UNIT II: -ENVIRONMENTAL BIOTECHNOLOGY

Industrial Wastewater is a type of waste (liquid, solid, gases) produced by industrial activity. It is characterized by high temperature, high concentration of biodegradable organic matter and suspended solids, high alkalinity or acidity, TDS, TSS, turbidity, BOD, COD Industrial waste is harmful to both human beings and environment.

Benefits of waste water treatment: -

- Used to develop method for the treatment of toxic compounds.
- Implementing the biomechanical treatment system used for degradation of toxic compounds.
- Improvement of public health, sanitation, soil integrity and the conservation of fresh water resources.

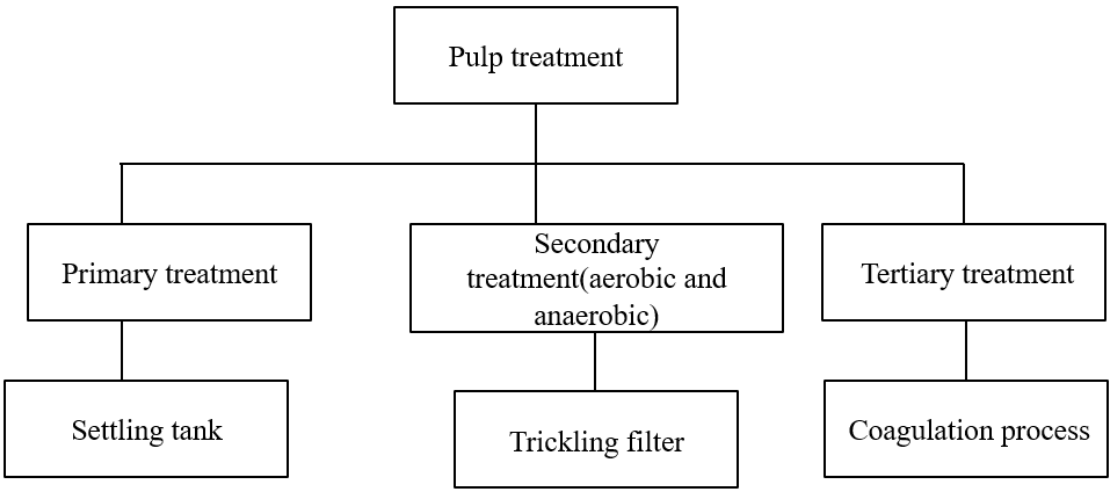
Type of industrial waste

1) Pulp
2) Dye
3) leather
4) solid waste management.

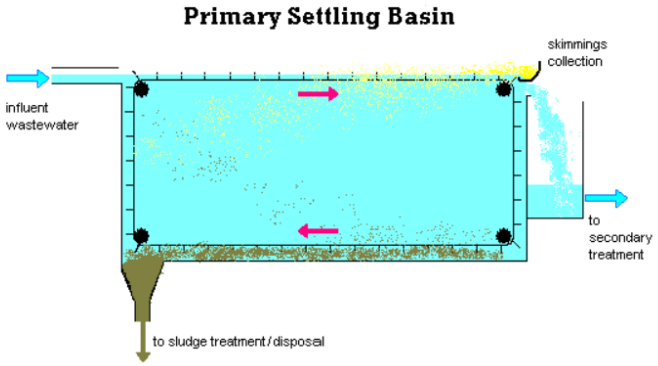
1) **Pulp treatment:** -Pulp is a raw material used for paper industry. The pulp is a black alkaline liquid which is produced during the process of pulping. This liquid consists of large number of total dissolved solids (TDS) and total suspended solids (TSS), high pH, high pigments, hydrocarbons, which causes acid rain. These wastes are toxic to human beings and environment so it is treated by following methods.

Characteristics of pulp Wastewater:-The waste water in pulp industry is usually alkaline in nature, and has high suspended solids, high total solids, high COD(Chemical Oxygen Demand) and relatively low BOD (Biological Oxygen Demand). The analysis of waste water from a typical pulp and paper industry is the following:

1. pH value: 8.0 - 9.0
2. Total solids: 1500 - 2500 mg/l
3. Suspended solids: 600 - 1500 mg/l
4. COD: 300 - 2500 mg/l
5. BOD: 150 - 1000 mg/l

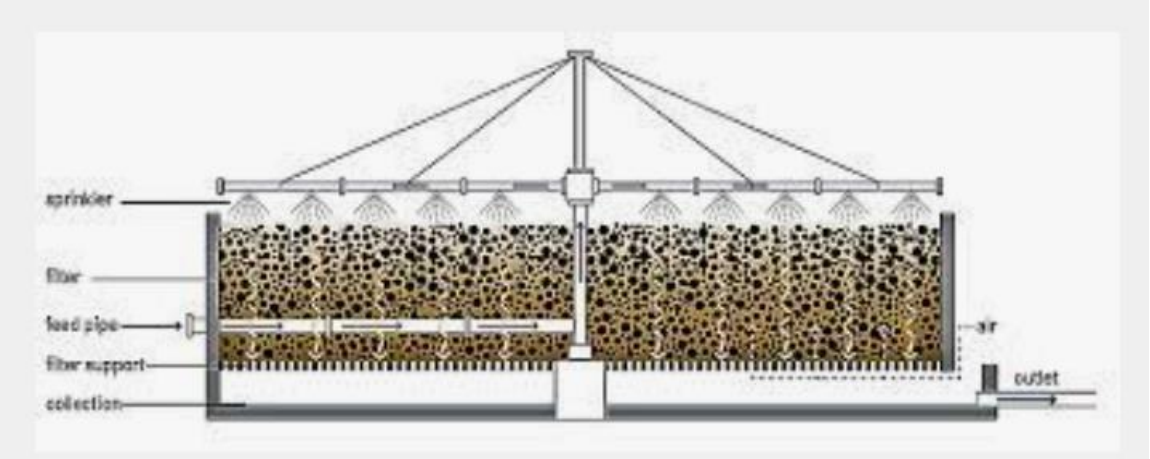


1)Primary treatment: **-Settling tank (primary settling basin):** Preliminary treatment is used to remove screenings and grit that enters a wastewater treatment plant from a sewered system. Preliminary treatment will have little effect on pathogens in the liquid wastestream. Primary treatment (also called primary sedimentation) is a sanitation technology that removes suspended solids and floating organic material (called scum) to reduce the suspended solids load for subsequent treatment processes. The removal of pathogens during primary treatment is not high.It is a vessel in which solids settle out of water by gravity. The settleable solids are pumped away (as sludge), while oils float to the top and are skimmed off. Primary tank is designed to overcome this problem the effluent is allowed to passthrough the settling tank. In the settling tank the solids settle down based on the specific-gravity. The solids with greater specific gravity than water settles at the bottom and removed as sludge, while the particles lighter specific gravity rise to the surface which is skimmed off.



2) Secondary treatment: -Chlorinated organic compounds, chlorophenol derivatives, halo aromatic hydrocarbons are removed by this method. Secondary treatment is carried out in trickling filters in presence of microorganisms (Aerobic bacteria and Anaerobic bacteria).

