Wastes and residues currently constitute a large source of biomass. These include solid and liquid municipal wastes, manure, lumber and pulp mill wastes and forest and agricultural residues with the exception of feed stocks of low water content, most of this biomass cannot be directly utilized and must undergo some form of the transformation prior to being utilized as a fuel. Biological processes for the conversion of biomass to fuels include ethanol fermentation by yeast or bacteria and methane production by microbial consortia under anaerobic condition. Waste disposal and pollution are inextricably linked. The term pollution describes both the act of polluting and the consequences of that act. Waste describes unwanted residues that are usually perceived to be of negative value. Pollution can be defined as the introduction into the natural environment by humans of substances, materials or energy that cause hazard to human health, harm to living resources and ecological system, damage to structure and amenities or that interfere with the legitimate uses of the environment. It is implicit in the definition that pollution only describes situations where unwanted effects occur. The most of majority of the waste disposal situations involve pollution of one kind or another. Waste results from consumption, depending on the product or resource in question; it is its mode of consumption that dictates the fraction that is wasted during use and the fraction that only becomes waste upon system redundancy.

The term ‘waste’ means that is unwanted and one can do without. With industrialization and development in all spheres of life, man has created wastes and without realizing the consequences have allowed its accumulation in quantities that have reached a proportion that demands its immediate and systemic disposal for maintaining a healthy and safe environment. Wastes can be categorized into two types: biological and non-biological. Wastes of biological origin, due to their high organic contents, are easily biodegradable whereas, non-biological ones like plastics and polymers fall under non-biodegradable category. Biodegradable wastes include organic waste e.g. kitchen waste, vegetables, fruits, flowers, leaves from the garden, and paper. While non biodegradable waste can be further segregated into (a) Recyclable waste, plastics, paper, glass, metal, etc. (b) Toxic wastes – old medicines, paints, chemicals, bulbs, spray cans,
fertilizers and pesticides containers, batteries, shoe polish etc. (c) Soiled – hospital waste such as cloth soiled with blood and other body fluids. Large amounts of agricultural, domestic and industrial wastes generated as a result of development, have potentially detrimental effects both on the environment and on human health. The importance of protecting the environment and restoring environmental damage cannot be overemphasized.

Energy occupies the topmost position in the world agenda. The crisis in the world, the bleak prediction regarding our fossils fuel reserves and an increase in concern for a healthy environment have all lead to a search for clean and unconventional sources of energy that can sustain human growth. Prices, security and climate changes are three major concerns that are related to energy supply and usage. Alternative fuels such as hydrogen and biodiesel are being investigated for further sustainable transport options that reduce dependence on petroleum and other conventional non-renewable fuels. Hydrogen is poised to become an important future energy carrier. One pathway to sustainable hydrogen generation is biological hydrogen production by microbial fermentation. This process uses readily available wastes, thus solving another environmental problem of waste stabilization, as well as presently unutilized bioresources. The critical factor in this process is the amount of hydrogen that can be produced /mole of substrate. In order to make this process practical, yield of hydrogen must be high.

Hydrogen is thought to be an ideal fuel of the future, which would, not only reduce global warming but also protect ecological balance by eliminating the emission of gaseous pollutants in air. Biological hydrogen production has an edge over its chemical counterpart mainly because it is environmentally benign.

Other overriding issues in the future of biological energy system are the overall efficiencies of converting biomass to useful fuels, the economics of such processes, their environmental impacts, their competitiveness with thermo chemical conversion processes for biomass (combustion, gasification), their resources potential, and perhaps most important, their compatibility with evolving economic and political structures. Biofuels would for example, complement solar electricity in the renewable energy mix of the future or if catastrophe economic and population collapse is to be avoided, we must not only curb populations and consumption, but must also develop and implement more efficient and environmentally benign
technologies, available to the large populations currently not enjoying the benefits of our technological economy. Biological energy system can play an important role in this transformation of the human economy and condition, necessary for our survival through the 21st century.

