BIOLOGY

for NEET & BOARD

MICROBES IN HUMAN WELFARE

Key Features

1. All-In One Study Material (For Boards/Medical/Olympiads).
2. Concise, Conceptual & Trick – Based Theory.
3. NTA Based Solved Multiple Choice Questions With Answers.

Microbes In Human Welfare

Chapter – **10**

India's First Trick Based Study Material

1 MICROBES IN HOUSEHOLD PRODUCTS

Dairy Products :

- Lactic acid bacteria **(LAB)** like *Lactobacillus* are added to milk. It converts lactose sugar of milk into lactic acid.
- Milk can be changed into curd, yoghurt and cheese. The starter used in preparation of milk products actually contains millions of LAB.

(a) Curd : Indian curd is prepared by inoculating cream and skimmed milk with *Lactobacillus acidophilus* at a temperature of about 40°C or less. Curd is more nutritious than milk as it contains a number of vitamins, especially Vit B_{12} and organic acids.

(b) Yoghurt : [CURDS]

For production of curds or yoghurt pasteurized milk is inoculated with a mixture of **Streptococcus thermophilus** and **Lactobacillus bulgaricus** and its lactose is fermented by keeping it at 40°C. The peculiar or characteristic taste and flavour of curds are due to presence of lactic acid and acetaldehyde. Curdling or coagulation of milk is also caused by lactic acid and which is formed. In India, curds are not generally commercially produced but in developed countries large scale manufacture of yoghurt is done. In U.S.A alone about 75 lakh kilogram of yoghurt is manufactured every year.

(c) Butter milk : It is acidulated product which is formed by inoculating skimmed milk with starter culture of *Streptococcus cremoris, S. lactis, Lactobacillus acidophilus, Leuconostoc* species at 22°C for 18 hours.

(d) Sour Cream : It is inoculated with *Streptococcus lactis* for producing lactic acid and

Leuconostoc cremoris for imparting the characteristic flavour.

(e) Cheese : It is partially degraded concentrate of milk fat and casein, manufactured by activity of microorganisms. There are several hundred varieties of cheese which are prepared by selected types of microorganisms. The quality and characteristic taste of cheese is determined by the biochemical activities of specific microorganisms. **Cheese** consists of milk curd that has separated from whey or liquid part. Cheese is of three types - **soft** (50-80% water), **semi-hard** (about 45% water) and **hard** (less than 40%

water). The method of preparing cheese with the help of microbes was known in Asia and Europe long before Christ. **Large holed Swiss cheese**

is ripened with the help of CO_2 producing bacterium called ***Propionibacterium sharmanii***. **Roquefort cheese** or blue cheese uses *Penicillium roquefortii,* while **Camembert cheese** employs *Penicillium camembertii* for ripening.

Bread :

- Selected strains of *Saccharomyces cerevisiae* (Baker's yeast) grown on molasses are used for this purpose.
- The kneaded flour is kept at a warm temperature for a few hours. It swells up. The phenomenon is called **leavening.**
- Leavening is caused by secretion of three types of enzymes by yeast. They are **amylase, maltase** and **zymase.**
- The leavened dough is baked. Both carbon dioxide and ethyl alcohol evaporates, making the bread porous and soft.

Dosa, Uppma and Idli :

- These are fermented preparations of rice and black gram. The two are allowed to ferment for 3- 12 hours with *Leuconostoc* and *Streptococcus* species of bacteria.

Other Foods :

- **Tempeh** (Indonesia) **Tofu** (Japanese) and **Sufu** (Chinese) are fermented foods obtained from **soya sauce,** is brown flavoured salty sauce fermented from soyabean and wheat.
- Tender **bamboo shoots** can be used as vegetable directly as well as after fermentation. Several types of **sausages** are prepared by fermentation and curing of fish and meat.

Toddy (Toddy palm-Caryota urens) :

- The unopened spadices of palm are tapped to obtain toddy. It is a refreshing drink which can be heated to produce jaggery or palm sugar. Toddy left for a few hours undergoes fermentation with the help of naturally occurring yeast to form beverage containing about 6% alcohol.

2 MICROBES IN INDUSTRIAL PRODUCTS

- Fermentive activity of microbes is used industrially to obtain a number of products. Production on an industrial scale, requires growing microbes in very large vessels called **fermentors.** The two common ones are alcoholic fermentation and antibiotics.
  - ✓ Microorganisms are used in industry to produce a range of chemical compounds, enzymes and drugs – this is because:
  - ✓ They are small and so can be grown economically in fermenters on either solid or liquid media

- ✓ They have a fast growth rate and so can produce large yields in short periods of time
- ✓ They can be genetically modified with relative ease to synthesise non-natural products of interest
- ✓ May be able to tolerate unusual growth conditions (e.g. extremophiles can grow in high temperatures or acidic conditions)

- When grown in fermenters, microorganism rate of growth will eventually become limited by metabolic waste products
  - ✓ The fermentation reaction can be periodically halted to replenish nutrients and remove wastes (batch culture)
  - ✓ Alternatively, nutrient and waste levels can be dynamically adjusted to maintain the reaction indefinitely (continuous culture)

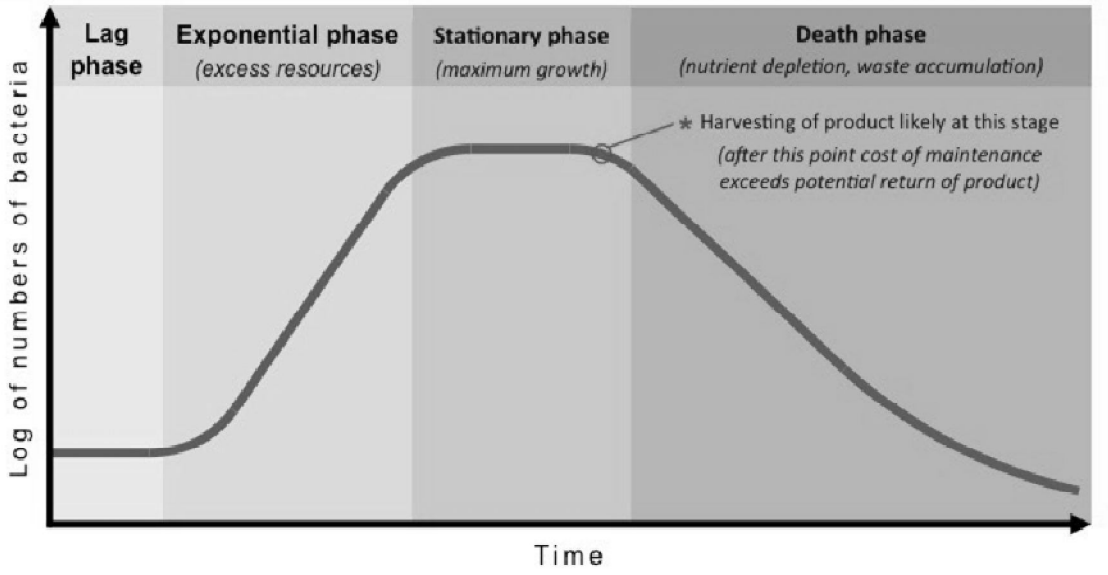


Fig: Microorganism Growth Rate (Fixed Container)

A **fermenter** is an enclosed and sterilised vessel that maintains optimal conditions for the growth of a microorganism

- The microorganism undergoes fermentation to produce large quantities of a desired metabolite for commercial use

Product can be collected from a fermenter after a fixed amount of time (batch cultivation) or ongoing (continuous cultivation)

- In batch cultivation, the microorganism goes through all the stages of growth prior to the collection of product
- In continuous cultivation, the microorganism is maintained at a peak rate of growth (exponential phase)

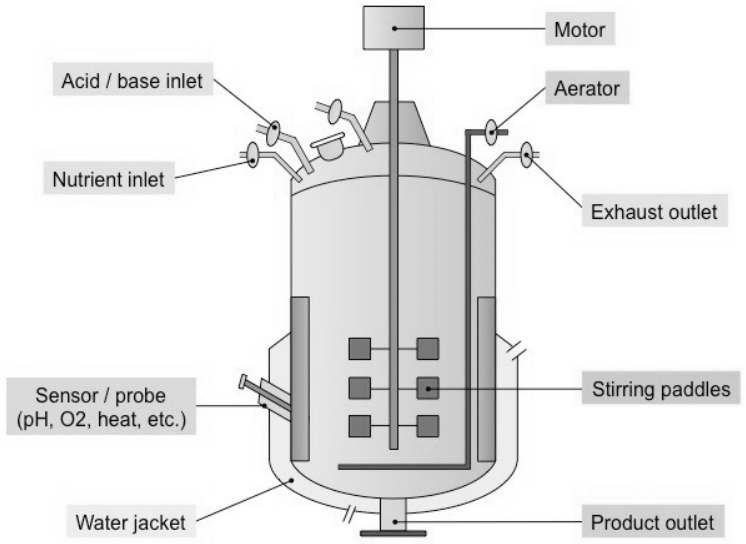


Fig: Diagram of a Standard Fermenter

Probes and sensors are used to monitor condtions within the fermenter in order to maintain optimal levels of microbial growth