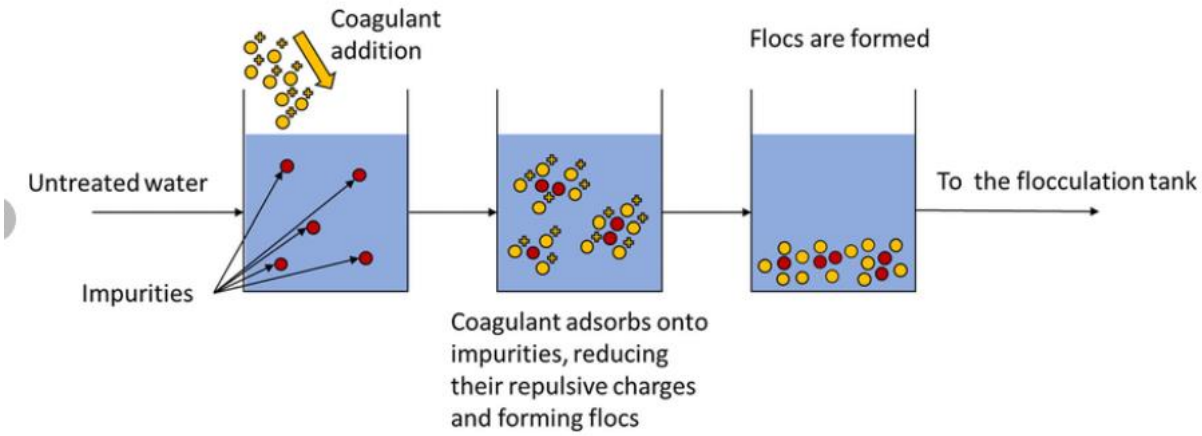
Prescence of bacteria like Arthrobacter, Pseudomonas, flavobacterium. Trickling filter process can define as the biological system, which tends to separate or degrade the maximum organic and inorganic waste (**up to 85%**) out of the primary or raw sludge via the slime layer. The designing of a trickling filter unit includes a support structure, pebble or plastic filled media and rotary distributor.It works under the **aerobic conditions** and makes the use of aerobic microbes so that they can exploit or oxidize the organic matter into a simpler form. The **filter bed** is placed below the pebble filled media, which aids the separation of secondary effluent out of waste activated sludge. The final effluent is released from the outlet pipe and further treated with disinfectants like chlorine, UV, ozone etc. to make it safe for disposal. Chlorophenolic derivatives, halo aromatic hydrocarbons are degraded by this method.



Trickling filter

Treatment of fungi: -The effluent consists of lignin, dyes, aromatic compounds. These compounds are treated by fungi like white rot fungi. This fungus has the capacity has the capacity to degrade the lignin as it contains lignin peroxidase, which catalyzes the several side chains of lignin and lignin related compounds.

3)Coagulation process: -Coagulation is a process in which the colloidal particles accumulate as the larger particles and settle down as precipitate. Coagulation helps to remove the turbidity and halides, extract metals. Coagulation has become a popular method of reducing the TSS.This process involves destabilizing the charged particles in the solution. Because of their similar electrical charges, the particles repel one another and prevent them from settling quickly. To destabilize this electrical charge, an opposite charge must be applied to the solution, enabling the colloids and other minerals to aggregate. Aluminium sulfate (alum), ferric sulfate or sodium aluminate are used in co-agulation process.



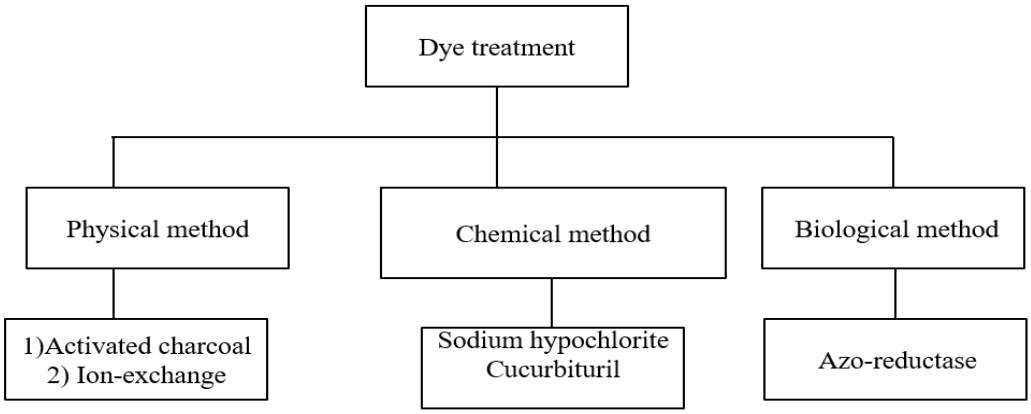
Method of coagulation

3)Dye treatment: - Dyes are such substances with considerable colouring capacity are widely employed in the in the production of consumer products, including paints, textile, printing inks, pharmaceutical, food, cosmetics, plastics, photographic and paper industries.

The waste water has high pH value, high concentration of suspended solids, chlorides, nitrates, metals like manganese, sodium, lead, copper, chromium, iron, and high BOD and COD value, and high salt concentration

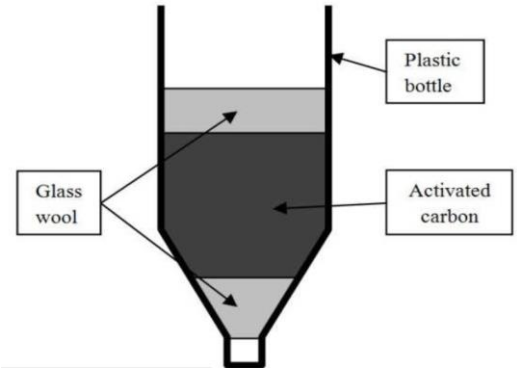
Characteristic of Dye waste:

pH	10
BOD	363
COD	1781
Dye concentration	700
Chloride	15,867
Sulfate	1400
NH_4	17
Total dissolved solids	55.02-61.9
Total suspended solids	47.07



Physical treatment

1)Activated charcoal: - Activated carbon is commonly used to adsorb natural organic compounds, taste and odour compounds, and synthetic organic chemicals in drinking water treatment. has high carbon content and a high internal porosity. The efficiency of the filter is high. The charcoal is used in both granular and powder form. Activated carbon is an effective adsorbent because it is a highly porous material and provides a large surface area to which contaminants may adsorb. it also removes turbidity and solids removal, and biological stabilization. The charcoal's porous texture has a negative electrical charge, which causes it to attract positively charged molecules, such as toxins and gases. The charcoal is easily available material and hence the project is economical.



2)Ion-exchange: - Ion exchange is an exchange of ions between two electrolytes or between an electrolyte solution and a complex. Ion exchange can be a process of purify- cation, separation and decontamination of aqueous or ion containing solutions. They are usually ion ex- change resins, zeolites, clay or soil humus. They are primarily either cation exchangers that exchange positively charged ions or anion exchangers that ex- change negatively charged ions. Both cation and anion dyes are removed from the effluent by ion-exchange method.

When the dyes are allowed to pass through ion -exchange where the purification takes place. The removal of anion dyes like eosin-Bango red, Eri chrome black T indicator is removed by ion-exchange method. Ion exchange resins are kept in a specially built tank forming a bed, which is usually 30-65 inches deep. In the process, water enters through the top of the exchangers, spreads uniformly over the surface of the resin, ion exchange takes place and the treated water is collected from the bottom.