Solid wastes can be classified into different types depending on their source: (a) Household waste is generally classified as municipal waste, (b) Industrial waste as hazardous waste, and (c) Biochemical waste or hospital waste as infectious waste. Waste disposal is one of the major problems being faced by all nations across the globe. The daily per capita solid waste generated in India ranges from about 100 g in small town to 500 g in large towns. It takes anywhere between three and seven days for the waste to be disposed from the time of its generation. The treatment and disposal of waste by conventional methods is a time consuming as well as energy intensive process and adds to environmental pollution.

Municipal solid wastes consist of household domestic waste, sanitation residue, and wastes from streets. This garbage is generated mainly from residential and commercial complexes. Garbage is mainly divided in four categories (a) Organic waste: kitchen waste, flowers, leaves, vegetables, fruits. (b) Toxic waste: old medicines, paints, chemicals, bulbs, spray cans, fertilizers, and pesticides containers, batteries, shoe polish. (c) Recyclable: paper, glass, metals, plastics. (d) Soiled: hospital waste such as cloth soiled with blood and other body fluids.

Municipal solid waste (MSW) is solid waste from residential, commercial, institutional, and industrial sources, but it does not include such things as construction waste. The amount of waste generated is increasing by becoming unmanageable. The local corporations have adapted different methods for the disposal of waste – open dump, landfills, sanitary landfills, and incineration plants.

The most obvious environmental damage caused by solid wastes is aesthetics, that is, the ugliness of street litter and the destruction of the beauty of the countryside by uncontrolled dumping of the solid wastes. More serious, however, and often unrecognized, is the contamination of surface and ground water sources by leachates from the refuse dump. Purtrescible organic wastes cause odor nuisances. Hazardous solid wastes can cause death or
injury to human and animal life, fire hazard, explosions, and danger of concentration in the food chain.

The fate of hydrogen biotechnology is presumed to be dictated by the stock of fossil fuels and state of pollution in future. The goal of scientific community is to find out the way of hydrogen production by which hydrogen can be generated in sufficient amount at a reasonable cost. The method of hydrogen production should be acceptable by the society and should be environmental friendly. Energy supply and environment protection are two crucial issues for the sustainable development of global prosperity. (Armor JN 1999) stated different, largely catalytic approaches for hydrogen production. Hydrogen was produced by catalytic process involving multiple steps with different types of catalysis. Over 80% of the energy consumed today in the world is derived from fossil fuels (D. Das and TN Veziroglu, 2001), which will eventually become depleted in the not too distant future. In addition, burning of fossil fuels contributes severely to the climate change, environmental deterioration, and the threatening of public health (D, Levin et al., 2004). For over two decades, environmental engineers have successfully commercialized anaerobic technology for the treatment of wastewater and solid wastes. In these processes, organic pollutants and wastes are converted into methane through a series of chain reactions by distinct groups of anaerobic microorganisms. Complex organics are first hydrolyzed and fermented into fatty acids, which are then further converted into acetate and hydrogen, both of which are lastly converted into methane. As compared to the aerobic waste/wastewater treatment processes, the methanogenic process offers several intrinsic advantages: (a) saving the energy that is otherwise needed for aeration, (b) lowering sludge yield, and (c) producing a readily useable fuel—methane. Over 2000 full scale methanogenic wastewater treatment systems have since been installed worldwide (H. H. P. Fang et al, 2001). Recently a new anaerobic process has been developed to convert organic pollutants into hydrogen, instead of methane. Various researchers are employing different physical, chemical and biological techniques to produce hydrogen. Out of these various methods, biohydrogen production is getting more attention as it is environmental friendly process. Hydrogen is favored over methane for two reasons. First, hydrogen has a wider range of industrial applications as compared to methane. It can be used for the syntheses of ammonia, alcohols, and aldehydes, as well as for the hydrogenation of edible oil, petroleum, coal, and shale oil, whereas methane is mostly used as fuel. Second, hydrogen is an ideal fuel,
producing only water upon combustion. Hydrogen gas has been deemed to be the fuel of the future, and it is believed that a hydrogen fuel based economy would be less polluted than a fossils fuel based economy. Hydrogen gas an energy carrier has been proved to be one of the best fuels for transportation, the most versatile, the most efficient and also one of the most and safest fuel. The combustion of hydrogen produces only water vapor without CO, CO$_2$, hydrocarbons or fine particles, and since it can be produced without causing any environmental problems, hydrogen as a future fuel has been drawing more and more attention (Veldez Vezquez et al 2005).