- Motorised stirring paddles function to distribute heat and materials evenly within the reaction chamber
- An external water jacket can be used to absorb excess heat and maintain a constant viable temperature
- An aerator can introduced compressed air into the chamber, while a defoamer can hinder the formation of foam
- Acid/base inlets allow for the regulation of pH levels within the chamber (formation of product may alter pH)
- Nutrient inlets and exhaust outlets allow for the introduction of sugars or the removal of metabolic wastes.
- The necessary adjustments required for continued growth may differ between batch cultivation and continuous cultivation.
- In both cases, conditions within the fermenter must remain aseptic to prevent contamination of the microorganism

Fermented Beverages/Alcoholic Fermentation

- Yeast species used in alcoholic fermentation are *Saccharomyces cerevisiae* (Brewer's Yeast), *S. ellipsoidens* (Wine Yeast), *S. sake* (Sake Yeast) and *S. pireformis* (Ginger Beer/Ale Yeast).
- The nutrient medium is barley malt 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
- Wine and beer are produced without distillation, whereas whisky (50% alcohol)

brandy (65- 70% alcohol), rum (40% alcohol) and gin about (40% alcohol) are produced by distillation of fermented broth.

Antibiotics (means against life in Greek)

- An antibiotic is a substance produced by a microorganism, which in low concentration inhibits the growth and metabolic activity of pathogenic organisms without harming the host.
- This is among the **most significant discoveries of twentieth century.**
- First antibiotic is generally associated with the name of **Alexander Flemming** (1928) when he discovered **Penicillin** from *Penicillium notatum.*
- The antibiotic was however, commercially extracted by efforts of **Ernst Chain** and **Howard Florey**. The chemical was extensively used in treating wounded American soldiers in world war II**. Flemming, Chain and Florey were awarded Nobel Prize in 1945.** Bulk of antibiotics are obtained from three groups of microorganism *i.e.,* Eubacteria, actinomycetes and fungi
- Antibiotics have greatly improved our capacity to treat deadly diseases like plague, whooping cough, diphtheria, leprosy etc. So with reference to human beings these are **pro-life.**

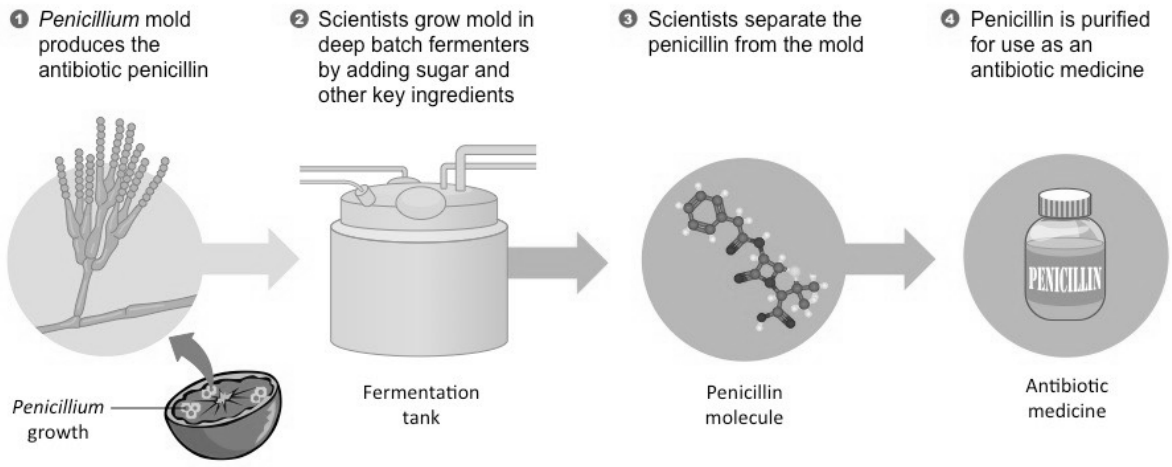


Fig: Production of Penicillin via Batch Fermentation

The antibiotic penicillin can be mass produced via the use of deep-tank batch fermentation

- Large industrial fermenters are constructed that have the capacity to hold thousands of litres
- *Penicillium* mold is grown in the deep-tank batch fermenters following the addition of sugars and other key ingredients
- The production process typically lasts 6 – 8 days, with the fermenter drained at the end of the fermentation cycle
- Penicillin is separated from the solution and purified via downstream processing to improve its antibiotic potential

Chemicals, Enzymes and Other Bioactive Molecules

- Bioactive molecules are those molecules which are functional in living systems or can interact with their components. A number of them are obtained from microbes like organic acids, enzymes, cyclosporin A and statins.

Some other common products of yeast fermentation are –

[i] Beer – It is produced from **Hordeum Valgare** [Barely] malt and alcohol content is 4-8%

[ii] Wine – Produced from **grapes**, alcohol content is 10-20%.

[iii]Brandy and Whisky – Produced by distillation of wine and alcohol content is 43-57 %

[iv]Gin – Produced from **European Rye-Secale cereale.**

[v] Rum – Produced from **Molasses** of Sugarcane and alcohol contents is 40%

Note – Another yeast which supplies nutritional rich food for Man animals is **Torulopsis utilis**

Industrial utilization of biotechnology involve three steps –

[i] Laboratory scale process

[ii] Pilot plant scale

[iii]Manufacturing unit

The development from laboratory scale to manufacturing unit is "Scaling up to industrial production". Micro-organisms can be grown in bioreactors in two ways :

(a) Support growth system – In this method microorganisms are growing as a thin layer or film in the **solid medium.**

(b) Suspended growth system – By suspending cells or mycelia in the **liquid** medium it is called suspended growth system

[iii]Manufacturing unit –

During the designing, bioreactor used for the process are often of very large size so that it can accommodate huge amount of medium.

Downstream Processing –

After completion of the biosynthetic stage, the product has to be subjected through a series of processes before it is ready for marketing as a finished product. The processes include separation and purification, which are collectively referred to as downstream processing. The product has to be formulated with suitable preservatives. Such formulation has to undergo through clinical trials as in case of drugs. Strict quality control testing for each product is also required. The downstream processing quality control testing vary from product to product.

(A) *Organic Acids*

- Certain microbes have the ability to convert carbohydrates into organic acids, this capability of microorganisms is applied in the industrial production of some

commercially important organic acids. A few very important organic acids are as follows.

1. **Acetic acid :** It is prepared from fermented alcohols with the help of acetic acid bacteria, *Acetobacter aceti.* Alcoholic fermentation by yeast is anaerobic process, but the conversion of alcohol to acetic acid is aerobic one. It is used for the prepration of **vinegar.** It is also used in pharmaceuticals, colouring agents, insecticides and plastic industries.

2. **Citric acid :** It is obtained through the fermentation carried out by fungi *Aspergillus niger* and *Mucor* species on sugary syrups. Yeast *Candida lipolytica* can also be employed, provided its nutrient medium is made deficient of iron and manganese. Citric acid is employed in dyeing, engraving, medicines, inks, flavouring and preservation of food and candies.

