Microbial biomass

Biomass is dead organic matter. Examples: kernels of corn, mats of algae, stalks of sugar cane are divided into two following types

A. Woody- these are coconut, oil palm, poplar, pine and generally burned to heat space or heat water to produce steam to generate electricity via a turbine generator. These can be utilized directly through biomass

B. Non-Woody- Thaes are other than wood like corn, sugar cane, soybeans, algae. Generally processed to produce different liquid biofuels, Indirect biomass

Bioenergy is the world's largest contributor to the renewable and sustainable energy sector, and it plays a significant role in various energy industries. Over-utilization of traditional fossil fuels has caused severe energy shortages and environmental damage. The combustion products of fossil fuels, like SO2, NOx, CO, CO2, etc., are harmful to the environment. It is therefore necessary to develop renewable and environment-friendly energy sources.

History of biofuel

1. Ancient times-late 1800s – People use biomass materials (which today include plants and plant-derived materials, manure and even garbage) in the form of burning wood for cooking, warmth and steam production. By the late 1800
2. Wood was being replaced by coal as the primary means of steam generation.
3. 1826 – Ethanol was first prepared synthetically through the independent efforts of Henry Hennel in Britain and S.G. Sérullas in France.
4. Technologies have developed an engine that ran on ethanol and turpentine.
5. 1980s – After investing heavily in renewable fuels in the 1970s, Brazil kept the program alive during the 1980s. With its robust ethanol program, Brazil developed an extensive ethanol industry.
6. By the mid-1980s, ethanol-only cars accounted for almost 90 percent of all new-auto sales in Brazil, making the country the biggest alternative fuel market in the world.

Definition of biofuel- Any fuel whose energy is obtained through a process of biological carbon fixation. A chemical process that converts carbon dioxide into a hydrocarbon molecule (a source of energy) that would be found in a living organism. Biofuels are converted into two categories they are first generation and second-generation biofuels

A. **First generation biofuels**: These biofuels are produced from the sources like sugarcane and corn starch. The sugars are fermented to produce bioethanol which can be directly used in a fuel cell to produce electricity or can be used in place of gasoline.

$$\text{Starch + Microbes} \xrightarrow[\text{pH}]{\text{Temperature}} \text{CO2 + Ethanol (Biofuel)}$$

B. **Second generation biofuels**: These biofuels are produced from the agriculture and municipal waste which Is a non-food-based biomass Examlpe biogas.

$$\text{1.Polymers} \xrightarrow{\text{Anaerobic bacteria}} \text{Monomers}$$

$$\text{2. Monomers} \xrightarrow{\text{Acidogenic bacteria}} \text{Organic acids}$$

$$\text{3. Organic acids} \xrightarrow{\text{Methanogenic bacteria}} \text{Methane} + CO_2 + \text{other gases}$$

C. **Microbial biomass** M**icrobial biomass (bacteria and fungi) is** a measure of the mass of the living component of soil organic matter. **The microbial biomass decomposes plant and animal residues and soil organic matter to release carbon dioxide and plant available nutrients.** For The Future. Microbial biomass refers the total amount of organic matter present in micro-organisms.

   A. It decomposes plant and animal residues and soil organic matter.
   B. It is used in microbial biofuel conversion and microbial electrochemical systems.
   C. It can serve as an alternative for the fossil fuel.
   D. Mainly derived from biomass or bio waste
   E. These fuels can be used for any purposes, but the main use for which they have to be brought is in the transportation sector.
   F. The most important feature of biomass is that they are renewable sources of energy unlike other natural resources like coal, petroleum and even nuclear fuel.
   G. Some of the agricultural products that are specially grown for the production of biofuels are:
      a. United States- switchgrass, soybeans and corn
      b. Brazil-sugar cane
      c. Europe- sugar beet and wheat
      d. China- cassava and sorghum
      e. Asia- miscanthus and palm oil
      f. India- jatropha

Catagories of microbial biomass- Recent advances have shown that some microbial species such as: yeast, fungi and microalgae can be used as potential sources for biodiesel as they can biosynthesise and store large amounts of fatty acids in their biomass

