**Role of Microorganisms Its Biochemistry In Biofuel Production**

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Biofuels are those which are derived from biomass by Various processes. Biofuels can be different liquid fuels or biogases. Biofuels are gaining increased, public and scientific attention, due to various reasons like scarcity of non-renewable energy sources like natural gas, coal etc, hike in oil prices and need for increased energy security. Biofuels provided 1.8% of the world's transport fuel in 2008. Investment into biofuels production capacity exceeded $4 billion world wide in 2007 and is growing rapidly, much due to their Significance as renewable sources of energy. There are different biofuels like Bioethanol, Biodiesel and Biogas.

1. Bioethanol is an alcohol which is biologically made by fermentative breakdown of the sugar, starch compounds of plant materials. With advanced technology being developed, cellulosic biomass, such as trees and grasses, are also used as substrates for ethanol production. Ethanol can be used as a fuel for vehicles in its pure form, but it is usually used as a gasoline additive to increase octane and improve vehicle emissions. Bioethanol is widely used in the USA and in Brazil. Thus biologically produced alcohols like, ethanol, propanol and butanol serve as biofuels.
2. Biodiesel is made from vegetable oils, animal fats or recycled greases. Biodiesel can be used as a fuel for vehicles in its pure form, but it is usually used as a diesel additive to reduce levels of particulates, Carbon monoxide, and hydrocarbons from diesel powered vehicles. Biodiesel is produced from oils or fats using trans esterification reaction and is the most common biofuel in Europe. Its chemical name is fatty acid methyl ester.  
   Oils are mixed with sodium hydroxide and methanol and the chemical reaction produces biodiesel and glycerol. Substrates or Feedstocks used for biodiesel production are animal fats, Vegetable oils, soya, rapeseed , jatropa, mahua, mustard, flax, sunflower, palm oil, hemp, pongamia and algae. Pure biodiesel is by far the lowest emission diesel fuel.
3. Biogas typically refers to a gas produced by anaerobic and biological breakdown of organic matter. Biogases are basically of two types based on production process and products in the gas. One type of biogas is produced by anaerobic digestion or fermentation of biodegradable materials such as plant biomass, manure, sewage, municipal waste, green waste, etc, This type of biogas comprises primarily of methane and Carbon-dioxide: The other principal type at biogas is wood gas which is produced by gasification of wood or other biomass. This type of biogas is comprised primarily of nitrogen, hydrogen and carbondioxide, with trace amounts of methane. Biogas can be compressed, similar to natural gas, and used to power motor vehicles. For example in UK biogas is estimated to have the potential to replace around 17% of vehicle fuel.

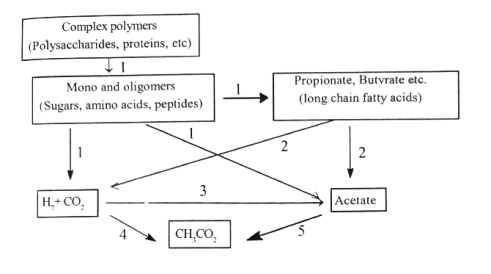
**History:**

* Jan Bapita Van Helmont in 17th century first identified that inflammable gases could evolve from decay ingorganic, matter.
* Count Alessandro Volta concluded in 1776 that there was a direct correlation between the amount of decaying organic matter and the amount of flammable gas produced.
* Sir Humphrey Davy in the year 1808, determined that methane was present in the gases produced during the anaerobic degradation of Cattle manure.
* Adigester was built as early as 1840 in the city of otago, New Zealand.
* Methane's practical and commercial value was first, recognised in England, in 1890s. A specially designed septic tank was used to generate gas for the purpose of lighting.
* The Gobar Gas Research Station was founded in 1960 as the newest of a long series of Indian research efforts Started sometime in the1930s.
* First - generation biofuels' are those which are made from sugar, starch, vegetable oil or animal fats, using conventional technology. The basic feedstocks for the production of first generation biofuels are Often seeds or grains such as wheat, which yields starch that is fermented into bioethanol, or sunflower seeds, which, are pressed to yield Vegetable oil that can be used in biodiesel. Biogas is also produced by the biological and anaerobic breakdown of organic matter and hence can be called a biofuel but not typically first generation fuel.