It can be used directly in the internal combustion engines, or used to produce electricity through fuel cells. Due to the greenhouse gas effect of carbon dioxide in order to circumvent the increase in the net carbon dioxide concentration in the atmosphere, hydrogen should be produced from sustainable resources such as biomass and sunlight, rather than fossil fuels. Biological hydrogen production from renewable resources offers an appropriate alternative. Many energy experts believe that hydrogen will replace fossil fuels as the next generation of energy (Hoffmann, 2001). Some even predict that a new economy empowered by hydrogen will fundamentally change the nature of our market and political and social institutions, just as coal did for the 19th century and petroleum for the 20th century. Furthermore, methane is a greenhouse gas with 21 times the heat-trapping effect of carbon dioxide. Although it constitutes only 0.00017% of the atmosphere, methane accounts for 0.47 W/m$^2$ of radiative forcing, which is about 19% of the total global greenhouse gas forcings today. Hydrogen is commercially produced by either electrolytic or thermo chemical process, both of which are energy intensive. Yet, in nature, hydrogen may be produced biologically by autotrophs as well as heterotrophs (Das and TN Veziroglu, 2001; Hallenbeck and Benemann, 2002). Autotrophs, such as algae, use carbon dioxide as a carbon source, whereas heterotrophs use organic matter as a carbon source. From an environmental engineering point of view, heterotrophs are of more concern because they can be used to degrade organic pollutants and thus clean up the environment. Heterotrophs produce hydrogen by either phototrophic or non phototrophic (often called “dark”) fermentation of organic matter; depending on whether light is the energy source: most studies have been related to non phototrophic fermentation. The scarcity of information related to phototrophic fermentation is due to two reasons: (a) It is difficult to control light penetration and its uniform distribution, and (b) the process is likely not cost-effective unless the free sunlight can be used as the light source.
However, environmental engineers are more interested in using mixed cultures for wastewater/waste treatment for practical reasons. A mixed culture system would be cheaper to operate, easier to control, and would have a broader choice of feedstock (Valdez-Vazquez et al., 2005). Biological hydrogen production processes are found to be more environmentally friendly and less energy intensive than thermochemical and electrochemical processes. Dark fermentation of biomass or carbohydrate-based substrates presents a promising route for biological hydrogen production compared to photosynthetic or chemical ones. Both mesophiles and thermophiles can produce hydrogen as a byproduct during anaerobic growth on carbohydrates. The superior production rates and the better profile of fermentation by-products seen with extreme thermophiles, however, make their application for hydrogen production economically and technically more interesting. The mixed culture that can be acclimatized at the thermophilic temperature range especially to enhance the growth of hydrogen producing bacteria still has various types of microbes other than hydrogen-producers. In order to screen these hydrogen-producing bacteria, the hydrogen consumers are suppressed by employing methods of load-shocking or heat shocking. The physiological differences in the two groups of microorganism form the basis of these two mechanisms for preparation of efficient hydrogen producing seeds. Spore-forming hydrogen-producing bacteria can form protective spores, with resistance to high temperature, and extreme acidity and alkalinity. Methanogens have no such capability, and can thus be eliminated by heat-treatment or extreme pH treatments. Similarly most methanogens are also limited to growth under organic load-shock condition. During organic load-shock, methanogenic substrates like formate, H₂, CO₂ and volatile fatty acids (VFAs) tend to accumulate. The accumulation of methanogenic substrate can inhibit of methanogens. We need to look into various factors including temperature, pH and other substrate and product (hydrogen itself) concentrations like acetic acid, butyric acid, propionic acid etc which effects the mixed culture growth on and the hydrogen production yield and net hydrogen production ability.