1. **Bacterial Biomass**- Microbes produce ethanol microbes are being used. It is produced from lignocellulose which is a mixture of cellulose, hemicellulose, and lignin. Cellulase is the enzyme which breaks down the cellulose. Several microbes are being studied by the scientists for the sources of this enzyme in the environment. It is found in the unusual environments like stomach of termites and near the volcanoes.
2. **Fungal Biomass**- Fungus that is found everywhere in the soil is *Trichoderma reesei*. It feeds by secreting cellulase. Genetic modifications were done for the fungus to produce still larger amounts of cellulase and convert straw to glucose. This can be converted to ethanol
3. **Algal Biomass**- Microalgae can produce lipids, proteins and carbohydrates in large amounts over short periods of time. These products can be processed into both biofuels and valuable co-products However, the production of lipids, proteins and carbohydrates may be limited by available sunlight due to diurnal cycles and the seasonal variations; thereby limiting the viability of commercial production to areas with high solar radiation

Why Mcf- Increasing demand of energy throughout the world.

1. Less environment pollution.
2. Depletion of fossil fuel reserve.
3. Rise of biotechnology for green energy Microbial biomass (bacteria, algae and fungi) is a measure of the mass of the living component of soil organic matter. The microbial biomass decompose plant and animal residues and soil organic matter to release carbon dioxide and plant available nutrients. The role of microbial biomass to its application in microbial biomass cultivation, microbial biofuel conversion and microbial electrochemical systems. The potential for the production of biofuels or other valuable byproducts from biomass has recently been intensively investigated. The mass cultivation of microbial biomass we can solve our fuel problem because the microbial biomass is an enormous source of bioenergy and it is a renewable energy

Producing biofuel from biomass -Biomass energy can be converted into liquid biofuels generally in two methods:

A. **Method I-** Sugar crops or starch are grown and through the process of fermentation, ethanol is produced.

B. **Method II-** Plants are grown which naturally produce oil, such as jatropha and algae

a. These oils are heated to reduce their viscosity after which they are directly used as fuel for diesel engines

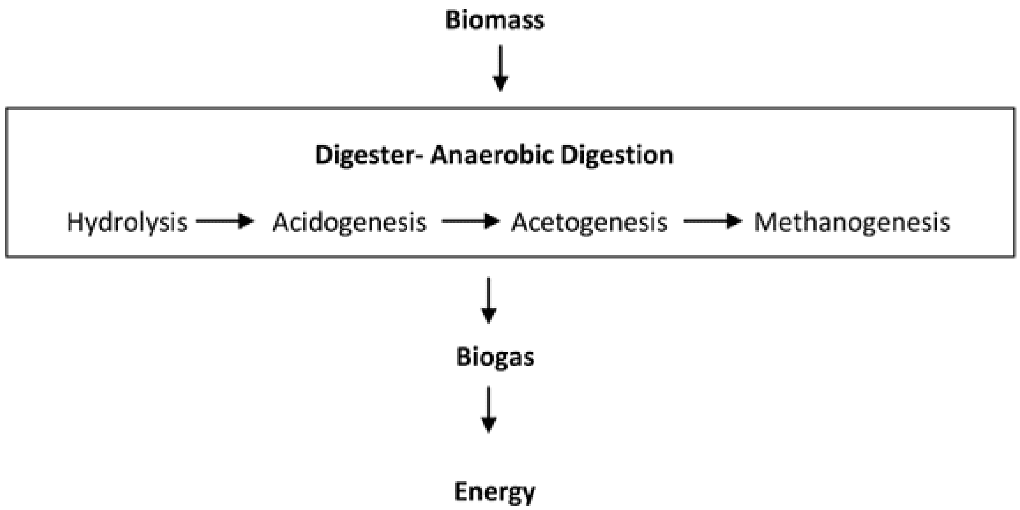
b. This oil can be further treated to produce biodiesel which can be used for various purposes

Classifications of biomass energy by microbes

1. **Thermochemical -** It can be direct *combustion* for immediate heat or p*yrolysis In this process the b*iomass is heated either in the absence of air or by the partial combustion of some of the biomass in a restricted air or oxygen supply. Products are extremely varied, consisting of gases, vapours, liquids and oils, and solid char and ash.
2. **Biochemical –** Biochemical conversion processes make use of the enzymes of bacteria and other microorganisms to break down biomass. In most of the case, microorganisms are used to perform the conversion process: anaerobic digestion, fermentation, and compostingIt is divided in two parts

A. Aerobic digestion- In the presence of air, microbial aerobic metabolism of biomass generates heat with the emission of CO2, but not methane. This process is of great significance for the biological carbon cycle, e.g. decay of forest litter, but is not used significantly for commercial bioenergy.

