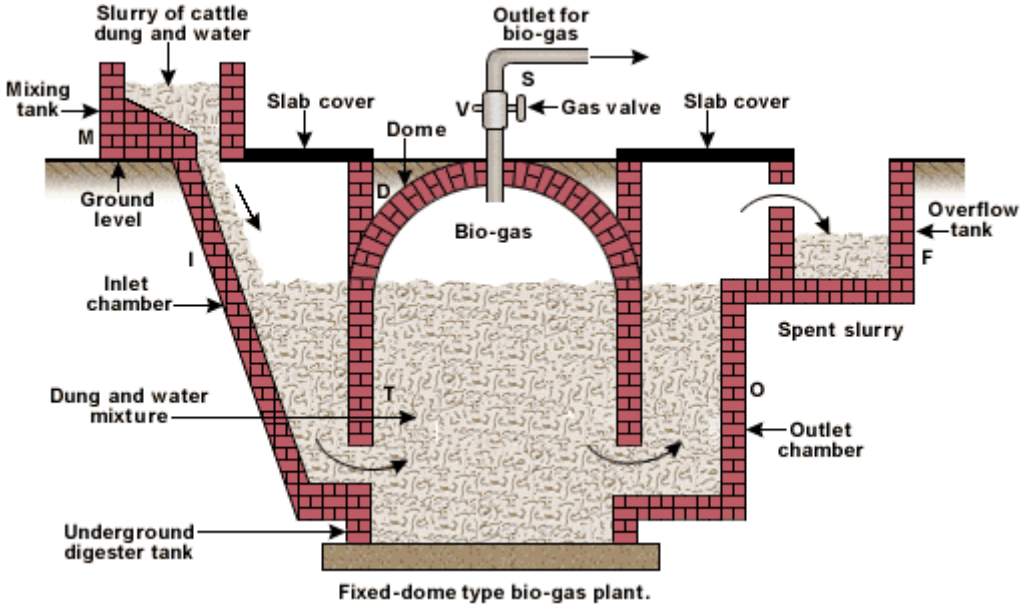
   e.g. *Syntrophomonas wolfei* and *S.wolinii*

3. Methanogenic bacteria: This group converts acetate, carbon dioxide and hydrogen into CH_4. E.g. *Methanosarcina barkeri* and *Methanobacterium omelianski*



4. Acetogenic bacteria: This group of bacteria oxidize H_2 by reducing CO_2 to acetic acid. Which is then used up by the methanogens to generate methane.





Fixed-dome type bio-gas plant.

4.2 Advantages of biogas

a) It is cheaper than other fuels
b) It is ideal for small scale production
c) It helps to clean environment because waste materials can be used as substrate
d) Aseptic condition are not required
e) Recovery of methane is spontaneous
f) Renewable source of energy

4.3 Disadvantage of biogas

a) It has low heat value
b) It has low yield of biogas
c) Large scale production is not economical
d) It contains some gases as impurities

5.0 Microbes as bio-fertilizers and bio control agents

- India is the 3rd largest producer and consumer of chemical fertilizers and pesticides. Chemical fertilizers are mostly synthesised from fossil fuels. They increase salt loading of ground water and causes pollution of surface bodies, pepticides are often persistent, induce resistance and ecological imbalance. Therefore, there is stress on

fertilizers and pesticides of biological origin. Fertilizers of biological origin are of two types, manure and bio fertilizers, Pesticides of biological origin includes bio herbicides and bio insecticides

5.1 Manures

Manures are semi-decayed organic substance which are added to the soil in order to maintain soil's fertility.

Manures improves the following properties of soil

i) Fertility

ii) Aeration

iii) Water holding capacity

iv) Crumb structure

5.1.1 Types of manure

a) Farm yard manure (FYM): It consists of a mixture of cattle dung, farm refuse, fallen leaves, and twigs etc. which are dumped in heaps to undergo decomposition by which dark amorphous manure is obtained.

b) Composite manure/compost: It consists of rotten vegetable matter, garbage, sewage sludge and animal refuse which is often enriched with small amount of chemical fertilizers like ammonium sulphates, super sulphates, lime etc. during decomposition.

c) Vermicomposting: It is composting of organic matter with the help of earthworms. Worm castings are rich in phosphorus, nitrogen calcium and other minerals

d) Green manure: It is ploughed back young leguminous crop like *Sesbania aculeate* (= s. *cannabina, Daincha*), *Sesbania rostrata* (both root and stem nodules), *Crotalaria juncea* (Sunn Hemp), *Cyamopsis tetragonoloba* (Cluster bean/Guar), *Melilotus parviflora* (M. *indica*, sweet clover/Senji), Trifolium alexandrinum (Egyptian clover/Berseem) etc. Green manure not only maintains soil

form but also increases its nitrogen content, increasing yield by 30-50%, Green manure also helps in checking weed growth and reclaimation of saline and alkaline soils.