Cation exchange resins Strong acid cation resins have H+ ions as the exchanger which exchange ions with the cation present as TDS in untreated water. For regeneration, a dilute acid solution is passed through the exhausted bed which leads to cations exchanging with the H+ ions present in the acid.

Anion exchange resins Strong base anion resins have OH- ions as the exchanger which exchange ions with the anions present as TDS in untreated water. For regeneration, a solution of dilute NaOH is passed through the exhausted resins which is exchange back the anions with OH- ions present in the base

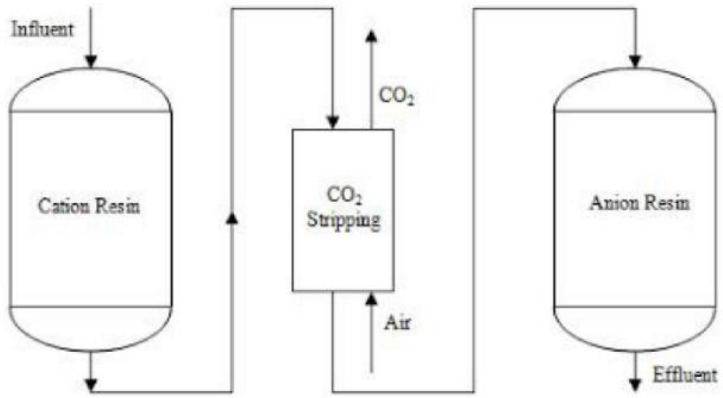


Figure 1 : An ion exchanger

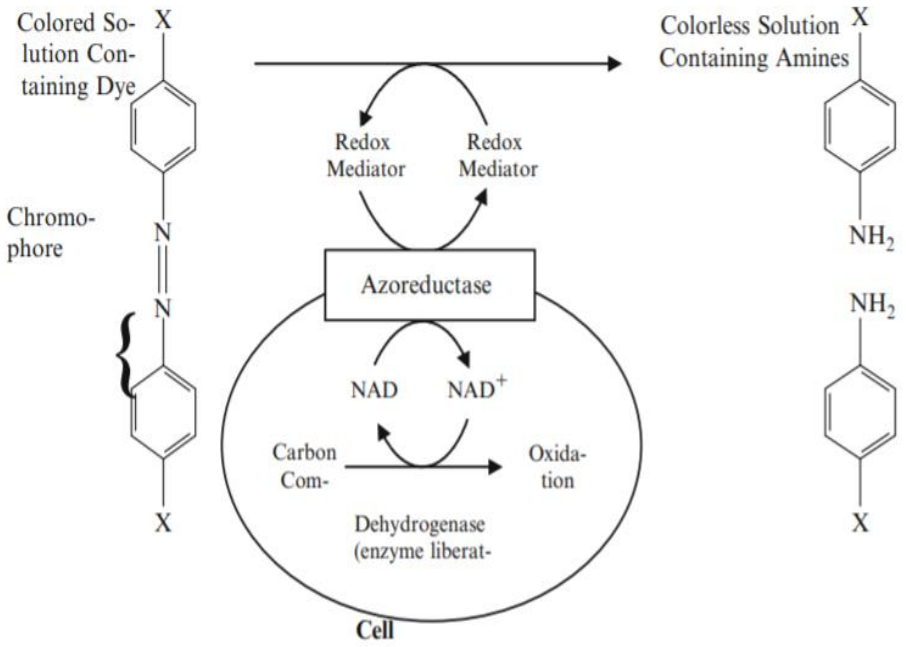
2)Chemical method

Sodium hypo chloride: Sodium hypo chloride attacks the amino group of the dye molecule by the cl^+. This initiate and accelerates the cleavage of azo bond. This method is not suitable for dispersive dyes. An increase in the decolorization is seen as the cl concertation. SH is a bleach or disinfectant, and is capable of killing pathogens like bacteria, viruses, fungi and mycobacterium. Just like chlorine, when sodium hypochlorite is released in water, it produces hypochlorous acid. This acid then reacts with pathogens in the water, like bacteria, viruses and protozoa, and deactivates them, preventing them from being able to reproduce or pose a risk to human health.

Cucurbituril: This is a member resembles pumpkin which has the capacity to adsorb the dyes which is due to the formation of insoluble cucurbituril-dye-aggregation since the adsorption occurs fastly. Cucurbituril was investigated regarding its potential as a sorbent for the removal of reactive dyes from model solutions and authentic wastewaters. The solubility of cucurbituril is low in pure water but increases in the presence of salts. When dyes sorbing onto cucurbituril are present, solubility is drastically decreased compared to dye-free media. Sorption efficiency depends on salt concentration and salt species

3) Biological method: -

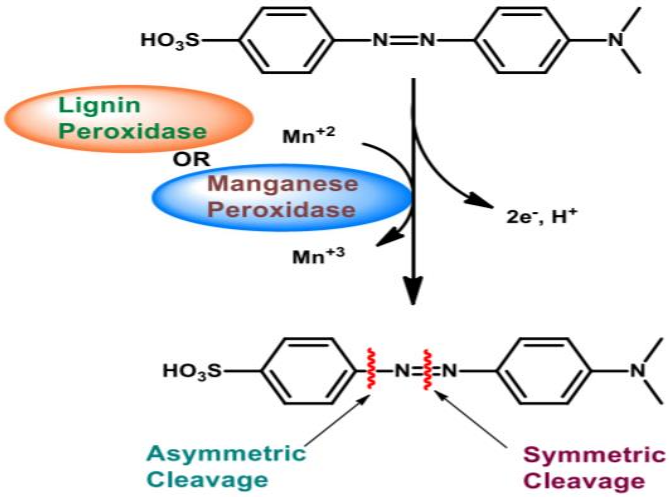
1) Azo-reductase: -Aspergillus-niger: -Azo- reductases are diverse flavoenzymes widely present among microorganisms and higher eukaryotes. They are mainly involved in the detoxification of azo dyes. These enzymes are present in the bacterial cell wall or cell membrane which requires NADPH or NAD as electron donors for the reduction of the azo dyes. It is found in clostridium erbactrium, azoreductase are highly sensitive to oxygen and present in the gut of the Human beings.