B. Anaerobic digestion- In the absence of free oxygen, certain microorganisms can obtain their own energy supply by reacting with carbon compounds of medium reduction level to produce both CO_2 and fully reduced carbon as CH_4. The process (the oldest biological ‘decay’ mechanism) may also be called ‘fermentation’, but is usually called ‘digestion’ because of the similar process that occurs in the digestive tracts of ruminant animals. The evolved mix of CO_2, CH_4 and trace gases is called *biogas* as a general term, but may be named *sewage gas* or *landfill-gas* as appropriate.



Four main stages or path way of Anaerobic Digestion

a. **Hydrolysis-** In anaerobic digestion, hydrolysis is the essential first step, as Biomass is normally comprised of very large organic polymers, which are otherwise unusable. Through hydrolysis, these large polymers, namely proteins, fats and carbohydrates, are broken down into smaller molecules such as amino acids, fatty acids, and simple sugars.

$$(C_6H_{10}O_5)_n + nH_2O = n(C_6H_{12}O_6)$$

b. **Acidification/acedogenesis**- Acidogenesis is the next step of anaerobic digestion in which acidogenic microorganisms further break down the Biomass products after hydrolysis. These fermentative bacteria produce an acidic environment in the digestive tank while creating ammonia, H_2, CO_2, H_2S, shorter volatile fatty acids, carbonic acids, alcohols, as well as trace amounts of other byproducts. While acidogenic bacteria further breaks down the organic matter, it is still too large and unusable for the ultimate goal of methane production

$C_6H_{12}O_6 + 2H_2O = 2CH_3COOH+4\ H_2 +2\ CO_2$, ***Bacteroids clostridium***

$C6H12O_6 + 2H_2 = 2CH_3CH2COOH+42H_2O$ ***Butyrivibrie eubacterium***

$C6H12O_6 = CH_3(CH_2)COOH+2\ CO_2 + 2H_2$ ***Biffidobacterium lactobacillus***

$C_6H_{12}O_6 = 2CH_3CHOHCOOH$

$C_6H_{12}O_6 = 2CH_3CH_2OH + 2\ CO_2$

c. **Acetogenesis and dehydrogenation**- **A**cetogenesis is the creation of acetate, a derivative of acetic acid, from carbon and energy sources by acetogens. These microorganisms catabolize many of the products created in acidogenesis into acetic acid, CO2 and H2. Acetogens break down the Biomass to a point to which Methanogens can utilize much of the remaining material to create Methane as a Biofuel.

$CH_3CHOHCOOH + H_2O = CH_3COOH + CO_2 + 2H_2$ ***Desulfovibrio syntrrophobactor***.

$CH_3CH_2OH + H_2O = CH_3COOH + 2H_2$ ***Wolini syntrophomus***

$CH_3CH_2CH_2COOH + H_2O = 2CH_3COOH + 2H_2$

$CH_3CH_2COOH + 2H_2O = CH_3COOH + CO_2 + 3H_2$ ***Desulfovibrio syntrrophobactor***

d. Methanogenes- Methanogenesis constitutes the final stage of anaerobic digestion in which methanogens create methane from the final products of acetogenesis as well as from some of the intermediate products from hydrolysis and acidogenesis

$4H_2 + CO_2 = CH_4 + 2H_2O$ ***Methanobacterium forcicum***

$2CH_3(CH_2)_2OH + CO_2 = 4CH_3COOH + CH_4$ ***Methanobravibacter***

$2CH_3(CH2)_2COOH + 2H_2O + CO_2 = 4CH_3COOH + CH_4$ ***Ruminantium methanospirilum***.

3. Agrochemical- Occasionally, liquid or solid fuels may be obtained directly from living or freshly cut plants. The materials are called exudates and are obtained by cutting into (tapping) the stems or trunks of the living plants or by crushing freshly harvested material.