Citric acid (citrate) is widely used as a flavour enhancer, a preservative in manufactured foods and an antioxidant

- It is produced as an intermediate of the Krebs cycle under aerobic conditions
- Citric acid is mass produced by continuous fermenter systems from cultures of the fungus *Aspergillus niger*
- Carbohydrates are continuously introduced into the fermenter in order to maintain the citric acid production
- Iron (Fe^{2+} ions) is excluded from the mixture in order to slow the further conversion of citric acid within the Krebs cycle
- As citric acid accumulates it is extracted as part of the medium that is being continuously withdrawn from the fermenter

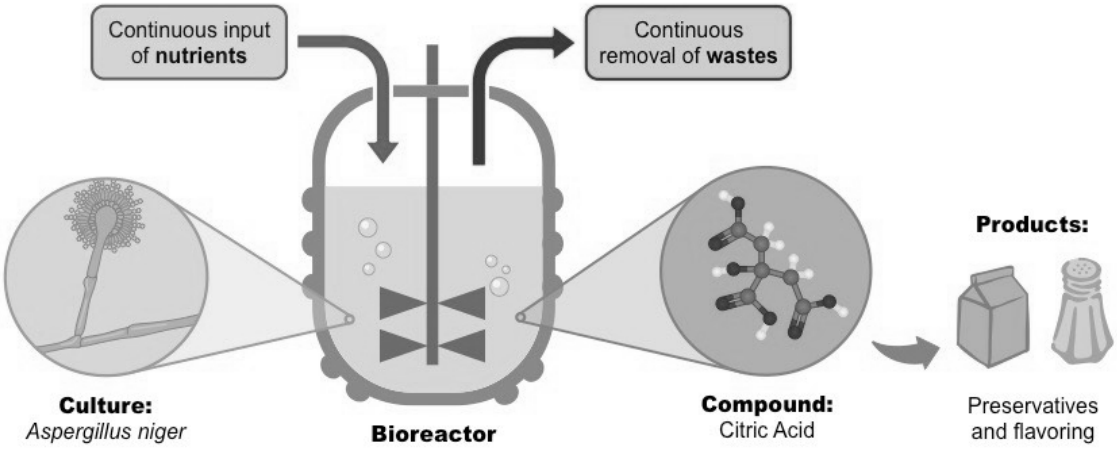


Fig: Production of Citric Acid via Continuous Fermentation

3. **Gluconic acid :** The acid is prepared by the activity of *Aspergillus niger* and *Penicillium chtysogenum.* Gluconate is used widely as a source of calcium for infants,

cows and lactating mothers.

4. **Lactic acid :** It was first acid to be produced by industrial fermentation. It is commercially produced from fermentable carbohydrates such as corn and potato starch, molasses and whey by using the bacteria - *Lactobacillus bulgaricus* and *L. delbrueckii.*

(B) *Enzymes: Hardly 1.0-1.5% of the total known enzymes are employed in industry and medicine. Total known enzymes 2,200 and only 1–1.5% are used*

(i) Rennet – Manufacturing "**Cheese**"

Old days cheese had been prepared either using the layer of stomach of Goat or Sheep OR the sap of **Fig tree,** containing special enzyme–**Ficin.** In 1874 a Danish Chemist – **Christian Hansen** extracted pure rennet enzyme from **Calf's stomach** for industrial production of cheese. First of all diastase enzyme was identity by payen and persoz (1933)

I. Unripened cheese – Ripened from out side soft.

II. Ripened cheese – it is hard and ripened externally as well as internally.

Manufacturing cheese involve following steps.

(i) Milk is inoculated with starter culture of bacteria – **Streptococcus lactis** or **S.cremoris** and warmed at 38°C. If higher temperature [50°C or more] then **S**. **thermophilus** combined with **Lactobaccilus lactis, I.bulgaricus** or **I.helveticus is used.**

(ii) When a certain **acidity** reached in milk by the activity of species of bacteria then rennet enzyme is added. Curdling of milk occurs within half an hour to one hour.

(iii) The curd is removed and liquid separates out which is called **whey** [contain 93% water and 5% Lactose]. Lactose of whey is used for the manufacture of **Lactic acid** – First fermented acid. If the cheese is used at this stage is called **cottage cheese** (unripened stage).

(iv) The salts mixed with cottage cheese and put into the frames and pressed so as to allow removal of whey. Salts hastens the removal moisture and prevent the growth of undesirable microbes. The frames are removed as soon as the cheese has set sufficiently to maintain its shape.

- The ripening period varies from 1-6 months but which is very tasty and nutritious. This is hard and ripened cheese contains about 20-30% fats, 20-35% proteins and small amount of minerals and vitamins
- [Cheese which prepared at homes with the help of lemon juice is called **Raw cheese**]
- Nearly 400 varieties of cheese available which can be classified into following type –

	Type of Cheese	**Micro Organisms used**	**Reaction**
1.	**Soft**		
	(A) Camembert	Penicillium camemberti Brevibacterium	Ripened by action of microorganisms on the surface of curd
	(B) Limburger	Streptococcus liquifaciens Brevibacterium	

2.	**Semi-hard** (A) Roquefort (B) Blue	Penicillium requeforti	Combination of surface and interior growths
3.	**Hard** (A) Swiss (B) Cheddar	Propionibacterium sp Geotrichum	Inoculating the organisms throughout the curd

(ii) Proteases – This enzyme obtained from **Aspergillus orizae** and **Bacillus subtilis,** Bacillus **licheniformis** and utilized from the formation of **detergents** in detergent industry [For removing proteinous strains on clothes]. The bottle juices are clarified by the use of pectinases and protease.

(iii) Amylases – It works on starch and used in Beer, Bread and Textiles industries.

(iv) Amylase, Gluco amylase and Gluco amylase and Gluco isomerase – By the action of all these enzymes **corn** (maize) **starch** transformed into **fructose corn syrup**. This syrup is more sweeter than sucrose and used in beverage industry to flavour **soft drinks** and in baking industry to sweeten biscuits and cakes.

(5) Tissue Plasminogen Activator [TPA] or Streptokinase – The enzyme utilized in medicinal field. Streptokinase produced by the bacterium. Streptococcus and modified by genetic engineering is used as a clot buster for removing clots from the blood vessels of patients who have undergone myocardial infraction leading to heart attack.

Uses of Enzymes

(1) Detergents

(i) **Proteases**

(ii) α-**Amylase**

(iii)**Cellulases**

(iv)**Lipases**

(2) Leather Industry

(3) Wool industries

(4) Glucose from Cellulose

(5) Food, Dairy, juice and Beverages Industries

(6) Production of Glucose Syrup

• Bioactive molecule, **cyclosporinA,** that is used as an immunosuppressive agent in organ-transplant patients, is produced by the fungus ***Trichoderma polysporum*. Statins** Produced by the yeast *Monascus purpureus* have been commercialized as blood-cholesterol lowering agents. It acts by competitively inhibiting the enzyme responsible for synthesis of cholesterol.

(C) *Cyclosporin A*

- It is an eleven membered cyclic oligopeptide obtained through fermentive activity of fungus, *Trichoderma polysporum.*
- It has antifungal and anti-inflammatory properties. It is used as an immunosuppressive agent in organ-transplant patients.

(D) *Statins*

- They are products of fermentation activity of yeast *Monascus purpureus* which resemble mevalonate and is competitive inhibitor of □-hydroxy-□-methylglutaryl CoA reductase or HMG COA reductase.
- This competitively inhibits cholesterol synthesis. It is used as cholesterol lowering agent

3 VITAMINS

Definitions : -

- Vitamins are complex organic substances found in various food and required for specific metabolic reaction in cells.
- The term vitamin first of all used by **Funk** and first of all **Vit-B_1** also isolated by him. **Vit-C** isolated by **A.S. Gyorgy.** The **first vitamin** to be produced by fermentation was **vit-C** by A.S. Gyorgy also **Vit-A** was isolated by **Mc Collum** and **Vit-D** by **Millanby.**
- Micorbes are important commercial sources of several vitamins. Some examples are given here : -

1. **Riboflavin (vitamin B_2) : -** This found in cereals, vegetables and Brewer's yeast was first produced in 1938. It is a crystalline, bitter, odourless, yellowish brown chemical and is essential for growth and reproduction in animals. The main sources of Riboflavin are **fungi–Ashbya gossypii** and the yeast like **Eremothecium ashybyii.** By the use of original wild strain of mould Ashbya gossypi production of Vit B_2 in increase 100-300 time more.

2. **Cobalamin or Vitamin B_{12}** : - First isolated from **liver extract** in 1948. It is a compound which contains cobalt, and is now obtained in a cobalt rich substrate (eg. corn sugarcane molasses or starch) by microbes such as **Propionibacterium frendenreichii, Bacillus megatherium.**

   Vitamin B_{12} is used to supplement animals feed, and in man for increasing appetite and for treating Anaemia. By the use of Mutant strains of **Pseudomonas denitrificans** production of Vit-B_{12} increase 50,000 times more.

3. **Ascorbic Acid (Vitamin C)** : - It is manufactured from L. Sorbose which is commercially produced from D-Sorbitol by Biological dehydrogenation brought about by different species of **Acetobacter.**

4 ANTIBIOTICS

The term was coined by **Selman Waksman** (1942)

Definition : -

"An antibiotic is a substance produced by a microorganism which in low concentration inhibits the growth and metabolic activity of pathogenic organism without harming the host."