Biological hydrogen production is a viable alternative source; biohydrogen gas production from renewable source also known as “green technology” has received considerable attention in recent years. Biohydrogen production can be realized by anaerobic and photosynthetic microorganism using carbohydrate and non-toxic material. Under anaerobic condition, bacteria produced hydrogen as a byproduct during conversion of waste organic material into organic
acids which are then used for methane and hydrogen generation. Production of clean energy source and utilization of **Domestic solid waste** materials make biological hydrogen production a novel and promising approach to meet the increasing energy needs as a substitute for fossil fuels.

**LITERATURE CITED:**

Household solid waste includes domestic waste, bulky waste, and garden waste. Household wastes covers 20% of total waste generated: about 2.8 million ton household waste is generated per year (James and James 2002). The main treatment methods for household solid waste are incineration, composting, landfill, and anaerobic digestion. (Baere; De 2000). Anaerobic digestion is a treatment method that converts the waste in anaerobic reactor to biogas. Garden waste and 40% to 45% of domestic waste are organic materials and this is the reason which makes them good materials for anaerobic digestion. Due to the improvement in collection and separation system at household level, more and more household solid waste is treated in biogas plants. In general, waste disposal methods include: (1) transportation of waste to low lying areas/landfills, (2) burning of waste on site or in the incinerators, (3) composting, (4) briquetting, (5) recycling of waste matter, (6) microbial treatment, aerobic and anaerobic, etc. Each of these methods has its own advantages, and can be employed to certain types of wastes. It is important to take an integrated approach for complete disposal of wastes. Wastes and residues currently constitute a large source of biomass which includes solid and liquid municipal wastes, manure, lumber and pulp mill wastes, forest and agricultural residues etc. With the exception of feed stocks of low water content, most of this biomass cannot be directly utilized and must undergo some form of transformation, prior to being utilized as a fuel. Biological processes for the conversion of biomass to fuels include ethanol fermentation by yeast or bacteria, and methane production by microbial consortia under anaerobic conditions. Bacterial fermentation mechanisms for hydrogen production under either dark or light conditions is currently of importance in terms of environmental issues and the utilization of organic wastes such as waste effluent of the food and fermentation industries, pre-treated sewage sludge and market garbage.

Many agricultural and food industry wastes contain starch and/or cellulose which are rich in terms of carbohydrate contents. Complex nature of these wastes may adversely affect the
biodegradability. Starch containing solid wastes are easier to process for carbohydrate and hydrogen gas formation. Starch can be hydrolyzed to glucose and maltose by acid or enzymatic hydrolysis followed by conversion of carbohydrates to organic acids and then to hydrogen gas. Cellulose containing agricultural wastes requires further pre-treatment. Agricultural wastes should be ground and then delignified by mechanical or chemical means before fermentation. Cellulose and hemicellulose content of such wastes can be hydrolyzed to carbohydrates which are further processed for organic acid and hydrogen gas production. It was reported that there is an inverse relationship between lignin content and the efficiency of enzymatic hydrolysis of agricultural wastes.

Glucose, an easily biodegradable carbon source is present in most of the industrial effluents and can be obtained abundantly from agricultural wastes. Theoretically bioconversion of 1 mol of glucose yields 12 mol of hydrogen gas (H₂). According to reaction stoichiometry, bioconversion of 1 mol of glucose into acetate yields 4 mol H₂/mol glucose, but only 2 mol H₂/mol glucose is formed when butyrate is the end product.

\[
1. (a) C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2. \\
\text{Acetic acid}
\]

\[
(b) 2CH_3COOH + 4H_2O \rightarrow 8H_2 + 4CO_2.
\]

Hence 1 mol of Glucose produces 12 mol of H₂.

(1) C₆H₁₂O₆ → 2CH₃CH₂CH₂COOH + 2CO₂ + 2H₂

**Butyric acid**

The highest hydrogen yield obtained from glucose is around 2.0–2.4 mol/mol. Production of butyrate rather than acetate may be one of the reasons for deviations from the theoretical yield. The partial biodegradation of glucose could be another reason for lower yields (H.H.P. Fang et al 2002a). However, even when more than 95% glucose was degraded, the yield could be less than 1.7 mol H₂/mol glucose. Therefore, utilization of substrate as an energy source for bacterial growth is the main reason for obtaining the yields lower than theoretical estimations.