**Biogas-Methane**:

1. Biogas is a type of biofuel produced typically from biogenic material by anaerobic and biological breakdown of it. Based on method of production they are of two types:  
   i) one type of biogas is produced by anaerobic digestion or fermentative breakdown of biodegradable materials such as biomass, manure, energy crops etc. It is a clean and efficient fuel which is a mixture of methane, carbondioxide, hydrogen and hydrogen Sulfide.
2. The other type of biogas is wood gas which is Created by gasification of wood or other biomass! It is a mixture of nitrogen, hydrogen, carbonmonoxide and trace amounts of methane. Biogas is practically produced as i) Landfill gas (LEG) or ii) Digester gas.  
   i) Landfill gas is produced by decomposing Wet organic waste under anaerobic conditions in a landfill. The waste is covered and mechanically compressed by the weight of the material that is, deposited from above. This material prevents oxygen exposure thus facilitating anaerobic microbial growth and degradation. This gas builds up and is slowly released into the atmosphere if the landfill site has not been engineered to capture the gas, or the gas can be collected.  
   ii) Digester gas is the biogas that is produced by utilizing anaerobic digesters. The digester can be fed with energy crops such as maize silage or biodegradable wastes including sewage sludge and food waste. The composition of biogass varies depending upon the origin of the anaerobic digestion process. Landfill gas typically has methane concentrations around 50% while digester gas can produce biogas with 55-75% CH4 also if purification techniques are used. A biogas plant is the name often given to an anaerobic digester that treats farm wastes or energy crops.

Microbial Conversion of organic matter to methane is carried out by action of various groups of anaerobic bacteria. It is an attractive method of waste treatment, and resource recovery. Three basic and important aspects about this process are: (a) The most important bacteria involved in biogas production are anaerobes which are slow growing (b) A greater degree of metabolic specialization is observed in these anaerobic microorganisms. (C) Most of the free energy present in the substrate is found in the terminal product methane. As less energy is available for the growth of organism,. less microbial biomass is produced and so disposal of sludge after the digestion is not be a major problem.  
Complex polymers are broken down to Soluble products by enzymes produced by fermentative bacteria that are grouped as Group 1 which ferment the Substrate to short - chain polymers like  
polysaccharides, peptides, fatty acids, hydrogen and Carbondioxide. Fatty acids longer than acetate are metabolized to acetate by obligate hydrogen - producing acetogenic, bacteria that belong to  
Group 2. The major products at the end of this degradation are hydrogen, carbondioxide and acetate, which can be converted to either acetate by hydrogen -oxidizing acetogens of Group 3 or methane by carbon dioxide reducing, hydrogen - oxidizing methanogens of Group 4 • Acetate is also converted to methane by aceticlastic methanogens belonging to Group 5• The degradation of complex polymers by, different groups of organisms is represented in the figure given below.



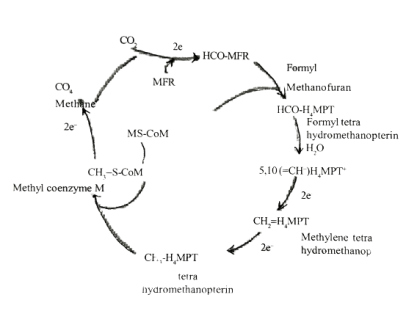
Degradation of Complex Polymers by Different Organisms

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| S.NO | Reactions | Products |
| 1 | Glucose + 3H2O | 3CH4 + 3HCO3- + 3H+ |
| 2 | Glucose + 4H2O | 2CH3COO- + 2HCO3- + 4H+ + 4H2 |
| 3 | 2CH3COO- + H2O | CH4 + HCO3- + H+ |
| 4 | 4H2 + 2HCO3-  + H+ | CH4 + 3H2O |
| 5 | 4H2 + 2HCO3-  + H+ | CH3COO- + H2O |
| 6 | Butyrate + 2H2O | 2CH3COO- + 2H+ + 2H2 |
| 7 | Propionate + 3H2O | CH3COO- + HCO3- + H+ + 3H2 |
| 8 | Benzoate + 7 H2O | CH3COO- + HCO3- +3H+ + 3H2 |

Representative reactions occurring in atypical biogas Digester

**Microorganisms:** Microbial diversity in biogas digesters is very diverse and almost seventeen fermentative bacterial species have been reported to play important role for production of biogas. The nature of the substrate determines the type and extent of the fermentative bacteria present in the digester. For example there are more proteolytic organisms in poultry waste - fed digesters and other animals waste -fed digesters while cow dung - fed digesters supported higher amylolytic microorganisms. Some examples of fermentative organisms, are Bacteroides, succinogens, Butyrivibrio fibrisolvens. Some examples of cellulolytic organisms are Clostridium cellobioparum, Ruminococcus albus and Clostridium sp. Cattle dung - fed digesters had Ruminococcus flavefaciens, Eubacterium Cellulosolvens, clostridium cellulosolvens, Clostridium cellularorans, Elostridium thermocellum, Bacteroides cellulosolvens and Acetivibrio cellulolyticus. Most of these bacteria adhere to the substrate prior to extensive hydrolysis.Volatile fatty acids are the primary and predominant products of carbohydrate fermentation in biogas digesters. In addition to these a variety of products are also formed by the action of fermentative bacteria, though less in number, obligately hydrogen - producing acetogenic bacteria are one of the important groups in biogas digesters. These organisms oxidize the fatty acids that are longer than acetate to acetate and thereby release energy from the Substrate in the form of methane. Hydrogen – Consuming acetogenic bacteria are the minor groups involved in fermentative reactions in biogas digesters. Methanogens possess very limited substrates or metabolic end products like acetate or C1 Compounds which are H2 and CO2 formate, methanol, methyl-amines or CO, with methane being the end product of the reaction. Both carbondioxide - reducing and aceticlastic - methanogens play an important role in maintaining stability of the digester.