Alexander Flemming was first to produce and antibiotic named **penicillin** from **Penicillium notatum Waksman** and **Albert** discovered **Streptomycin** and **Actinomycin.**

Burkholder isolated **Chloromycetin.**

Antibiotics are of two types : -

1. **Broad spectrum Antibiotic** : - It is an antibiotic which can kill or destroy a number of pathogens that belong to different groups with different structure and wall composition.
2. **Limited spectrum (Specific) antibiotic** : - It is an antibiotic which is effective only against one type of pathogens.

Action of Antibiotics : - An antibiotic acts on pathogen by

(1) disruption of wall synthesis

(2) Disruption of plasmalemma repair and synthesis

(3) inhibition of DNA/RNA/Protein synthesis.

Good Antibiotic : -

A. Harmless to host with no side effect.

B. Harmless to normal micro-flora of Alimentary canal.

C. Ability to destroy pathogen as well as broad spectrum.

D. Effective against all strains of pathogen

E. Quick Action

Resistance of Antibiotics : -

Pathogens often develop resistance to exiting antibiotics so that never antibiotics are required to be produced.

The resistance is produced due to : -

i. Development of copious mucilage

ii. Alteration of cell membrane so that antibiotic cannot recognize the pathogen.

iii. Change to L-form by pathogen

iv. Mutation in pathogen.

Main sources of Antibiotic : -

The main sources of Antibiotics production are three types –

(i) Eubactrials – Most of this type of antibiotic is obtained from **Bacillus sps.** (70%) Bacillus subtilis produced more than 60 antibiotics and from **Pseudomonas species** 30%.

(ii) Actinomycetales [Ramified] – Streptomyces, Micromonospora and streptosporangium. From single species **Streptomyces griseus** more than 40 antibiotics have been obtained.

(iii)Fungi – Penicillium, Cephalosporins.

5 STEROIDS

- ✍ They are complex crystallisable lipids having a tetracyclic hydrocarbon core (one 5-carbon and three 6 carbon rings) a long side chain. They are constituents of hormones and some important biochemicals like cholesterol, progesterone, oestrogen, testosterone, cortisterone, and cortisone. Compounds of steroids are found in both animals and plants. The main important steroid which is found in animals i.e. **Cholesterol.** It is the main constituent of animals cell membrane and main point for the initiation of steroid in hormone inside the body. Steroids are used medicinally in correcting hormonal imbalance, Anabolic stimulants, Birth control pills
- ✍ antifertility drugs, Anti-inflammatories, relieving pain and suppressing immune response various steroids differ from one another in radicals like –OH = O, $–CO–CH_3$, $–COCH_2OH$.
- ✍ **Murray** and **Peterson** (1950) found that **Rhizopus stolonifer** could bring about hydroxylation required for steroid synthesis including removal of hydrogen (dehydrogenation) from specific carbons. Different microorganisms produce different steroids from progesterone like pregnane, cortexolone, Androsterone etc. The commercial conversion of **Cortixolone** to **Predinisolone** which is used as **Antiflammatory durgs** this involves first hydroxylation then dehydrogenation.

6 INTERFERONS

First of **all Issac** and **Linderman** observed that immunity due to the formation of special soluble substances, produced by viral infected cells. This small groups of protein is named - **Interferons**.

These are the proteins released by the cells in response to a viral infection which they help to combat. These interferon do not inactivate the virus, but they make the unattacked cells, less susceptible so they are prevented from the attachment of virus. They also prevent the viruses from taking over the cellular machinery. Interferon proteins have proved to be effective in treating influenza and hepatitis, but their role in cancer treatment is doubtful. Interferon are produced by **Charlse Weisman** through the **E.coli** strain produced by Recombinent DNA technology.

7 DEXTRINS

- ✍ It is a **plasma expander** having 6-10% solution of dextrins which is given in case of haemorrhage, shock and dehydration and plasma transfusion.
- ✍ Dextrins are soluble **polyglycans** or polymers of D-glucose. They are prepared either through partial hydrolysis of starch or partial polymerization of simple sugars through microorganism ***Leuconostoc mesenteroids*** or Enzyme dextran sucrase. The enzyme is more useful as dextran or dextrin of suitable molecular weight can be obtained more easily.

8 INSULINS

- ✍ It is a proteinaceous hormone having 51 Amino acids arranged in two polypeptides A and B having 21 and 30 Amino Acids, respectively and joined by S-S disulphide bridges.

- ✍ **Sir Edward sharpy-Shafer** (1916) was the first to note that diabetes of some persons was because of failure of some islands of pancreas to produce a substance which the called insulin (Derived from the latin, insula, meaning island).
- ✍ **Banting** and **best** (1921) were the first to isolate insulin from dog's pancreas and used it to cure diabetes in man.
- ✍ The source of insulin used for curing diabetes these days, is the pancreas of slaughtered **cattle** and **pigs**. Through this insulin effective in controlling diabetes, it results in certain undesirable effects.
- ✍ The first genetically engineered insulin obtained by **recombinant DNA technique** with the help of **E-Coli** was produced by the American firms, **Eli-Lilly** on July 5, 1983. It has been given the trade name **humulin** and has been approved for clinical use.

9 VACCINES

- ✍ Production of **antibodies** against of antigens inside the body is the basis of **immunity**. Process of inoculation of vaccines is called **vaccination**. Scientific base of vaccination established by **Louis Pasteur**. First vaccine was discovered by **Edward Jenner** for small pox.
- ✍ A vaccine contain either **weakened** or even **killed-attenuation pathogens** [serum suspension with virulence] which have still antigens to induce antibody production. All these vaccines are called **First generation vaccines.** They are produced by **conventional technique.**
- ✍ Recently new vaccines are produced called **second generation vaccines.** These are produced by **Recombinant DNA technology** [Genetic Engineering] e.g., **Herpese Virus and Hepatitis-B.**
- ✍ Latest vaccines produced synthetically or **synthesized vaccines** are called **third generation vaccines.**

AN IDEAL VACCINE :

1. It should not be tumerogenic or toxic or pathogenic, i.e., it should be safe.
2. It should have very low levels of side effects is normal individuals.
3. It should not cause problems in individuals with impaired immune system.
4. It should be effective in producing long lasting humoral and cellular immunities.
5. The technique of vaccination should be simple.
6. The vaccine should be cheap so that it is generally affordable. So far, such an ideal vaccine has not been developed.

10 MICROBES IN SEWAGE TREATMENT

- Sewage is collective noun used to represent municipal waste (both liquid and solid waste) generated in cities and towns which is carried off in sewerage
- It contains large amount of domestic water and waste including human and animal excreta, microbes and every things that enter sewerage system.

- Sewage or municipal waste should not be passed into rivers, streams and other water bodies, because it not only contains human excreta and other organic wastes but a number of pathogenic microbes.
- It is made less polluting by passing it through **sewage treatment plants** (STPs).
- Treatment of waste water is done by the heterotrophic microbes naturally present in the sewage.
- The various steps in sewage treatment are :

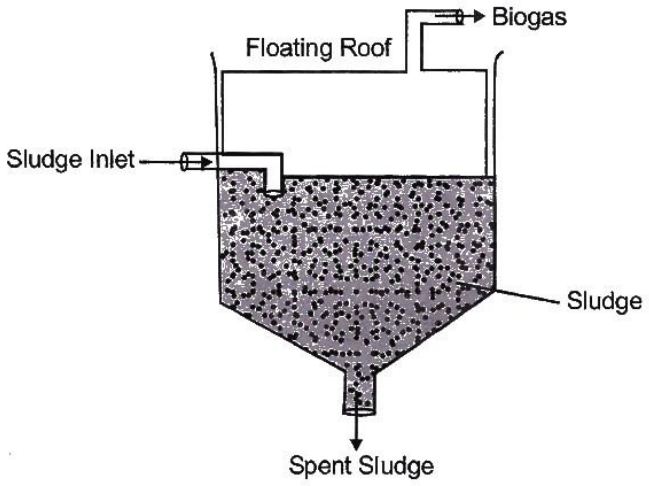
1. Primary Treatment :

- It is a **physical** process of removal of large and small particles from sewage through *sequential filtration and sedimentation.*
- Initially, floating debris is removed by sequential filtration, then the grit (soil and small pebbles) are removed by 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 collectively constitute *primary sludge.*
- Primary sludge can be used for preparing compost or manure directly. It can also be burnt.
- The waste water **(primary effluent)** after removing the primary sludge contains fine organic matter. It is passed for secondary treatment.