Mechanism of azo-dye reduction

2)Lignin peroxidase: -Lignin peroxidase, are topical owing to their high redox potential enzyme. Lignin peroxidase (Lip) from *Phanerochaete chrysosporium* is a heme-containing lignin-degrading oxidoreductase that catalyzes the peroxide-dependent oxidation of diverse

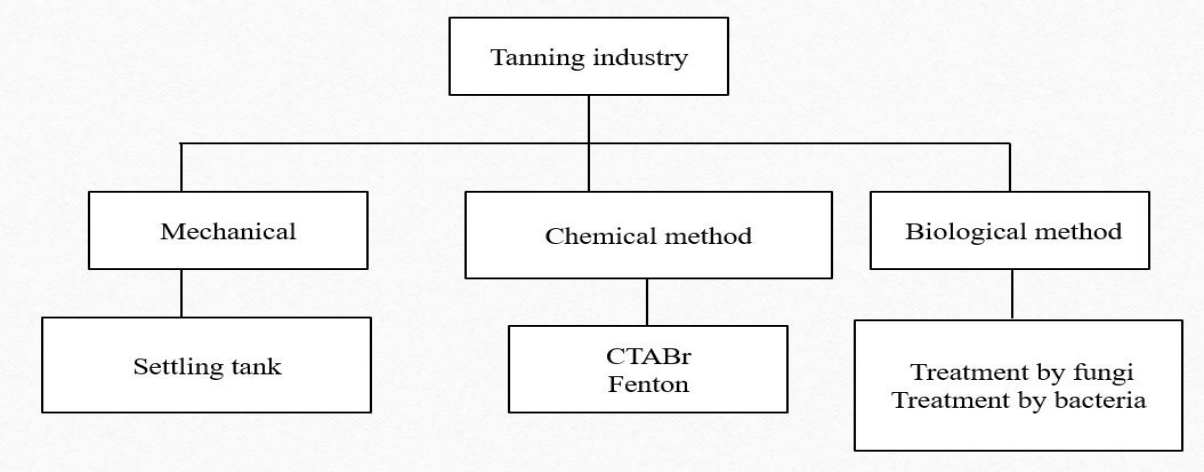
molecules, including industrial dyes.Lip catalyses catalyzes several oxidations in the side chains of the lignin and related compounds by one-electron abstraction to form free radicals.



Tanning industry: Leather Tanning is the process of converting raw hides or skin into leathers and on further treatment they are converted into leather products. The word Tanning is derived from 'Tannin' which is an acidic chemical compound. Chromium is an important heavy metal used in leather; thus, the leather industry contains the chromium. tannery industry plays a key role in the emission of poisonous effluents into the environment, according to 67.9% of respondents. Water a contains inorganic compounds (chromium, chlorides, ammonium, sulfides, sulfates, etc., among which **chromium** is the one with the highest concentrations and problematic) and a very high salt content (in the tanning process salts are used to the preservation of the skin, sulphides, chromium salt, etc.) which results in high levels of alkalinity, with a pH of around 10, of a mixed and homogenized wastewater.

Characteristics of Tannery waste water

1	pH	7.6
2	Colour	Dark brown
3	Turbidity	140 NTU
4	Total Suspended solids	1040
5	Alkalinity	2450
6	BOD	1520
7	COD	3640
8	Sulphide	210
9	Total Nitrogen	257
10	Ammonical Nitrogen	85
11	Total Chromium	10.0
12	Total Phosphate	16
13	Total Kjeldahl Nitrogen	144

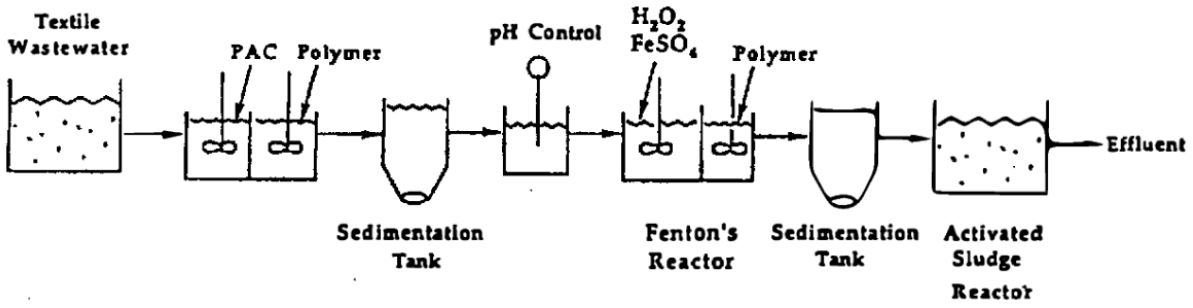


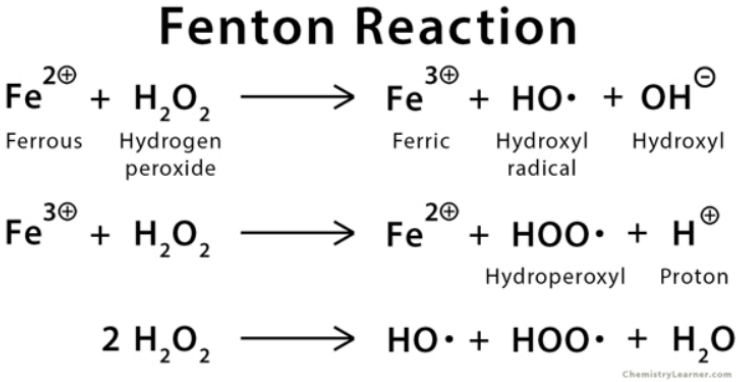
1)Mechanical treatment

Settling tank (primary settling basin) :-It removes settleable or floating solids only. It is a vessel in which solids settle out of water by gravity. The settleable solids are pumped away (as sludge), while oils float to the top and are skimmed off. Sedimentation tanks can also be adapted for secondary and tertiary processes, and can also be used to treat drinking water. Primary treatment of wastewater involves sedimentation of solid waste within the water. This is done after filtering out larger contaminants within the water. Wastewater is passed through several tanks and filters that separate water from contaminants. The resulting "sludge" is then fed into a digester, in which further processing takes place. This primary batch of sludge contains nearly 50% of suspended solids within wastewater.

2)Chemical treatment

Fenton reagent: -Suitable for the treatment of effluents which are resistant to biological treatment or poisonous to live biomass. The oxidation of organic substrates by iron (II) and hydrogen peroxide (typically iron (II) sulfate, $FeSO_4$) is called the Fenton reaction. The reagent used for this purpose is known as Fenton's reagent, which is accepted as one of the most effective methods for the oxidation of organic pollutants. Fenton's reagent is effective in treating various industrial wastewater components that include aromatic amines, a wide variety of dyes, pesticides, surfactants, and explosives. It is also used to treat many other water-polluting substances like phenols, formaldehyde, BTEX, and rubber chemicals