A. A well known similar process is the production of natural rubber latex. Related plants to the rubber plant *Herea*, such as species of *Euphorbia*, produce hydrocarbons of less molecular weight than rubber, which may be used as petroleum substitutes and turpentine.

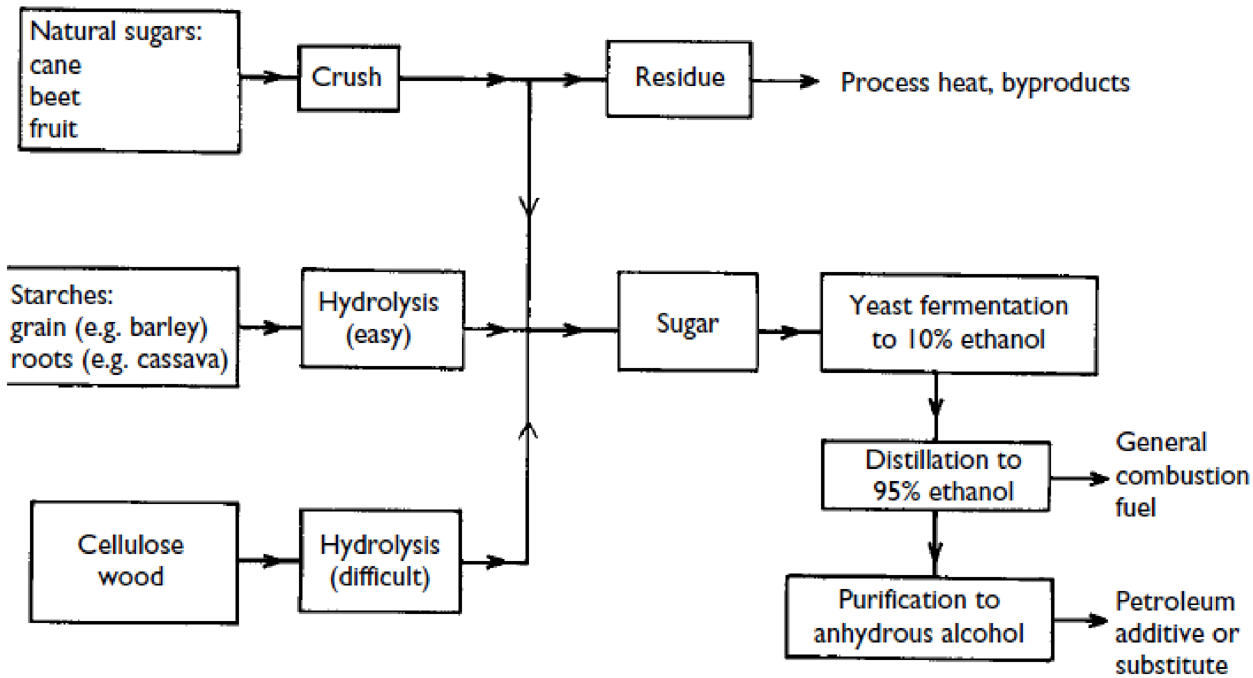
B. Concentrated vegetable oils from plants may be used directly as fuel in diesel engines; indeed Rudolph Diesel designed his original 1892 engine to run on a variety of fuels, including natural plant oils.

C. However, difficulties arise with direct use of plant oil due to the high viscosity and combustion deposits as compared with standard diesel-fuel mineral oil, especially at low ambient temperature ≤~5C.

D. Both difficulties are overcome by converting the vegetable oil to the corresponding ester, which is arguably a fuel better suited to diesel engines than conventional (petroleum-based) diesel oil**.**

4. Electrochemical conversions-Biomass can be directly converted to electrical energy via electrochemical (electrocatalytic) oxidation of the material. This can be performed directly in a direct carbon fuel cell, direct liquid fuel cells such as direct ethanol fuel cell, a direct methanol fuel cell, a direct formic acid fuel cell, a L-ascorbic Acid Fuel Cell (vitamin C fuel cell) and a microbial fuel cell. The fuel can also be consumed indirectly via a fuel cell system containing a reformer which converts the biomass into a mixture of CO and H_2 before it is consumed in the fuel cell.