A considerable progress has been made in the last decade in the development of continuous processes for hydrogen production utilizing mixed mesophilic microflora and solid wastes but low yields associated with the process acts as a major deterrent. Biological processes are carried
out largely at ambient temperatures and pressures, and hence are less energy intensive than chemical or electrochemical ones. A large number of microbial species, including significantly different taxonomic and physiological types, can produce hydrogen. Biological processes use the enzyme hydrogenase or nitrogenase as hydrogen producing protein. This enzyme regulates the hydrogen-metabolism of uncountable prokaryotes and some eukaryotic organisms including green algae. The functioning of nitrogenase, as well as hydrogenase, is linked with the utilization of the products of photosynthetic reactions that generate reductant from water. Various researchers have been working on the production of biohydrogen by different substrate materials, like wastewater, potato waste etc.

*Kusum Lata et al (2002)* have investigated fermentation of the organic content from vegetable market waste and tea waste in a packed digester for 24 h and 300 h respectively. The sequence of appearance of volatile fatty acids during digestion of both the substrates was found to be different. The rates of production of acetic acid and propionic acid were found to be higher than other volatile acids during digestion of both the substrates, although it was approximately higher for vegetable market waste compared to tea waste.

**PURE CULTURE:**

Pure cultures known to produce hydrogen from carbohydrates include species of *Enterobacter* (*Kumar and Das; 2001*), *Bacillus* and *Clostridium*. The latter two groups are characterized by the formation of spores in response to unfavorable environmental conditions such as lack of nutrients or rising temperature. The highest hydrogen yields per mole of hexose have been found for *Clostridium* sp. i.e. 1.61–2.36 mol/mol glucose (*Hawkes et al.; 2002*).

*Habibollah Younesi et al. (2008)* have studied the bioconversion of synthesis gas (syngas) to hydrogen by continuous stirred tank bioreactor (CSTBR) utilizing acetate as a carbon source. Anaerobic photosynthetic bacterium, *Rhodospirillum rubrum* catalyzed water-gas shift reaction and gas flow rates were 5 to 14 ml/min, agitation speeds were in the range of 150–500 rpm and pH and temperature of the bioreactor was set at 6.5 and 30° C. The liquid flow rate was kept constant at 0.65 ml/min, hydrogen production rate and yield were 16 ± 1.1 m mol g⁻¹ cell h⁻¹ and 87 ± 2.4% at fixed agitation speed of 500 rpm and syngas flow rate of 14 ml/min, respectively.
has been also shown that *Rhodobacter Sphaeroids* O.U.OO1 was capable of using several carbon sources present in dark fermentation (*Basar Ugyar et al. 2009*). The bacteria consumed acetate first, followed by propionate and then butyrate. It was also found that consumption rate of the main substrate significantly increased when the minor substrates were depleted. Hydrogen yields from fermentative hydrogen production can be increased with heat treatment of the inoculums, dissolved gas removal and varying the organic loading rate. Hydrogen yields are not maximized after heat pretreatment as compared to bromoethanesulfonate, iodopropane, or perchloric acid. Gas sparging increases hydrogen yield compared to un-sparged reactors. The higher hydrogen yields during dissolved gas removal and changes in organic loading rate (OLR) helps in improving hydrogen yields (*Jeremy T. Kraemer, David M. Bagley 2007*).