6.0 Bio fertilizers

Bio fertilizers are of three types- nitrogen fixing bacteria, nitrogen fixing cyanobacteria and mycorrhiza

1. Free living Nitrogen Fixing Bacteria
   They are bacteria of diverse nutritional status which are able to absorb molecular nitrogen (N_2) from soil air and convert the same into nitrogen salts (amino acids) Soil is enriched through exudation and formation of nitrates after their death and decay. Nitrogen added to soil is 10-25 kg/ha/annum. Bacterium like Azotobacter (aerobic) *closteridium* (anaerobic), *Beijerinckia* (aerobic), *Rhodopseudomonas, Rhodospirillum, Chromatium*, etc. comes in this group.
2. Phyllosphere and Rhizosphere nitrogen fixing bacteria:
   They are free living nitrogen fixing bacteria which form associative mutualism on the surface of leaves and roots of plants providing nitrogen to plants and getting nutrition through exudations. Example *Azospirillum lipoferum*, *Beijerinckia*
3. Symbiotic Nitrogen fixing bacteria
   Rhizobium leguminosarum and other species forms mutually beneficial association with root nodules of legumes. A root nodule has a growing point, vascular strand and reddish pigment leghaemoglobin but lacks root cap and root hair. Its central infection zone has large cells with group of bacterioids covered by membrane lined by leghaemoglobin. In sesbania, the stem nodules contain another bacterium called *Aerorhizobium caulinodans*. Root nodules of non-legume plants (e.g. casuarina, alnus = Alder) possess Frankia while leaf nodules of Ardisia have Xanthomanas. Frankia can fix nitrogen in the soil as well. Nodules produced by it do not have leghaemoglobin. Instead, they have hopanoids for O_2 scavenging. Plants having symbiotic bacteria have higher protection content. No external nitrogen fertilizers is required. Presence of phosphorus is essential for nitrogen fixation
4. Free living nitrogen fixing cyanobacteria
   They increase nitrogen content of moist soil and water bodies e.g. Anabaena, Nostoc, Tolypothrix, Aulosira fertilissima is most active nitrogen fixer of rice fields.
5. Symbiotic nitrogen fixing Cyanobacteria

Azolla pinnata is a small aquatic fern incoculated to rice fields of south-east Asian countries. It contains symbiont Anabaena in its leaf cavities. Increase in yield is more than 50%

6. Mycorrhiza
   Mycorrhiza is a mutually beneficial relationship between fungus and roots of higher plant. The shape is irregular or coralloid with wooly covering but no root hairs or root cap. Mycorrhiza helps in absorption of water, minerals from organic matter and protection from soil borne pathogenic fungi
   (i) Ectomycorrhiza: the fungus forms a mantle on outside and intercellular hyphae in cortex. Host secretes nutrients in intercellular spaces. E.g. eucalyptus, oak, pine
   (ii) Endomycorrhiza : Fungus sends hyphal tips into cells as vesicles and arbuscules (branched masses). Hence VAM or vesicular-arbuscular mycorrhiza. Intercellular hyphae and external hyphal present but fewer e.g. grasses, orchids

7.0 Bioherbicides

- Bioherbicides are organism which destroy weeds without harming the useful plants. The first bio herbicide was a mycoherbicide, which was based on a fungus *Phytophthora palmivora*. First herbicide, developed in 1981, is used to control the growth of milk weed in citrus orchards. Biological control of weed may involve either utilization of insects, which would feed selectively on a weed or use of certain microorganisms, which produce diseases in weeds and eliminate them
- Bio herbicides be categorized as

(i) Predators herbivores: In India and Australia, cochineal insect (*Cactoblastis cactorum*) was used for the control of cacti (Opuntia) Similarly in USA, chrysolina beetles were used to control *Hypericum perforatum* (Klamath weed) and beetle *Zygogramma bicolorate* is used for controlling *Parthenium hysterophorus* (the congress grass)

(ii) Smoother crops: The crops, which do not allow the weeds to grow nearby, are called smoother crops. Examples are sweet clover, soya beans alfa-alfa, sunflower, rye, sorghum. These crops eliminate weeds through the chemicals. Therefore, crop rotation with these smoother crops will reduce the incidence of weeds