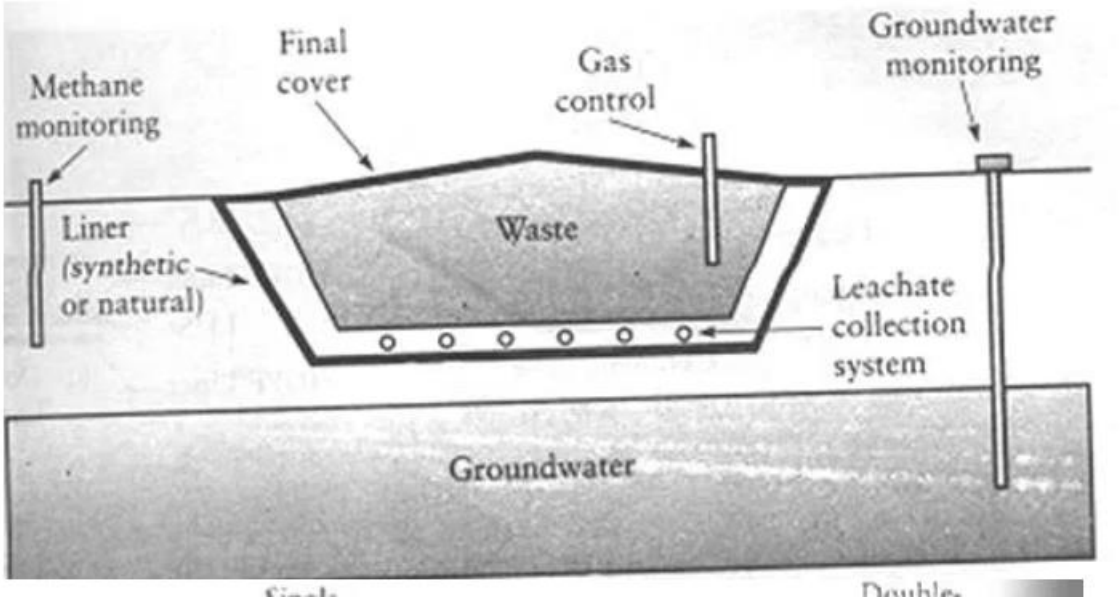




Treatment by bacteria: -The bacterial cell wall is composed of peptidoglycans, n-acetylglucosamines, peptide chains. These peptidoglycans have the capacity of binding to metals, by the electrostatic force of attraction. Thus, reducing the chromium content present in activated sludge under anerobic condition. microorganisms efficiently used in the treatment of tannery wastewater. Cr-tolerant species include Bacillus species with ability to reduce hexavalent chromium to its trivalent form. Streptomycin sp., Pseudomonas aeruginosa, P. fluorescens, Micrococcus sp, Streptomyces as well as yeasts like Pichi guilliermondii and Aspergillus species. Mechanism of Cr-tolerance or resistance of selected microbes is of particular importance in the bioremediation of contaminated tannery wastewater

4) Solid waste management: Solid waste is generated due to various activities that like residual and commercial, agricultural, Whatever the origin, solid waste must be managed systematically to ensure environmental best practices There are five functional components of the waste management system as outlined below:

1.Sanitary Landfill:- Landfills are the physical facilities used for the disposal of residual solid wastes in the surface soils of the earth. The safe and reliable long-term disposal of solid waste residue is an important component of integrated waste management. Solid waste residue are waste components that are not recycled, that remain after processing at materials recycle facility, or that remain after the recovery of conversion products and/or energy. Garbage is basically spread out in thin layers, compressed and covered with soil or plastic foam. Modern landfills are designed in such a way that the bottom of the landfill is covered with an which is usually made of several layers of thick plastic and sand. This liner protects the groundwater from being contaminated because of leaching or percolation.



Advantage: If landfills are managed efficiently, it is an ensured sanitary waste disposal method.

SEGREGATION: -Segregation or Waste sorting is the process by which waste is separated into different elements. Waste segregation means dividing waste into dry and wet. Waste can also be segregated as

1. Biodegradable

2. No biodegradable

2**. Incineration**: -This method involves the burning of solid wastes at high temperatures until the wastes are turned into ashes. Incinerators are made in such a way that they do not give off extreme amounts of heat when burning solid wastes. Incinerators that recycle heat energy through furnace and boiler are called waste-to-energy plants. These waste-to-energy systems are more expensive to set up and operate compared to plain incinerators because they require special equipment and controls, highly skilled technical personnel, and auxiliary fuel systems. This method of solid waste management can be done by individuals, municipalities and even institutions. The good thing about this method is the fact that it reduces the volume of waste up to 20 or 30% of the original volume.

Advantage: The volume of combustible waste is reduced considerably by burning waste. In the case of off-site pits, it is an appropriate method to minimize scavenging.

Disadvantages: It can cause smoke or fire hazard and also emits gaseous pollutants

3. Recovery and Recycling: -Recycling or recovery of resources is the process of taking useful but discarded items for the next use. Plastic bags, tins, glass and containers are often recycled automatically since, in many situations, they are likely to be scarce commodity. Traditionally, these items are processed and cleaned before they are recycled. The process aims at reducing energy loss, consumption of new material and reduction of landfills. The most developed countries follow a strong tradition of recycling to lower volumes of waste.

Advantage: Recycling is environmentally friendly.

Disadvantages: It is expensive to set up, and in most emergencies, there is limited potential.

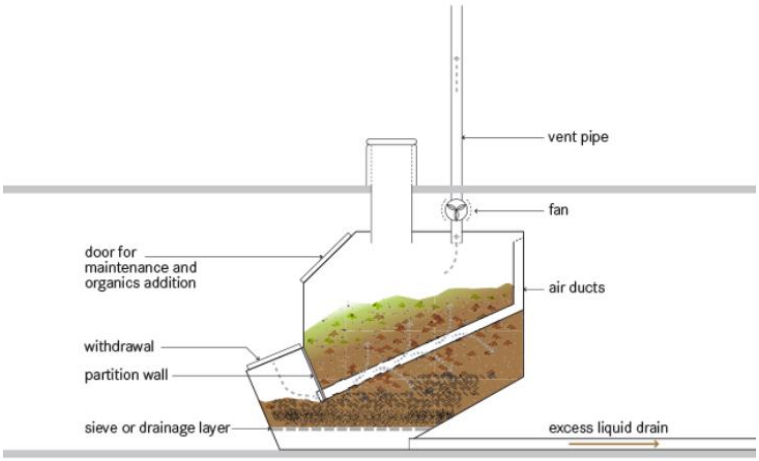
4. Composting:-Due to a lack of adequate space for landfills, biodegradable yard waste is allowed to decompose in a medium designed for the purpose. Only biodegradable waste materials are used in composting. It is a biological process in which micro-organisms, specifically fungi and bacteria, convert degradable organic waste into substances like humus. This finished product, which looks like soil, is high in carbon and nitrogen. Good quality environmentally friendly manure is formed from the compost that is an excellent medium for growing plants and can be used for agricultural purposes.

Advantage: Composting is environmentally friendly as well as beneficial for crops.

5. Pyrolysis: -This is a method of solid waste management whereby solid wastes are chemically decomposed by heat without the presence of oxygen. It usually occurs under pressure and at temperatures of up to 430 degrees Celsius. The solid wastes are changed into gasses, solid residue of carbon and ash and small quantities of liquid.

Advantage: This will keep the environment clean and reduce health and settlement problems.

Disadvantages: The systems that destroy chlorinated organic molecules by heat may create incomplete combustion products, including dioxins and furans. These compounds are highly toxic in the parts per trillion ranges. The residue it generates may be hazardous wastes, requiring proper treatment, storage, and disposal.



COMPOSTING

Eco-friendly products: -Eco-friendly products are those products that will not pollute the earth which can be recycled or conserved, products with natural ingredients, products containing organic elements. Eco-friendly are not harmful to the environment

Advantages of eco-friendly products

- Do not harm the environment
- To minimize carbon footprint or the emission of greenhouse gases
- To maintain ecological balance
- To reduce exploitation of resources
- Save energy
- can be recycled and reused

Biomass is renewable organic material that comes from plants and animals. Biomass was the largest source of total annual U.S. energy consumption until the mid-1800s. Biomass continues to be an important fuel in many countries, especially for cooking and heating in developing countries. The use of biomass fuels for transportation and for electricity generation is increasing in many developed countries as a means of avoiding carbon dioxide emissions from fossil fuel use. In 2021, biomass provided nearly 5 quadrillion British thermal units (Btu) and about 5% of total primary energy use in the United States.