Biofuels from Microorganisms- Recently, research has focused on whether microbial systems can be exploited for the biosynthesis of a wide range of liquid biofuels. Which are following.



1. **Ethanol-** *Ethanol*, C2H5OH, is produced naturally by certain micro-organisms from sugars under acidic conditions, i.e. pH 4 to 5.

A. **Directly from sugarcane**- Usually commercial sucrose is removed from the cane juices, and the remaining molasses used for the alcohol production process (These molasses themselves have about 55% sugar content. But if the molasses have little commercial value, then ethanol production from molasses has favourable commercial possibilities, especially if the cane residue (bagasse) is available to provide process heat. In this case the major reaction is the conversion of sucrose to ethanol:

$$C_{12}H_{22}O_{11} + H_2O \xrightarrow{\text{yeast}} 4C_2H_5OH + 4CO_2$$

B. **From sugar beet**- Sugar beet is a mid-latitude root crop for obtaining major supplies of sugar. The sugar can be fermented, but obtaining process heat from the crop residues is, in practice, not as straightforward as with cane sugar, so ethanol production is more expensive.

C. **From starch crops**- Starch crops, e.g. grain and cassava, can be hydrolyzed to sugars. Starch is the main energy storage carbohydrate of plants, and is composed of two large molecular weight components, amylose and amylopectin. These relatively large molecules are essentially linear, but have branched chains of glucose molecules linked by distinctive carbon bonds. These links can be broken by enzymes from malts associated with specific crops, e.g. barley or corn, or by enzymes from certain moulds (fungi). Such methods are common in whisky distilleries, corn syrup manufacture and ethanol production from cassava roots. The links can also be broken by acid treatment at pH 1.5 and at 2 atmospheres pressure, but yields are small and the process more
expensive than enzyme alternatives. An important by-product of the enzyme process is the residue used for cattle feed or soil conditioning.

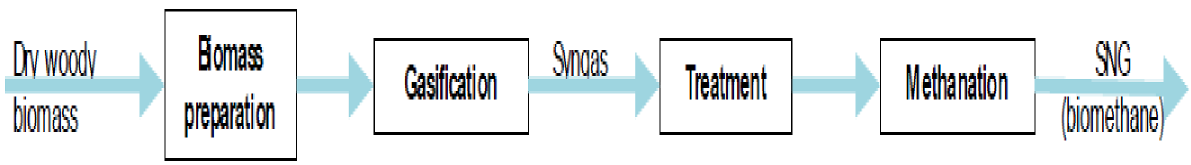
D. **From cellulose**- Cellulose comprises about 40% of all biomass dry matter. Apart from its combustion as part of wood, cellulose is potentially a primary material for ethanol production on a large scale. It has a polymer structure of linked glucose molecules, and forms the main mechanical-structure component of the woody parts of plants. These links are considerably more resistant to breakdown into sugars under hydrolysis than the equivalent links in starch.
Cellulose is found in close association with lignin in plants, which discourages hydrolysis to sugars. Acid hydrolysis is possible as with starch, but the process is expensive and energy intensive. Hydrolysis is less expensive, and less energy input is needed if enzymes of natural, wood-rotting fungi are used, but the process is slow. Prototype commercial processes have used pulped wood or, more preferably, old newspaper as input. The initial physical breakdown of woody material is a difficult and expensive stage, usually requiring much electricity for the rolling and hammering machines. Although not yet generally applied commercially, these processes may allow ethanol from biomass to compete commercially with fossil petroleum.

2. **Butanol**- Biobutanol is produced from the same agricultural feedstocks as bioethanol (i.e. corn, wheat, and sugar beet and sugarcane), it is a good proposition for global farmers. This would provide another marketing opportunity for key agricultural residual products, thus enhancing value to farmers. The process for its production is very complicated and difficult. It produced by the anaerobic conversion of carbohydrates by strains of *Clostridium acetobutylicum* into acetone, butanol and ethanol as clostridia secrete numerous enzymes that facilitate the