(iii) Mycoherbicides: Devine and Collego are fungal spores, which are sprayed over weeds for their elimination. These spores are identical for marketing, because they can tolerate adverse conditions and can remain viable for long periods

(iv) Transgenic plants: Transgenic plants are genetically engineered plants, which develop resistance against pests. They contain genes of smoothers crops. Pest and herbicide resistance e.g. transgenic tomato resistant against horn worm larva

(v) Vegetables: Certain weeds such as Amaranthus, chenopodium etc can be eliminated or made useful by using them as vegetable or fodder

8.0 Bio-insecticides

Living organisms or their products used for insect control are called bio-insecticides

(1) Sporeine was first commercial bio-insecticide . It was developed in France in 1940s from the soil bacterium *Bacillus thuringineis* (Bt). This bacterium carries a gene coding for Bt-toxin proteins. One of these, thurioside is active against several insects through the damage of intestinal tract by inhibiting ion transport in the midgut e.g. heavy destruction of coffee plantation by insects. *Prophantis smaragdina* is controlled by thurioside.

(2) 'DOOM' is a mixture of *Bacillus papillae* and *Bacillus lentimorbus*, which has been commercially used for controlling Japanese beetles Popilliae.

(3) Ladybug (lady bird beetle) and praying mantis can control scale insect or aphid pests of vegetables, cotton and apple

(4) Vedalian beetle (*Radiola cardinalis*) has been found effective against cottony cushion scale (*Icerya purchasi*)

(5) Mycar is a product obtained from the fungus *Hirrutella thompsoni* and used to control citrus rust mite

(6) Predator bug (*Cystorhinus mundulus*) has been successfully used to control sugarcane leaf hopper in Hawaii.

(7) Bacillus sphaericus is toxic to larva of Anopheles mosquito

(8) Boverin is obtained from a fungus *Beauveria bassiana* and is used for controlling Colorado potato beetle (*Leptinotarsa decemlineata*) and coding moth

(9) The fungus *Entomophthora ignobilis* may be used for controlling green peach aphid

(10) The fungus coelomomyces is useful to control mosquito larvae

9.0 Sustainable agriculture and organic farming

Sustainable agriculture is an agriculture practice which uses renewable practice which uses renewable resources, causes minimum pollution and maintain optimum yield level. Sustainability of agriculture will be enhanced if there is reduction in use of nonrenewable resources and decrease in level of pollution. The various component of sustainable agriculture are bio fertilizers, bio pesticides, developing resistant varieties and single cell protein. The nonuse of artificial fertilizers and chemical pesticides and use of bio pesticides as well as bio fertilizers in agriculture is called organic farming

10.0 Integrated pest management (IPM)

IPM or integrated pest management is the practice of pest control through biological or natural methods and using the chemical pesticides to the minimum when it is essential. It consists of

(i) Sanitation: removal of crop residue and using it for maturing at a distance

(ii) Culture practices: Use of pest resistance varieties, crop rotation, mixed cropping and intercropping

(iii) Biological control: Allowing the natural predators, parasites and parasitoids of the pests to keep the population of the latter under check e.g. bugs and beetles

(iv) Natural pesticides: They are nonpollutant pesticides e.g. thurioside, azadirachtin, cinerin, pyrethrin

11.0 Microbes in industrial process alcoholic fermentation

- Louis Pasteur found for the first that beer and butter milk are produced due to activity of yeast and yeast like organism
- The nutrient medium is barley matt for beer, fermented rye malt for gin, fermented rice for sake, cashew apple for fenny, potato for vodka, fermented cereals for whisky, fermented molasses for rum and fermented juices for wines and brandy. Yeast does not possess sufficient diastase/amylase. Therefore, either 1% malt or Rhizopus is used when the nutrient medium consists complex carbohydrates as present in cereals and potato. Hydrolysis of starch is carried out in separate tank at high temperature ($55^{O}C$) for 30 minutes. The crushed food mixed with hot water for obtaining malt is called mash. The sweetened nutrient medium prior to alcoholic fermentation is called wort

1. Bioreacter/fermentation tank is sterilized with the help of steam under pressure. The liquid nutrient medium or wort is added into the tank and sterilized similarly. It is then allowed to cool
2. When the liquid nutrient medium is cooled down to appropriate temperature, it is inoculated with appropriate strain of yeast. Fermentation occurs in three ways

(i) Batch process
Bioreactor is very large capacity up to 2,25,000 litre of medium. Yeast and nutrient are allowed to remain there till maximum alcohol content is achieved (6-12%). It is called wash. The same is removed and the tank sterilized for the next batch

(ii) Continuous process
There is regular removal of a portion of fermented liquor/wash addition of more nutrient