Biomass contains stored chemical energy from the sun. Plants produce biomass through photosynthesis. Biomass can be burned directly for heat or converted to renewable liquid and gaseous fuels through various processes.

Biomass sources for energy include:

1. Wood and wood processing wastes—firewood, wood pellets, and wood chips, lumber and furniture mill sawdust and waste, and black liquor from pulp and paper mills
2. Agricultural crops and waste materials—corn, soybeans, sugar cane, switchgrass, woody plants, and algae, and crop and food processing residues, mostly to produce biofuels
3. Biogenic materials in municipal solid waste—paper, cotton, and wool products, and food, yard, and wood wastes
4. Animal manure and human sewage for producing biogas/renewable natural gas

Some of the advantages of biomass energy are:

1. **Biomass is always and widely available as a renewable source of energy.**
The organic materials used to produce biomass are infinite, since our society consistently produces waste such as garbage, wood and manure.

2. **It is carbon neutral:-**As a natural part of photosynthesis, biomass fuels only release the same amount of carbon into the atmosphere as was absorbed by plants in the course of their life cycle.

3. **It reduces the overreliance of fossil fuels:-**Not only is there is a limited supply of fossil fuels, but fossil fuels come with environmental baggage, including the release of large amounts of carbon dioxide into the atmosphere and the pollutants that result from removal, transportation and production.

4. **Is less expensive than fossil fuels:-**While fossil fuel production requires a heavy outlay of capital, such as oil drills, gas pipelines and fuel collection, biomass technology is much cheaper. Manufacturers and producers are able to generate higher profits from a lower output.

5. **Biomass production adds a revenue source for manufacturers.**
Producers of waste can add value by channelling their garbage to create a more profitable use in the form biomass energy.

6.Less garbage in landfills: -By burning solid waste, the amount of garbage dumped in landfills is reduced by 60 to 90 percent, and reduces the cost of landfill disposal and amount of land required for landfill.

Disadvantages of biomass: -

1. Biomass energy is not as efficient as fossil fuels:-Some biofuels, like Ethanol, is relatively inefficient as compared to gasoline. In fact, it has to be fortified with fossil fuels to increase its efficiency.

2. **It is not entirely clean**: -While biomass is carbon neutral, the use of animal and human waste escalates the amount of methane gases, which are also damaging to the environment.

Additionally, the pollution created from burning wood and other natural materials can be considered just as bad as that resulting from burning coal and other types of energy resources.

3. Can lead to deforestation: -Since wood is one of the most used source of biomass energy, vast amounts of wood and other waste products have to be burned to produce the desired amount of power. While currently there is enough wood waste already, there is a risk of deforestation in the future.

4. **Biomass plants require a lot of space.** While there are some downsides to biomass energy, more research and innovation is continuing to be devoted to the field as a more widely available, cheaper alternate and valuable substitute for traditional electricity and other energy sources.

Biochemical processes the bacteria and other microorganisms are used to transform the raw biomass into useful energy like methane and ethane gas. Following organic treatments are given to biomass

1) Fermentation of biomass (aerobic digestion)
2) Anaerobic digestion of biomass

Physical Properties

- Moisture Content
- Particle Size and Size distribution
- Bulk Density & Specific gravity
- Higher Heating/Calorific Value.

Properties of Biomass Chemical Composition

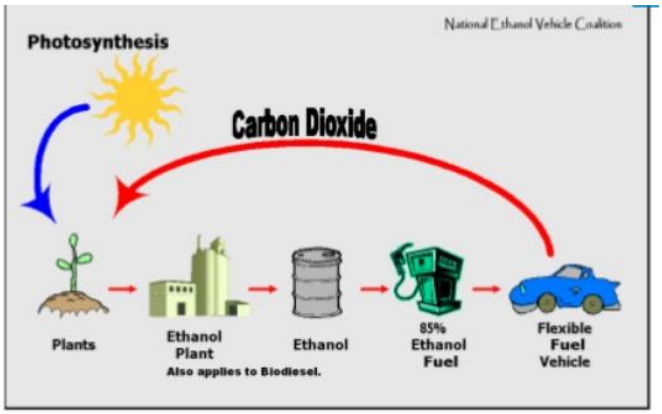
- Total Ash
- Solvent soluble
- Water Soluble
- Lignin
- Cellulose
- Hemi-cellulose

Advantages of Biomass:-

- Burns readily - has a convenient ignition temperature**.**
- Reduces burden on forests and fossil fuels
- Produces a clean fuel - helps in controlling air pollution
- Provides nutrient rich (N & P) manure for plants
- Controls water pollution by decomposing sewage, animal dung and human excreta

ALCOHOL AS FUEL

Alcohols are attractive alternate fuels because they can be obtained from both natural and manufactured sources. Methanol and ethanol are two kinds of alcohols.

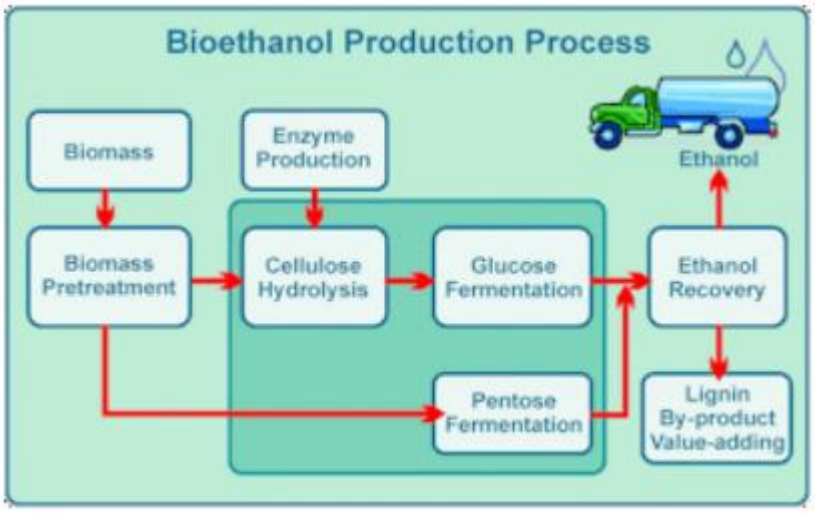


Ethanol from lignocellulosic (plant dry material) biomass is produced mainly via biochemical route.The three major steps involved in the biochemical method for ethanol production are

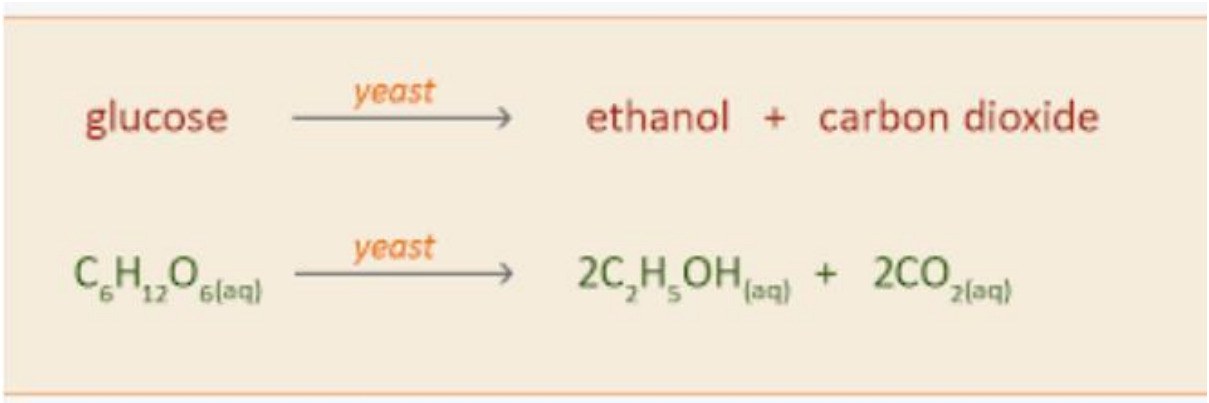
1. pre-treatment
2. enzymatic hydrolysis
3. fermentation.