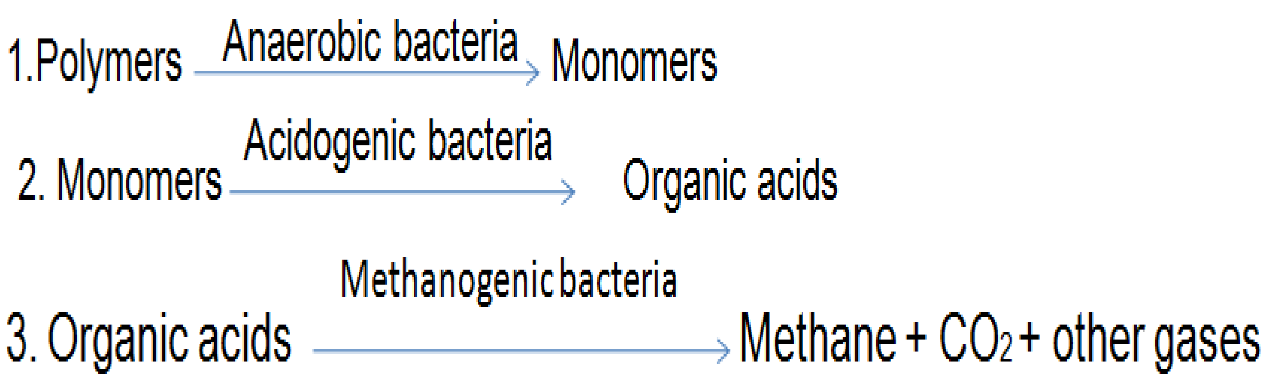
breakdown of polymeric carbohydrates into monomers. This fermentation is known as the acetoneebutanoleethanol fermentation or ABE fermentation", with a product ratio of 3:6:1

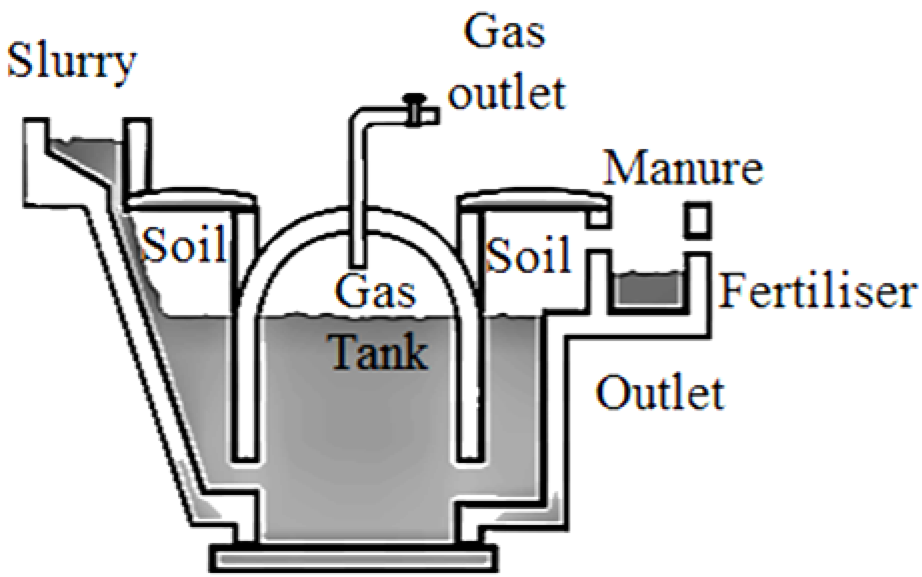
3. **Biomethane**- Methane produced from biomass is referred to as Bio-Methane, Green Gas, Bio-Substitute Natural Gas (Bio-SNG) or Bio-CNG when it is used as a transport fuel. Biomass energy is expected to make a major contribution to the replacement of fossil fuels. A number of concepts are available such as,

A. Production of biomethane from the bio-chemical conversion of biomass (biogas) and via thermo-chemical conversion of solid biomass tgrough gasfication temperatures between 500-1400°C so called Bio-SNG.