(iii) Feed batch process
It is intermediate between batch and continuous process. Here, the culture is continuously fed with fresh medium Immobilized yeast. Lately yeast is being used in immobilized

state in calcium alginate beads. The technique is 20 times more efficient

3. In case of beer, the fermented liquor having alcohol content of 3-6% is filtered, lightly hopped and pasteurized. In case of wines, 10-27% alcohol content is achieved through refinement and concentration. Fortification by direct addition of alcohol may also be carried. Distillation is done in other cases. E.g. gin(41%), rum (40%), brandy (60-70%). Rectified spirit is 95% alcohol. Absolute alcohol is 100% alcohol
4. By products of alcoholic fermentation are CO_2 and yeast. A number of other chemicals can be formed with the change of nutrient medium. pH and aeration -n propanol, butanol, amyl alcohol, pyruvic acid, succinic acid, lactic acid, caproic acid, caprylic acid, ethyl acetate, acetaldehyde, diacetyl, hydrogen sulphide etc
5. Beer: It contain 3-6% alcohol. Nutrient medium is barley grain which have been semi germinated and crushed to form malt. Before inoculation with yeast, the infusion of malt or wort is treated with hops (dried petals of vine *Humulus lupulus*) to provide flavor, colour and stability. Beer is allowed to age before pasteurization. Lager beer is prepared by carrying out ageing in the cold.
6. Wine: It is alcoholic beverage prepared from ripe fruits and fruit juices. Red wine is red due to colour from grape skin, Cider is apple wine. Alcohol content ranges from 10-20%. Sparkling wines are naturally carbonated due to continuation of fermentation after bottling. Champagne is the most popular sparkling wine other famous wines are port wines and sherry wines
7. Brandy : It has an alcoholic content of 60-70%. Brandy is prepared from distillation of generally grape wine but can also be mixture of distillates various fermented juices and wine
8. Whisky and other alcoholic drinks are prepared through distillation as the maximum content of alcohol in fermentation is 6-12%. Vodka has an alcoholic content 60-80%. Gin (40% alcohol) is flavored with juniper berries. Rum has 40% alcohol. The alcohol content of whisky varies with brand

12.0 Antibiotic synthesis by microorganisms

- Antibiotics are chemical substance produced by microorganism which can kill or inhibit the growth of disease causing microorganism. These also inhibit the metabolic activities of other microorganisms. The term 'antibiotic was defined by Waksman in 1942. A microorganism which produces an antimicrobial substance is called an antibiont. At present more than 7000 antibiotic sunstances are known and approximately 100 are available for medical use

- The first antibiotic was penicillin, a product of the moulds *Penicillium notatum* and *P.chrysogenum* discovered by Fleming in 1928.
- Sir Alexander Fleming noticed a halo (technically known as plaque or zone of inhibition) of bacterial growth around a contaminate blue-green mould on a staphylococcus plate culture. He concluded that the mould was releasing a substance that was inhibiting bacterial growth. He grew a pure culture of the mould and discovered that it was Penicillium notatum in 1928. *P.chrysogenum* is a strain was previously known as *Penicillium notatum*. *P.chrysogenum* has been used industrially to produce penicillin and nanthocillin X, to treat pulp mill waste and to produce the enzyme polyamine oxidase, phosphor gluconate dehydrogenase and glucose oxidase.
- Antibiotics are used (i) as medicine for treatment (ii) as preservatives in perishable fresh food articles (iii) As feed supplement especially poultry because they enhance growth

12.1 Microbes as nutritional supplements

- Yeast e.g. *Saccharomyces cerevisiae*, which is a deactivated test is known as nutritional supplement organisms. It is an excellent source of protein and vitamins, especially the B-complex. Vitamins required in metabolism. It is also required for minerals and cofactors essential for growth. Nutritional yeast is low in fat and sodium and fortified with vitamin B_{12}
- It has a nutty cheesy flavor that makes it popular as an ingredient in cheese substitutes. It is also used as topping for popcorn and in mashed and fried potatoes, as well as in scrambled eggs. It is available in the form of flakes, as a yellow powder and is similar in texture to cornmeal. Chlorella (green algae) and Spirulina (simple, one called organism) are also referred as source of protein
- Microbes are commercially used in various industries for the large scale production of various chemical substances such as organic acids, alcohol and enzyme. In the production of chemical substances, microbes are used for fermentation and oxidation reactions.