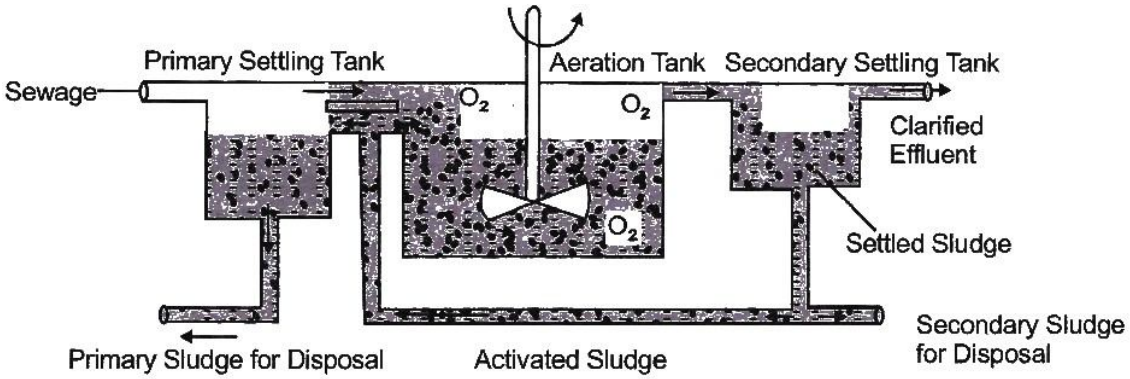
2. Secondary Treatment or Biological Treatment :

- It involves biological process of microbial degradation of organic matter.
- There are three main methods use of 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. It contains a large population of aerobic heterotrophic microbes, including bacteria and fungi.
- The microbes form **flocs** (masses of bacteria associated with fungal filaments).
- The BOD of the effluent **rises initially** and the treatment continues till the **BOD decrease** to a certain level.
- It is taken to secondary settling tank where the flocs undergo sedimentation.

- The sediment is called **activated sludge** (This can be the inoculant for next secondary treatment).
- The supernatant is allowed to pass into rivers and streams.
- Activated sludge is taken to **anaerobic sludge digesters** alongwith the primary sludge
- Here, anaerobic microbes act upon organic matter to first produce monomers and then organic acids.
- This 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 can be used as a source of energy.
- The spent sludge is used as manure, land fill or can be burnt. Pathogens present in the original sewage get killed during anaerobic digestion.



Anaerobic sludge digester



Activated sludge method of sewage treatment

3. Tertiary Treatment :

- It is **physiochemical process** in which chlorine gas, zirconium, ozone gas, perchlorate salts, UV rays or reverse osmosis etc are used to remove DDT, pesticides, pathogens

domestic use.

11 RIVER ACTION PLANS

- Prior to 1985, very few cities and towns had sewage treatment plants.
- The municipal waste water was discharged directly into rivers resulting in their pollution and high incidence of water borne diseases
- In order to protect the major rivers of India from sewage pollution, the Ministry of Environment and Forests, has initiated development of sewage treatment plants under the National River Conservation Authority, *e.g.*, Ganga Action Plan (GAP), Yamuna Action Plan, etc.

12 MICROBES IN PRODUCTION OF BIOGAS

- Biogas is a mixture of gases produced from degradable organic matter by activity of various anaerobic bacteria.
- The microorganisms involved in biogas production are mainly facultative as well as strict anaerobic bacteria.
- The most important among them are methanogenic archaebacteria, represented by *Methanobacterium.*
- The other bacteria involved are *Bacillus, Cellulomonas, Clostridium* and *Ruminococcus.*
- These bacteria are commonly found in anaerobic sludge formed during sewage treatment. Methanogens do occur in rumen of cattle where they act upon cellulose.

Composition of biogas :

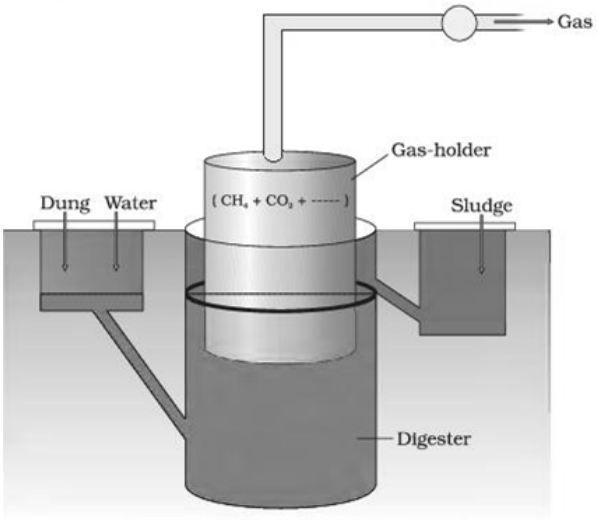
- The major component of biogas is methane (about 50 —70%) which is highly inflammable. The other gases are carbon dioxide (30 — 40%) and 10% mixture of other gases, viz., H_2, H_2S etc. Calorific value of biogas is 4429 kcal/m^3 at 50% methane content.

Substrates Useful in Biogas Production :

Animal wastes	Cattle dung and urine, Buffalo dung and urine, Goat/sheep dung and urine, slaughter house wastes
Aquatic plants	*Eichhornia* (water hyacinth), algae
By-products/wastes	Bagasse, bran, tobacco waste
Crop residues	Crop stubble, straw, weeds, fodder, cotton and jute sticks
Forest residues	Bark, branches, leaves, twigs
Human wastes	Night soil (human faeces and urine)
Urban solid wastes	Paper, domestic wastes etc

Commercial Production of Biogas :

- The technology for biogas production was developed in India by **IARI (Indian Agriculture Research Institute)** and **KVIC (Khadi and Village Industries Commission)**.
- A biogas plant has a large (10-15 ft. 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. The raw material used in biogas plants is cattle dung, night soil, farm refuse, water weeds *(e.g., Eichhornia)* and other organic wastes.
- It is converted into slurry with 90% water content and fed to digester.
- Cattle dung contains *Methanobacterium* and other methanogens which are normally present in rumen of cattle for aiding in digestion of cellulose.
- An inoculum can also be provided when a gobar gas plant is to be initiated.



A typical biogas plant

- Formation of biogas is a three step anaerobic process :

1. Solubilisation (Decomposition) :

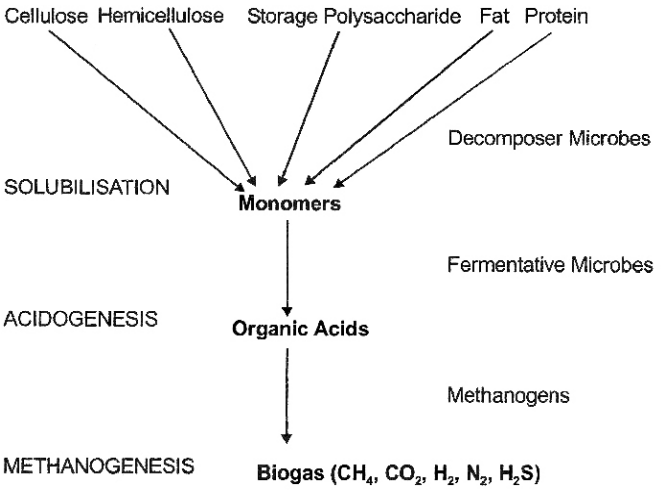
- Organic wastes are composed of lipids, proteins, cellulose, hemicellulose and lignin. In the first stage of biogas generation, facultative anaerobic decomposers are active.
- They secrete hydrolytic enzymes, *e.g.*, lipases, cellulases, proteases, peptidases.
- The enzymes breakdown the complex organic components into simpler and soluble substances. The latter are commonly called **monomers**.

2. Acidogenesis :

- Monomers are changed into organic acids with the help of fermentating microbes. The

most common organic acids produced during acidogenesis is acetic acid.

- Hydrogen and carbon dioxide are produced as by products.



Stages in biogas formation

3. Methanogenesis :

- Methanogens or methane producing bacteria become active.