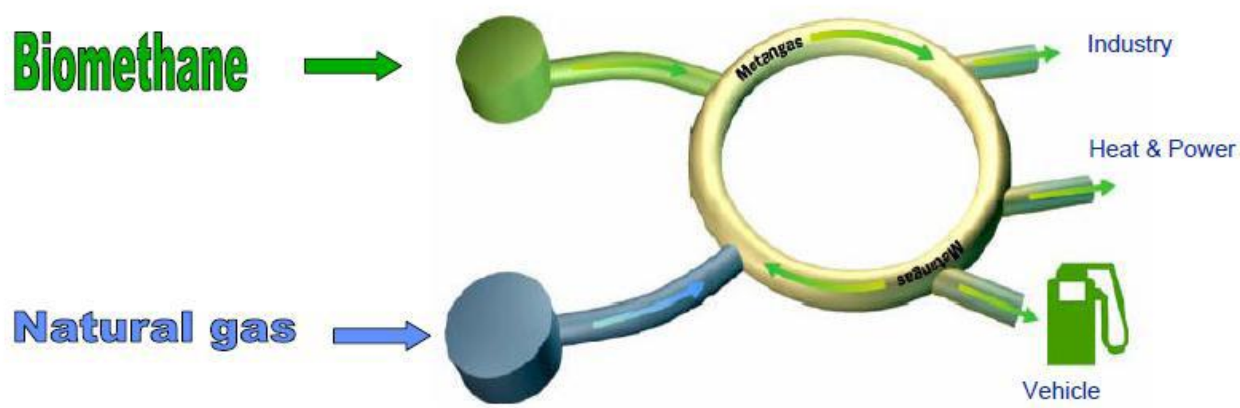
B. Anaerobic digestion processes are state of the art and well developed. Using the long time experiences of upgrading biogas the challenges of future biomethane supply plants will be optimisation of substrate supply, digestate utilisation as well as efficient gas grid access using optimised and new technologies





Floating type biogas plant

Uses of biomethane-



Biohydrogen is H_2 that is produced biologically. Interest is high in this technology because H_2 is a clean fuel and can be readily produced from certain kinds of biomass, including biological waste.[3] Furthermore some photosynthetic microorganisms are capable to produce H_2 directly from water splitting using light as energy source.[

Biohydrogen from biomass- Production of hydrogen from renewable biomass has several advantages compared to that of fossil fuels

Process routes of hydrogen-production- Biohydrogen production is considered **a development vital to a sustainable global clean energy supply and a promising alternative to conventional fossil fuels 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.

Biological hydrogen production by dark fermentation- This biological hydrogen production system includes **photosynthetic bacteria and nonphotosynthetic bacteria**. A variety of carbohydrates can be digested by Clostridium butyricum, which can degrade the carbohydrates and produce biohydrogen without light.

The main reactions driving hydrogen formation involve the oxidation of subtrates to obtain electrons. Then, this electrons are transfered to free protons to form molecular hydrogen. This proton reduction reaction is normally performed by an enzyme family known as hydrogenases.

In heterotrophic organisms, electrons are produced during the fermentation of sugars.Hydrogen gas is produced in many types of fermentation as a way to regenerate NAD^+ from NADH. Electrons are transferred to ferredoxin, or can be directly accepted from NADH by a hydrogenase, producing H_2. Because of this most of the reactions start with glucose, which is converted to acetic acid.[6]

A related reaction gives formate instead of carbon dioxide:

These reactions are exergonic by 216 and 209 kcal/mol, respectively.

Blue-green algae (cyanobacteria) are promising microorganisms for this. Advantages are hydrogen evolution is separated from oxygen evolution. It can also produce relatively higher hydrogen yields. Furthermore, by-products can be efficiently converted to hydrogen. **Genetically modified algae could be efficient producers of hydrogen and biofuels**. Algae are a promising source of biofuels: besides being easy to grow and handle, some varieties are rich in oil similar to that produced by soybeans. Algae also produce another fuel: hydrogen

a. **Production by algae-** The biological hydrogen production with algae is a method of photobiological water splitting which is done in a closed photobioreactor based on the production of hydrogen as a solar fuel by algae. Algae produce hydrogen under certain conditions. In 2000 it was discovered that if *C. reinhardtii* algae are deprived of sulfur they will switch from the production of oxygen, as in normal photosynthesis, to the production of hydrogen.

b. **Halobacteria- Two sp. Of this bacteria H. holobium and H. cutirubrum are known which can produces large amount of H2 gas. In high concentration of salt and low amount of O2 they produce H2**

c. **Bacteria-**