12.2 Solvents, acids, enzymes and Other bioactive chemicals

1. Solvents
   Acetone, methyl alcohol and butanol are important industrial solvents. Which have been produced through fermentation of sugar and starch by *Clostridium acetobutylicum* and *C.saccharoacetobutylcium*. Glycerol is formed by adding sodium sulphite to alcoholic fermenter. It prevents ethanol formation. Instead glycerol is formed
2. Organic acids
   (i) Acetic acid: it is both microbial and synthetic in origin, Vinegar is 10-13% ripened acetic acid. Initially molasses or

other sugar solution is allowed to undergo alcoholic fermentation by yeast. It is an anaerobic process. As soon as 10-20% alcohol is formed, the liquid is filtered to remove yeast. It is aerated and inoculated with *Acetobacter acetic*. The latter produces acid.

$$C_6H_{12}O_6 \xrightarrow{Yeast} C_2H_5OH + 2CO_2$$

$$C_2H_5OH + O_2 \xrightarrow{acetobacter\ acetic} CH_3COOH + H_2O$$

When 10-30% acetic acid content is achieved, the liquid is filtered. The filtrate is allowed to ripen till the disagreeable smell disappears. It is pastured and called vinegar. The acid is concentrated and vinegar is used as souring agent, preservative and condiment. Acetic acid is used in plastics, pharmaceuticals, colouring agents, insecticides, production of solvents and flavoring agent.

(ii) Citric acid: *Aspergillus niger*, *Mucor* species can ferment sugar to produce citric acid. Citric acid is preservative and flavoring agent. It is also used in medicines, engraving, dyeing and inks

(iii) Lactic acid: First organic acid is to be fermented, lactic acid is obtained by activity of bacteria like *streptococcus lacti*, fungus Rhizopus etc. Over a variety of nutrient media like hydrolysed corn or potato starch, molassess, sulphates liquor etc. Lactic acid is used in cleansing, flavoring, preserving pickles, curing of meat. It is used as mordant in tanning, dyeing, printing of wool and preparation of plastics

(iv) Other organic preparations:

(a) Gallic acid is obtained with the help of Aspergillus niger. It is employed in ink making.

(b) Gluconic acid is manufactured through the agency of Penicillium purpurogenum, for use as calcium gluconate (providing calcium to infants, lactating mother and treating milk fever in high milk yield cows and in preparation of pharmaceuticals

(c) Butyric acid is formed by activity of *Clostridium butyricum*. It is the acid that occurs in rancid butter

3. Enzymes

(i) Pectinase: They are obtained from fungi grown on pectin containing media. Eg. Aspergillus niger, Pectinases are used in enhancing juice extraction from fruits, cleansing of fruit juice, removing bitterness retting of fibres and fermentation of green coffee

(ii) Proteases: They are obtained from both bacteria (e.g. *Bacillus subtilis, B. lichenformis*) and fungi (e.g. *Aspergillus oryzae*). Proteases are used in enhancing tenderness of meat, extraction of fish liver oil, clarification and chill proofing of alcoholic drinks, synthesis of glue, cleaning of hides and in detergents for removing protein based strains

(iii) Lipase they are lipid digestive enzymes that are obtained by growing *Candida lipolytica, Aspergillus niger* etc lipases are used in flavouring cheese, hydrolyzing oils for soap manufacture and in detergent formulations for removing fat based stains

(iv) Amylases they are starch digesting enzymes which are obtained from *Aspergillus oryzae, B. diastaticus* etc. The enzymes are used in softening and sweetening of bread, desizing fibres, cleansing starch related stains, clearing starch related stains in utensils and clothes

(v) Streptokinase: It is also called tissue plasminogen activator or TPA which is obtained from haemolytic streptococcus species and modified genetically to be useful in human beings. It is used as clot buster or dissolving blood clots in vessels of patients having undergo mycocardinal infaction or heart attack

4. Cyclosporin A
   It is an 11=membered cyclic oligo-peptide. It is obtained from fungus *Trichoderma polysporum* or *Tolypocladium inflatum* cyclosporin A has immunosuppressive property as it inhibits activation of T-cell response to transplanted organ
5. Statins
   They are inhibitor of enzyme HmG can reductase of liver which forms mevalonate required for cholesterol synthesis. Statin are synthesised by activity of yeast Monascus purpureus
6. Vitamins
   (i) Vitamin C: Acetobacter is helpful in dehydrogenation of D-sorbital and its conversion to L-sorbose. Which is precursor of vitamin –C or L-ascorbic acid
   (ii) Vitmin B_{12} : Organism like *Propionibacterium frendeureichii*, *Streptomyces oliaceus* are used on nutrient medium made of starch, corn syrup or molasses. The cells are harvested and autolysed to separate the vitamin.