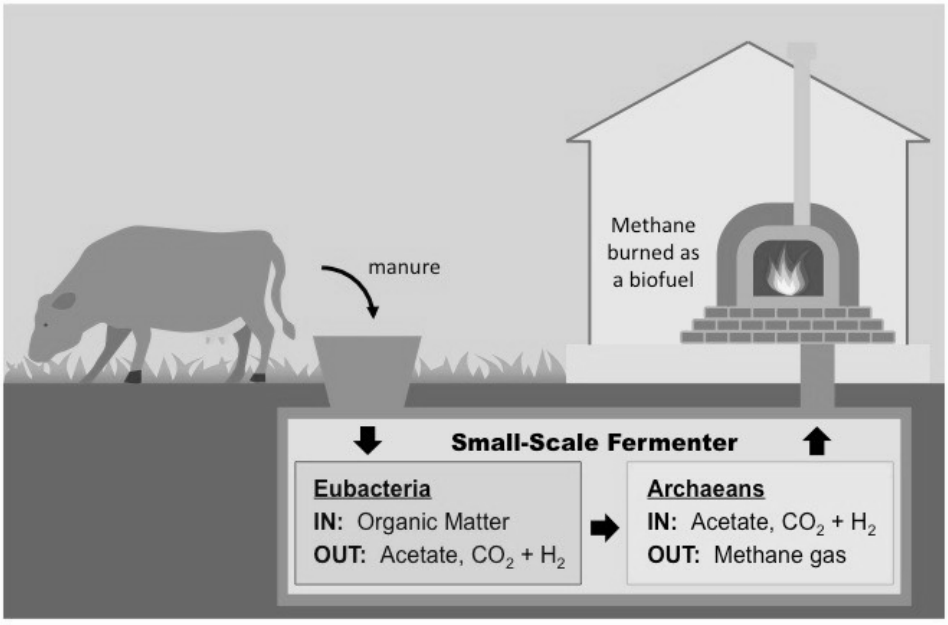


Fig: Overview of Biogas Production

13 MICROBES AS BIOLOGICAL AGENTS

BIOLOGICAL PEST CONTROL OR BIOPESTICIDE

Biopesticides are those biological agents that are used for control of weeds, insects and pathogens. The micro-organisms used as biopesticides include viruses, bacteria, fungi protozoa and mites.

One example is the soil bacterium, *Bacillus thuringiensis*. Spores of this bacterium produce the insecticidal Cry protein. Therefore, spores of this bacterium kill larvae of certain insects. The

commercial preparations of *B. thuringiensis* contain a mixture of spores, Cry protein and an inert carrier. This bacterium was the first biopesticide to be used on a commercial scale in the world.

The very familiar beetle with red and black markings the Ladybird and Dragonflies are useful to get rid of aphids and mosquitoes, respectively. An example of microbial bio-control agents that can be introduced in order to control butterfly caterpillars is the bacteria Bacillus thuringiensis (often written as Bt). These are available in sachets as dried spores which are mixed with water and sprayed onto vulnerable plants such as brassicas and fruits trees, where these are eaten by the insect larvae. In the gut of the larvae, the toxin is released and the larvae gets killed. The bacterial disease will kill the caterpillars, but leave other insects unharmed.

Fungal pathogens are attractive bio-control agents for weed control in view of their host specificity and ease in production and inoculation in the field where,

A biological control being developed for use in the treatment of plant disease is the fungus ***Trichoderma.*** *Trichoderma* species are free-living fungi that are very common in the root ecosystems. They are effective biocontrol agents of several plant pathogens

Baculoviruses are pathogens that attack insects and other arthropods. The majority of **baculoviruses** used as biological control agents are in the genus Nucleopolyhedrovirus. These viruses are excellent candidates for species-specific, narrow spectrum insecticidal application. They have been shown to have no negative impacts on plants, mammals, birds fish or even on non-target insects. This is especially desirable when beneficial insects are being conserved to aid in an overall **integrated pest management (IPM) programme,** or when an ecologically sensitive area is being treatment.

Difference between bio-pesticides and chemical pesticides

	Biopesticides	**Chemical pesticides**
1.	These do not harm non-target species.	Non-target species are also harmed.
2.	They do not pollute the environment.	Cause pollution by chemical farming; sometimes serious.
3.	No harmful residues remain in food, fodder and fibers	Harmful residues many often remain in food, fodder and fibres.
4.	Relatively Cheaper.	Relatively costlier
5.	Insects are expected not to develop resistance	Insects may become resistant, e.g. Heliothis has become resistant to most insecticides.
6.	to Biopesticides.	It is often not critical.
7.	Since they are highly specific, correct identification of the pest is essential.	often not required
8.	High specificity may often make the use of two or more biopesticides necessary. Performance may be variable due to the influence of biotic and a biotic factors of the environment.	This is not often the case.

1. **Biopesticides** are the organisms which are applied to destroy the pests. They are used to destroy the weeds as well as the insect pests. Two basic types are **bioherbicides** and **bioinsecticides**.

2. **Transgenic plants** are genetically engineered plants to develop resistance against pests. *e.g.,* transgenic tobacco and transgenic cotton.

3. **Smoother crops** are those which do not allow the weeds to grow nearby *e.g.,* barley, rye, *Sorghum,* millet, sunflower, alfalfa, soyabean, marigold etc. Smoother crops eliminate weeds through chemicals. Crop rotation with these crops will naturally reduce the incidence of weeds.

4. **Catch/ trap crops :** Around the major crop in the field some early growing crop is sown in strips which is termed as catch or trap crop. The pests get attracted towards the early grown trap crop and then can be easily killed by cutting and destroying the trap crop. A good example of trap crop is bhindi (okra) which is sown around the cotton field to attract the jassid and spotted bollworm. Sesame is also good trap crop to attract the red hairy caterpillar from the cotton field.

5. **Bioherbicides :** It involves the biological control of weeds by some living organisms. *e.g.,* use of insects feeding on a specific weed or use of micro-organisms which will cause diseases in weeds. Some of the common examples are given below :

(a) In India and Australia, the overgrowth of *Opuntia* (prickly pear cactus) was checked by the introduction of the **cochineal insect** *(Cactoblastis cactorum).*

(b) The first bioherbicide was mycoherbicide called **Devine,** derived from a fungus *Phytophthora palmivora* which controls the growth of milk weed vines in *Citrus* orchards.

(c) Another mycoherbicide called **Collego** has been derived from **conidia** of fungus *Colletotrichum gloeosporioides.* It controls the growth of northern Jointvetch *(Aeschynomene virginica* fam. Leguminosae) growing in rice fields.

(d) Extensive growth of *Hypericum perforatum* or kalmath weed was checked in USA by the introduction of *Chrysolina* beetles.

(e) Water hyacinth has been successfully controlled in Florida using the indigenous fungus

Cercospora rodmanii.

6. **Bioinsecticides :** These are non persistent, non toxic and biodegradable. They include

(a) Pathogens, parasite and predators :

- A well known example of biological control of an insect pest is the destruction of large populations of **aphids** (a pest on crucifers) by an insect called **lady bug** or **praying mantis** which feeds on the aphids.

- The hoover fly larvae (Syrphid larvae) are very effective in keeping the aphids (plant bugs) under check as they feed on the aphids only. Dragon flies are useful to get rid of aphids and mosquitoes.

- The mosquito larvae are easily controlled by rearing the larvicidal fish *Gambusia* (mosquito fish).

- The sugarcane scale insects are controlled by the coccinellid predators *(Cailochorus*

negriti and *Pharoscymnus homi),* the fluted scale insect *(lcerya purchasi),* a common pest on *Citrus* trees by the lady bird beetles *(Rodolia cardinalis)* and *Nephantis serinopa,* is a dangerous pest on coconut palms, by *Perisierola nephanticdis* and *Trichospilus pupivora.*

- Baculoviruses are pathogens that attack insects and other arthopods. NPV *(Nuclearpolyhedrovirus)* based insecticide has been found to eliminate bollworms which cause extensive damage to cotton. These are species specific and narrow spectrum.

- *Trichoderma* species are effective biocontrol agents of several plant pathogens. *Trichoderma* species are free-living fungi that are very common in the root ecosystems.

(b) Sterilisation strategy : Screw worm *(Cochliomyia hominivorax)* was eradicated by releasing sterile males.

(c) Insect hormone or Pheromones :

- The pheromones are those chemical messengers which help in communication, sending alarm signals, marking trails or for attracting males.

- Pheromones are secreted by females. Traps containing **pheromones** are placed in infested fields. Males attracted by the trap become unavailable for reproduction.

- In **confusion technique,** the pheromone containing papers are spread all over the field, so males can no longer locate the females.

- Introduction of moulting hormone **ecdysone** or juvenile hormones at inappropriate times results in the early death of insect pests.

(d) Natural insecticide :

- These are obtained from living organisms (Plants) *e.g.,* **rotenones** (obtained from the roots of *Derris elliptica),* **nicotine** (obtained from tobacco), **pyrethrum** and **cinerin** (pyrethroids, obtained from *Chrysanthemum cinerarifolium),* **azadirachtin** from margosa *(Azadirachta indica)* leaves, **thurioside** from mutant strains of a bacterium called ***Bacillus thuringiensis*** **(Bt)**.