Biomass is pre-treated to improve the accessibility of enzymes. After pre-treatment, biomass undergoes enzymatic hydrolysis for conversion of polysaccharides into monomer sugars, such as glucose and xylose. Subsequently, sugars are fermented to ethanol by the use of different microorganisms.

1)Pre-treatment is a critical step which enhances the enzymatic hydrolysis of biomass-treatment methods can be broadly classified into four groups – physical, chemical, physio-chemical and biological. Physical pre-treatment processes employ the irradiation processes to change only the physical characteristics of biomass. The physio-chemical process utilizes steam or steam and gases, like SO2 and CO2. The chemical processes employs acids (H2SO4, HCl, organic acids etc) or alkalis (NaOH, Na2CO3, Ca(OH)2, NH3 etc).

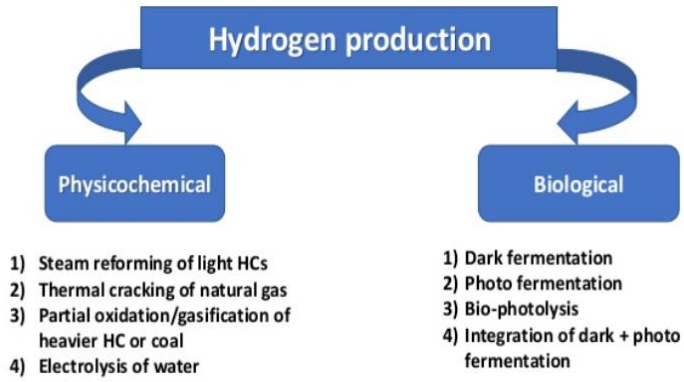


2)The acid treatment typically shows the selectivity towards hydrolysing the hemicelluloses components, whereas alkalis have better selectivity for the lignin. The fractionation of biomass components after such processes help in improving the enzymes accessibility which is also important to the efficient utilization of enzymes. The pre-treated biomass is subjected to enzymatic hydrolysis using cellulase enzymes to convert the cellulose to fermentable sugars. Cellulase refers to a class of enzymes produced chiefly by fungi and bacteria which catalyzes the hydrolysis of cellulose by attacking the glycosidic linkages.The functional proteins work synergistically in hydrolysing the cellulose into the glucose. These sugars are further fermented using microorganism and are converted to ethanol. The microorganisms are selected based on their efficiency for ethanol productivity and higher product and inhibitors tolerance. Yeast Saccharomyces cerevisiae is used commercially to produce the ethanol from starch and sucrose.



Biological production of hydrogen: -Biological hydrogen production biological production of hydrogen is carried out using microorganisms in an aqueous environment at particular temperature and pH. But the yield of hydrogen production is low as compared to other conventional methods but there is reduced emission of greenhouse gases by 57–73% using biological methods. Among different hydrogen production methods, biological methods are of great importance as they are less energy intensive. Biological hydrogen production stands out as an environmentally harmless process carried out under mild operating conditions, using renewable resources. Several types of microorganisms such as the photosynthetic bacteria, cyanobacteria, algae or fermentative bacteria are commonly utilized for biological hydrogen production

Methods involved in hydrogen production



Hydrogen produced through the action of living organisms is called biohydrogen. This is a type of biofuel, like bio-ethanol, bio-diesel or bio-gas or bio-oil. There are three classes of biofuels:-

1. First generation – made from food crops

2. Second generation – made from non-food crops or wastes
3. Third generation (advanced) - made using microbes

Advanced biofuels have several advantages over 1st and 2nd generation biofuels. Whereas first generation biofuels have caused increases in food prices, advanced biofuels would not. In comparison to second generation biofuels, advanced biofuels could capture sunlight energy 10 times more efficiently, meaning that smaller areas or land are needed to produce enough fuel.Biohydrogen is an example of an advanced biofuel (or third generation biofuel). In advanced biofuel technologies, microbes are grown in special bioreactors and provided with the energy and nutrients that they need including, sunlight, waste organic material, CO_2 from the air or from conventional gas plants. As they grow the microbes produce the biofuel.Among the advanced biofuels, biohydrogen is particularly attractive because of the excellent properties of hydrogen as a fuel and because biohydrogen is very easy to collect from the bioreactor. Conversely, biofuels such as bio-oils have to be purified from the microbial cells which is complex and expensive. Bio-hydrogen is hydrogen that is produced biologically. Clean fuel and rapidly produced from biomass from biological methods. Molecular H2 has the highest energy content per unit weight among the known gaseous fuels (143GJton−1). Hydrogen is the only carbon-free fuel which ultimately oxidizes to water as a combustion product. Does not contribute to greenhouse emission, acid rain or ozone depletion.

Methods of Bio hydrogen Production

1.Dark Fermentation

2.Photo Fermentation

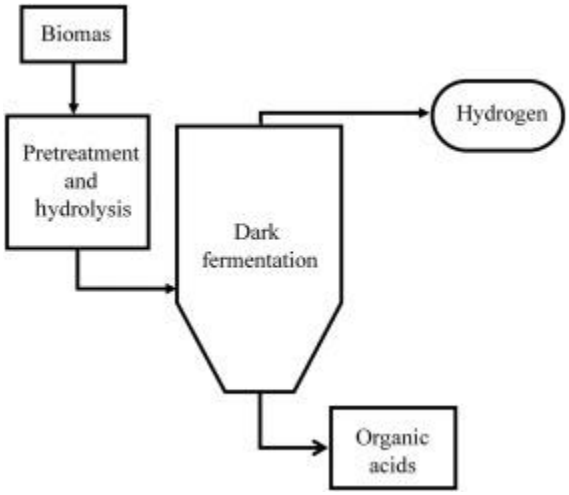
3.Direct Photolysis (algae)

4.Indirect Photolysis (cyanobacteria)

1.Dark Fermentation

Dark fermentation is a type of biological production of hydrogen. Dark fermentation is carried out by obligate anaerobes and facultative anaerobes in the absence of light and oxygen. In dark fermentation, bacteria act on the substrate and generate hydrogen. The

substrate for the dark fermentation is lignocellulosic biomass, carbohydrate materials like wastewater from industry, sugar-containing crop residues, and municipal solid waste. In the first step, the pre-treatment of the biomass greatly affects the efficiency of the dark fermentation. The other efficiency affecting parameters are the microorganism involved and the sugar content of the substrate. Fig. shows the process flow of the dark fermentation Fermentative conversion of organic substrate to biohydrogen. This method doesn't require light energy. The Gram positive bacteria of Clostridium genus is of great potential in biohydrogen production. Require wet carbohydrate rich biomass as a substrate. Produces fermentation end product as organic acids, Co2 along with biohydrogen.