The different methanogenic bacteria in digesters are Methanobacterium bryanti, Methanobacterium formicum, Methanobrevibacter ruminantium,Methanobrevibacter got tschalkii,Methanobacterium  
defluvii, Methanobacterium wolfei, Methanobacterium thermoautotrophicum, Methanococcus jannaschii etc...Methanogens are usually Coccoid or rod shaped. All methanogens belong to the group Archae and are anaerobic.



The Biochemical pathway of methanogenesis

**Raw materials used for biogas Production:**

Animal wastes are generally used as feedstock in biogas plants. The two most important parameters in the selection of particular plant feed stocks are the economic considerations and the yield of methane for fermentation of that specific feedstock. Dilferent feed stocks have been tested for biogas especially  
methane production by different research groups!Animal wastes have been used and studied in Kilograms whereas in case of plant materials different sizes were used of which 40mm size gave good results.Different feed stocks studied by one research group were fresh- water aquatics, forage grasses, roots and tubers, and marine species. The highest yield was reported from root Crops followed by forage grasses, and fresh-water aquatics, but marine species gave the  
lowest yield of methane. Another feed stock coir pith and Cattle waste at 3:2 ratio gave a better gas output and the methane content was in the range of 80-85% .

Different deoiled cakes like castor, neem, groundnut and coconut wore tested but they didnot give good yields of methane. This was so because accumulation of long-chain fatty acids and propionate after four weeks of digestion inhibited the methanogenic process. Banana peel gave better gas production. The use of cannabis for biogas production gave good yields.

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| S.No | Feed Stock | Methane yield in m3 Kg-1 |
| 1 | Cattle waste | 0.36 |
| 2 | Buffalo waste | 0.54 |
| 3 | Piggery waste | 0.18 |
| 4 | Chicken waste | 0.011 |
| 5 | Human waste | 0.028 |
| 6 | Fruit waste | 0.03 – 0.42 |
| 7 | Tomato processing waste | 0.42 |
| 8 | Banana peeling | 0.4 |
| 9 | Mirabilis leaves | 0.34 |
| 10 | Ipomia | 0.43 |
| 11 | Wheat straw | 0.25 |
| 12 | Rice straw | 0.37 |

Methane production from different feed stocks

**Factors affecting biogas production:**

\* Various factors such as biogas potential of feedstock, design of digester, inoculum, nature of substrate, pH, temperature, loading rate, hydraulic retention time (HRT), C:N ratio, volatile fatty acids (VFA), etc. influence the biogas production.

\* A pH between 7.0 and 7.2 is optimum, though the gas production was satisfactory between pH 6.6 and 7.6 also.

\* The temperatures were above 45oC. Temperature Slightly varied with the feedstock and the microbial Consortia used. Though there was higher production of biogas at 55°C; the process was unstable due to higher production of volatile fatty acids. The production reduced in winters due to low temperatures. Production of gas is most rapid between 29°C and 41°C or between 49°C and 60°C. This is due to the fact that two different types of bacteria multiply best in these two different ranges, but the high temperature bacteria are much more sensitive to ambient influences.

\* C:N ratio, 25-30:1 is optimum for biogas production. A higher ratio will leave carbon still available after the nitrogen has been consumed, Starving some of the bacteria of this element. These will die, returning nitrogen to the mixture, but slowing the process. Too much nitrogen will lead to accumulation of it at the end of digestion and this reduces the quality of the fertilizer produced by the biogas plant.

**Applications/uses of Biogass:**

* Biogas can be utilized for electricity production and water heating.
* If compressed, it can replace compressed natural gas for use in vehicles.
* Methane within biogas can be concentrated via a biogas upgrader to the same standards as fossil natural gas and becomes biomethane. This can be distributed through the local , gas distribution networks.Gas must be very clean to reach pipeline quality, and must be of the correct composition for the local distribution network to accept. Impurities like CO2, H2O  
  H2S and particulates must be removed.
* Domestic biogas plants convert livestock manure and night soil into biogas and slury, or fermented manure. This curbs land, soil and air pollution.

**Recent Developments:**

\* Research results indicated that biogas generation is increased when the particle size of organic material is small and especially less than 1mm, when there are metal ions in biomass.  
\*Research in other areas has focused on differentSubstrates, their composition and its effect on gas production. Water hyacinth (Eichornia crassipes) was found to be useful.  
\* Anaerobic digester design has continued to evolve over the years, but systems are generally variations around the theme of the floating-dome and the fixed - dome design.  
\* The addition of Magnesium and calcium salts increased methane generation in addition to reduced  
Slurry foaming.

\* Additives to stabilize fluctuating pH and to reduce concentration of Ammonia and hydrogen sulfide are also in use.