- Thurioside is a proteinaceous toxin and is effective against several insects such as moths, flies, mosquitoes and beetles which accumulate as crystals inside the bacteria during sporulation.

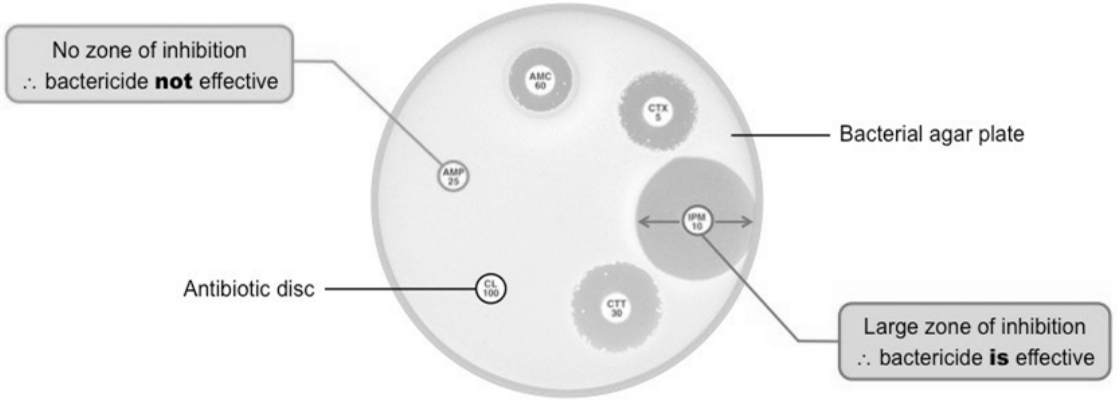


Fig: Effect of Bactericides on Bacterial Growth

Bactericides are substances which kill bacteria – they include certain types of disinfectants, antiseptics and antibiotics

- Whereas bactericidal agents will kill bacteria, bacteriostatic agents function by slowing their growth and reproduction

  The efficacy of a particular bactericide can be tested by examining its effect on bacterial growth in a sterile bacterial culture

- If a bactericide is effective against a particular bacterial strain, there will be no growth around the agent (zone of inhibition)
- The larger the zone of inhibition, the more effective the bactericidal agent is at killing the bacterial strain.
- The zone of inhibition will also be affected by the solubility of the bactericide (i.e. fast diffusion rate = large zone of inhibition)
- This is an important consideration when testing multiple bactericides on the same growth medium.
- Bioremediation is often supported by physical and chemical procedures that help filter the contaminant out of the environment
  - ✓ **Physical:** Detergents and dispersal agents may be deployed on oil spills to prevent the contaminants from aggregating
  - ✓ **Chemical:** Contaminants can alternatively be burnt, removed by dissolving or destroyed by chemical oxidation

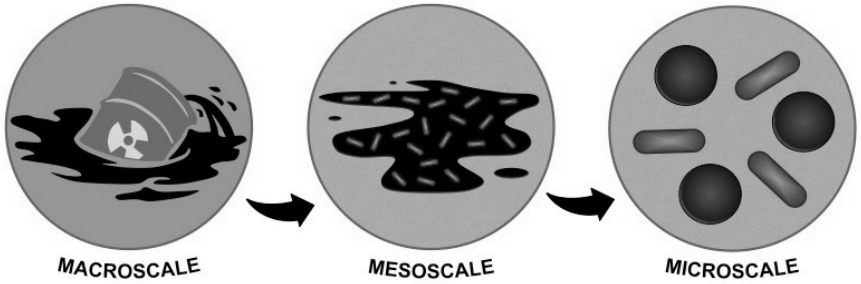


Fig: Bioremediation of an Oil Spill

Pollutant metabolism

A **pollutant** is a substance introduced into the environment that has an adverse effect upon a natural resource

- ✓ Certain pollutants may be disintegrated via the biological metabolism of microorganisms (biodegradation)
- ✓ Examples of pollutants that may be metabolised by microorganisms include:
- ✓ **Oil:** Hydrocarbons in crude oil may be metabolised by *Pseudomonas*
- ✓ **Benzene:** Aromatic hydrocarbons (e.g. benzene) may be metabolised by halophilic *Marinobacter*
- ✓ **Mercury:** Toxic methyl mercury may be converted into environmental mercury by *Pseudomonas*
- ✓ **Uranium:** Uranium can be converted by *Geobacter sulfureducens* into a insoluble form that can be easily collected

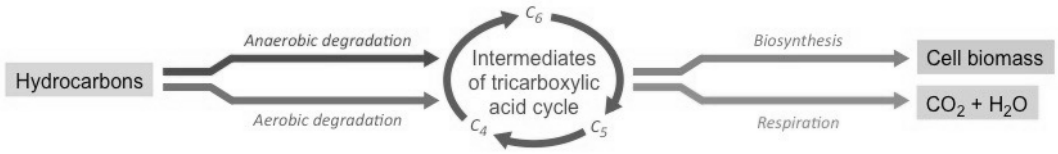


Fig: Hydrocarbon Metabolism by Chemoheterotrophs

Mercury Contamination

Mercury exists in three forms: as a metal (elemental mercury), as inorganic ions or as an organic form (methyl mercury)

- ✓ Elemental mercury is produced as an industrial pollutant and is converted to the other forms by aquatic bacterial communities
- ✓ Methyl mercury is the most toxic form and will accumulate within aquatic ecosystems polluted by elemental mercury
- ✓ *Pseudomonas* is capable of degrading the toxic methyl mercury to form elemental mercury and methane gas
- ✓ This makes *Pseudomonas* an important biological agent in the treatment and remediation of mercury pollution

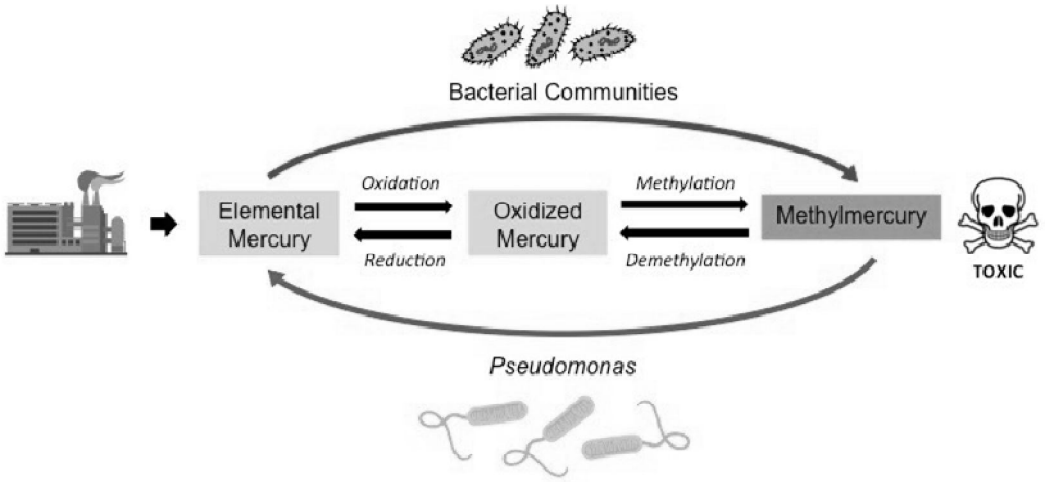


Fig: Mercury Conversion

Biofilm Formation

A biofilm is a cooperative aggregate of microorganisms associated with a surface and enclosed in a polymeric matrix ('slime')

- Biofilms can form on solid and liquid surfaces and may be composed of a single species or many species of microorganism
- Biofilm formation begins with the attachment of microorganisms to a surface through weak, reversible associations
- If not removed, the microorganisms form more permanent, irreversible attachments using adhesion structures (such as pili)
- The cells forming a monolayer will grow and divide, before producing an exopolymer matrix to enclose the developing colony

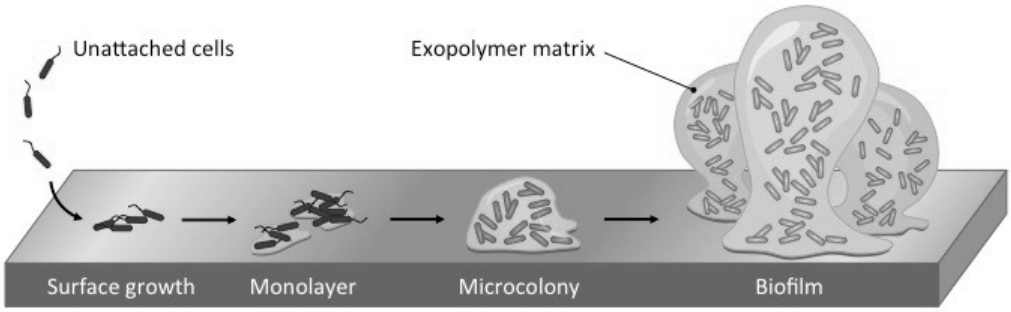


Fig: Biofilm Development

Use of biofilm:

Sewage is the fluid waste of human communities and is rich in organic matter that can be broken down by microorganisms

- Solids are *anaerobically* digested by saprotrophic microorganisms, while liquid effluent is cleaned by *aerobic* metabolism.