$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$$

Advantages

♦ It produces valuable metabolites as a butyric acid, propionic acid.

♦ It is an anaerobic process so no oxygen limitation.

♦ It can produce carbon during day and night.

♦ Variety of carbon sources can be used as a substrate.

2.**Photo Fermentation**

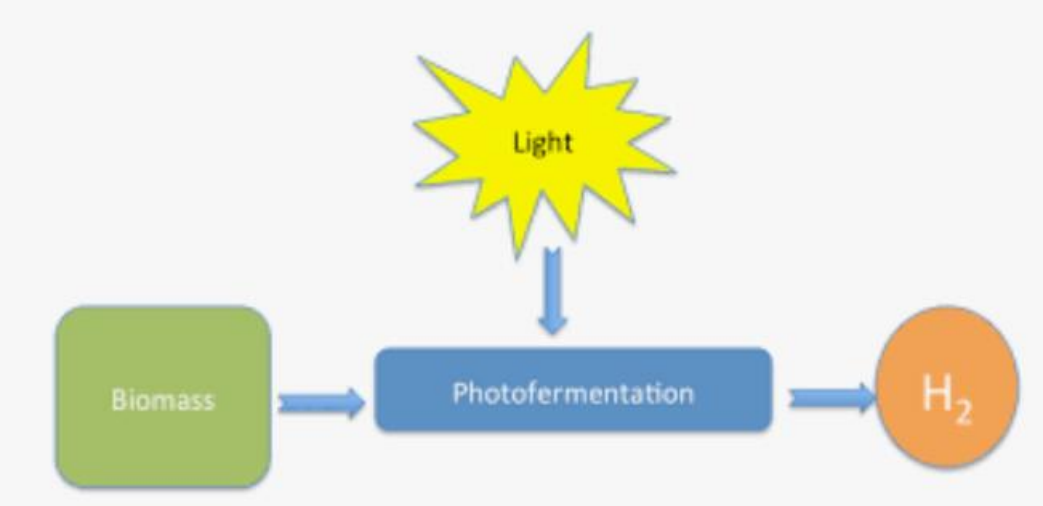
Photofermentative hydrogen production is a bioprocess in which photosynthetic purple nonsulfur bacteria grow heterotrophically on organic acids like acetic acid, lactic acid and butyric acid and produce hydrogen using light energy under anaerobic conditions. Two enzymes are specifically involved in hydrogen production, namely nitrogenase and

hydrogenase. While nitrogenases produce hydrogen under nitrogen-limited conditions acting as ATP-dependent hydrogenase, hydrogenases have the ability for both production and consumption of molecular hydrogen depending on the type of hydrogenase and physiological conditions. Photofermentation process can be achieved in a wide variety of conditions such as in batch or continuous mode, upon artificial or solar illumination, utilizing various carbon and nitrogen sources including food industry wastewater and dark fermentation effluents.Purple non sulphur bacteria genus rhizobacteria holds significant promise for production of hydrogen. Photo fermentation where light is required as a source of energy for the production of hydrogen by photosynthetic bacteria. Organic acids are preferred as a substrate. The light energy required in this process is up to the range of 400nm.

Mechanism $CH_3COOH + 2H_2 + Light$ ……..> $4H_2 + 2Co_2$

Production of hydrogen by photosynthetic bacteria takes place under illumination and in the presence of inert and anaerobic atmosphere for the breakdown of organic substrate to produce hydrogen molecules. Relatively higher achievable yield of H2, as a portion of substrate is used to produce organic acids. Anaerobes are capable of further breakdown of acids in to biohydrogen.

Drawbacks: - It can produce carbon during day only.



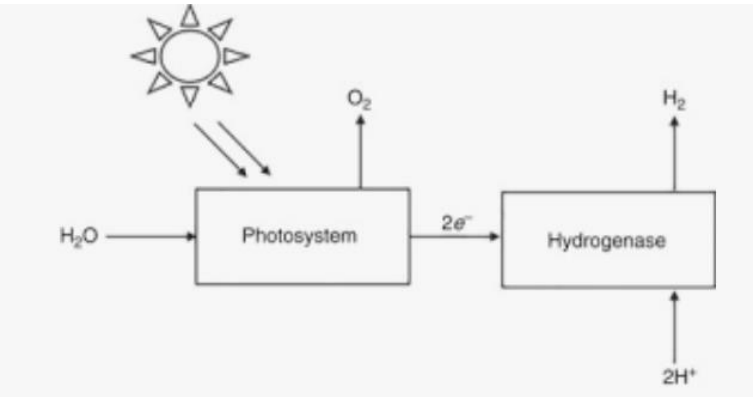
Photolysis is the splitting or decomposition of a chemical compound by means of light energy or photons. For example, the photolysis of the water molecule in photosynthesis occurred under the influence of light. When photons are absorbed, it causes the hydrogen to bind to an acceptor, subsequently releasing the oxygen. Etymology: from Ancient Greek - (“phōt”-),

(“phôs”), meaning “light” ” (lyúsis”), meaning
“decomposition”. Synonym: photodissociation; photodecomposition.

$$2H_2O \longrightarrow 4H^+ + 4e^- + O_2$$
$$4H^+ + 4e^- \longrightarrow 2H_2$$

Direct Bio-photolysis Direct photolysis **involves water-oxidation and a light-dependent transfer of electrons to the [Fe]-hydrogenase**, leading to photosynthetic hydrogen production.Solar energy and the photosynthetic system of algae converting water into chemical energy. One-stage direct bio-photolysis. Hydrogen production by direct photolysis follow these steps.

The light absorbed by (PSII) is used to generated electrons from water.The light absorbed by photosystem-I (PSI) is used to transport electrons to ferredoxin. overall reaction of direct bio-photolysis.



Direct pholysis

4)**Indirect bio-photolysis** :- Indirect photolysis is a two-step process where the formation of biohydrogen and oxygen are in separate reactions. The production of biohydrogen is from intracellular reserves such as glycogen and starch which are found in cyanobacteria and microalgae Cyanobacteria, also known as blue-green algae, is commonly used in this process. Indirect photolysis starts from cyanobacteria fixating carbon dioxide and using sunlight to produce cellular substance and oxygen The cellular substance is subsequently used for biohydrogen production

$$12H2O+6CO2+\text{light energy} \rightarrow C6H12O6+6O2$$

$$C6H12O6+12H2O+\text{light energy} \rightarrow 12H2+6CO2$$

Cyanobacteria possess [NiFe] hydrogenases instead of [FeFe] hydrogenases. This makes it more favourable for the production of biohydrogen. [NiFe] hydrogenases have lower oxygen sensitivity compared to [FeFe]. Cyanobacteria also possess nitrogenases which can work separately to catalyse the production of biohydrogen.Hence, oxygen sensitivity of hydrogenase can be reduced by spatially separating the fixation of carbon dioxide which produces oxygen and biohydrogen generation by nitrogenases.