- The aerobic conditions required for the saprotrophic decomposition of liquid effluent can be maintained by trickle filter beds.
- Trickling filter systems continually recirculate the effluent by spraying it over a bed of stones to maintain dissolved O_2 levels.
- A biofilm of saprotrophic bacteria is situated in the stone bed and feed on the organic matter within the sewage.
- Clean water filters out the bottom into another tank, whereby bacteria are removed and water is further treated (chlorination).

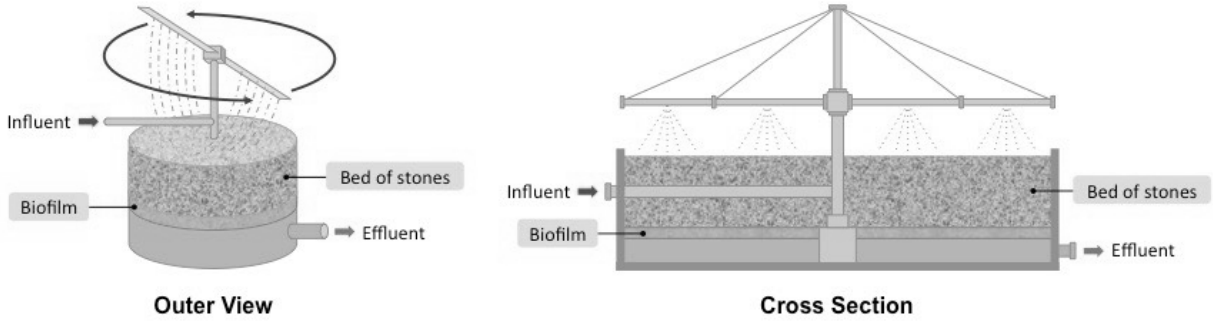


Fig: Sewage Treatment – Trickle Filter Beds

14 INTEGRATED PEST MANAGEMENT (IPM)

- Sustainable pest management is otherwise known as **Integrated pest management** i.e., integration of tactics for control of single pest on one or more crops.
- The overall objective of IPM is to create and to maintain situations in which insects are prevented from causing significant damage to crops

Biofertilisers

Micro-organisms (bacteria, fungi and cyanobacteria) employed to enhance the availability of nutrients like nitrogen (N), and phosphorus (P) to crops are called **biofertilisers.** You know that several micro-organisms fix atmospheric nitrogen and make them available to plants.

Examples of nitrogen-fixing micro-organisms are bacteria and cyanobacteria (blue-green algae); some of these are free-living, while others form symbiotic association with plant roots. **Rhizobia** form root nodules in legume crops and some cyanobacteria (blue-green algae) form symbiotic associated with the fern Azolla.

Biofertilisers are low-cost input and they do not pollute the environment. They also reduce the dependence on chemical fertilizers and also help to use **organic farming.**

15 MICROBES AS BIOFERTILIZERS

- **Organic farming** is the raising of unpolluted crops through the use of biofertilisers that provide optimum nutrients to crop plants.
- Organisms which can be used to improve the nutrient quality of soil through biological activity are known as **biofertilisers.**

- The main sources are bacteria, cyanobacteria and fungi. In paddy fields, cyanobacteria serve as an important biofertiliser.

(a) Symbiotic $\mathbf{N_2}$ fixing bacteria like *Rhizobium leguminosarum* fixes atmospheric $\mathbf{N_2}$ in root nodules of legumes.

(b) *Frankia* (Actinomycetes) in root nodules of non-legume plants *(e.g., Casuarina* and *Alnus).*

(c) Symbiotic cyanobacteria (blue green algae) like *Anabaena azollae* fixes atmospheric $\mathbf{N_2}$ in leaves of *Azolla* (water fern). *Azolla pinnata* (a pteridophyte) is used as an excellent fertilizer in rice field.

(d) *Anabaena cycadae* lives in coralloid root of *Cycas* (a gymnosperm).

(e) *Aulosira* is most active, non symbiotic nitrogen fixer in rice field in India.

(f) Free living nitrogen fixer like *Azospirillum* and *Azotobacter* enrich the nitrogen content in soil.

(g) **Mycorrhiza :** It is symbiotic association between the fungus and roots of higher plants (seed plants). Many members of the genus *Glomus* form mycorrhiza. The fungal partner absorbs phosphorus from soil and passes it to the plant. Plants having mycorrhizal associations show resistance to root-borne pathogens, tolerance to salinity and drought, and an overall increase in growth and development. It is of two types :

(i) **Ectomycorrhizae (Ectotrophic or Ectophytic) :** Hyphae of fungus only form mantle on the outer surface of the root, increasing absorption of water and minerals *e.g., Pinus,* oak etc. Mycorrhiza absorb and store nitrogen, phosphorus, potassium and calcium.

(ii) **Endomycorrhizae (Endotrophic or Endophytic) :** Fungal hyphae penetrate into cortex and cells of root *e.g.*, orchids, coffee and woody plants. These are also called as vesicular arbuscular mycorrhizae or VAM, because cortical cells swell and form vesicles or arbuscles. It has significant role in phosphorus nutrition in plants.

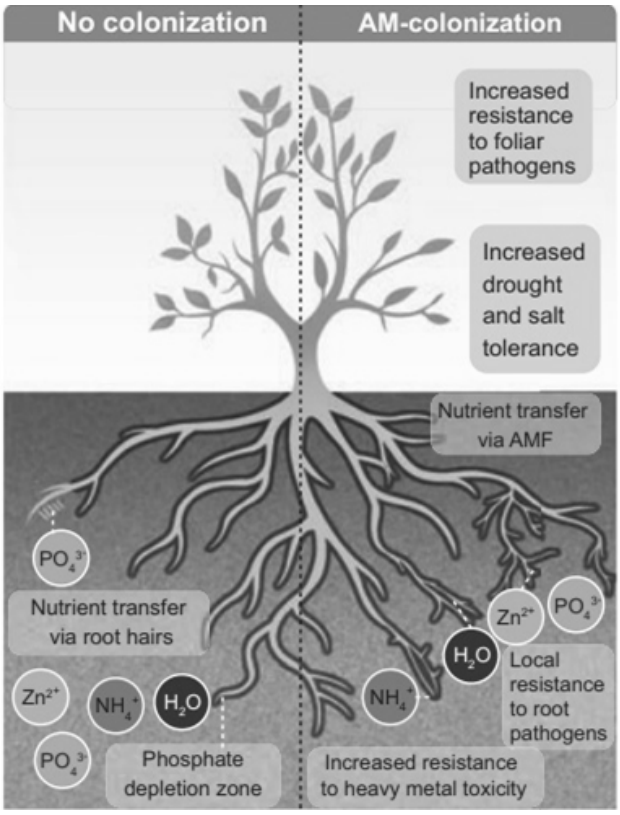


Fig: Classification of Biofertilizers

S.N	Groups		Examples
A	**N_2 fixing Biofertilize**		
	1.	Free-living	*Azotobacter, Clostridium, Anabaena, Nostoc,*
		Symbiotic	*Rhizobium, Anabaena azollae*
	3.	Associative Symbiotic	*Azospirillum*
B	**P Solubilizing Biofertilizer**		
	1.	Bacteria	*Bacillus subtilis, Pseudomonas striata*
	2.	Fungi	*Penicillium, Aspergillus.*
C	**P Mobilizing Biofertilizers**		
	1.	Arbuscular Mycorrhiza	*Glomus, Scutellospora.*
	2.	Ectomycorrhiza	*Amanita.*
D	**Biofertilizer for Micro nutrients**		
	1.	Silicate and Zinc solubilizers	*Bacillus.*
E	**Plant Growth Promoting Rhizobacteria**		
	2.	Pseudomonas	*Pseudomonas fluorescence*

Fig: Benefits Of AM Colonizers