*D. Levin et al. (2005)* have studied hydrogen production by *C. thermocellum* on various cellulosic sources, including alpha-cellulose, shredded filter-paper, and delignified wood fibers (DLC) and maximum yield of 1.95 moles $H_2$/mole glucose was obtained for delignified wood fibers under substrate-sufficient condition. Proportion of substrates converted into acetate was highest under limited substrates condition and shifted away by 50% under excess condition by the end of growth. In unstirred cultures, development of high hydrogen concentrations, which resulted in end-product inhibition, and decreasing pH was found responsible for this shift. They finally concluded that *C. thermocellum* exhibits significant potential for efficient conversion of cellulosic waste biomass to hydrogen. *Kaushik Nath et al. (2006)* have studied fermentative hydrogen production carried out by *Enterobacter cloacae* DM11. Using glucose as the substrate, effects of initial substrate concentration, initial medium pH, and temperature to produce biohydrogen has been studied. At an initial glucose concentration of 1.0% ($m/v$), the molar yield of hydrogen was 3.31 mol (mol glucose)$^{-1}$. At higher initial glucose concentrations, both the rate and cumulative volume of hydrogen production decreased. The pH of 6.5 ± 0.2 and a temperature of 37 °C was found most suitable with respect to maximum rate of production of hydrogen in batch fermentation. Effect of the addition of $Fe^{2+}$ on hydrogen production was studied. It had a marginal enhancing effect on total hydrogen production. The main factors are inoculums, substrates, reactor type, nitrogen, phosphate, metal ion, temperature and pH which influence fermentative hydrogen production (*Jianlong Wang and Wei Wan; 2009*).
Walid M. Alalayah et al. (2008) have investigated the effect of environmental factors, initial substrate concentration, initial pH and temperature on hydrogen production using *Clostridium sacchroperbutylacetonicum* N1-4 (ATCC 13564). Microbial consortium of facultative anaerobes *Enterobacter cloacae* IIT-BT 08, *Citrobacter freundii* IIT-BT L 139 and *Bacillus coagulans* IIT-BT S1 were used for hydrogen production. Hydrogen yield at the consortium ratio (1:1:1) was 41.23 ml H$_2$/g COD and microbial consortia from sewage sludge was an attempt to augment the hydrogen yield from sludge (Shireen Meher Kotay and Debabrata Das; 2006).

X.Wang et al., (2009) have given the biochemical kinetics of fermentative hydrogen production by newly isolated strain of *Clostridium butyricum* W5. The amount of hydrogen produced in batch process was 45.54 m mol/L at 10h and peak production rate was 7.61mmol/l/h. The optimum conditions for biological hydrogen production from food waste by *Clostridium butyricum* KCTC 1875 at the optimum initial pH and fermentation temperature were 7.0 and 40°C, respectively. When pH of the fermentation was controlled to 5.5, a maximum amount of hydrogen could be obtained. Under these conditions, about 2,737 mL of hydrogen was produced from 50 g COD/L of food waste for 24 h, and the hydrogen content in the biogas was 38%. Hydrogen production rate and yield were about 108 mL/L h and 128 mL/g COD degraded, respectively (Jung Kon Kim et al;2008)

**MIXED CULTURE:**

Various studies have been carried out to identify the microbial communities present in mixed cultures used for hydrogen production. The microbial species present in a hydrogen producing culture (CSTR, pH 5.5, 36°C, 6.6 h HRT, sewage sludge inoculum) with glucose was analyzed (H.H.P.Fang et al.; 2002 b). It was found that 64.4% of the clones present were *Clostridiaceae*, with 43.8% being most closely related to *Clostridium cellulosi*, 12.5% most closely related to *Clostridium acetobutylicum* and 8.3% most closely related to *Clostridium tyrobutyricum*. 18.8% of all clones were affiliated with *Enterobacteriaceae*, and 3.1% with *Streptococcus bovis*. A study of granular sludge (26°C, pH 5.5, 6 h HRT, and sucrose as substrate) showed 69% of the
clones associated with four *Clostridium* species and 13.5% with *Sporolactobacillus racemicus* in the *Bacillus/Staphylococcus* group.

*Hawkes et al* (2007) examined the hydrogen produced by the mixed and pure cultures. The heat treated sludge inoculum deactivated methanogens and contained hydrogen producing bacteria. They showed that heat treated aerobically digested sewage sludge in scale up reactor was feasible for hydrogen production by preventing methanogenic activity using short hydraulic retention time (HRT) (8-12h) and pH below 6.0 at 30°C. *I. Shihwu Sung et al.* (2003) investigated selective growth of hydrogen-producing bacteria (e.g. heat selection and pH control and hydraulic loading) in a mixed culture environment and enriched naturally available mixed seed (e.g. compost, soybean soils, anaerobic digester sludge, etc.) to microbial culture rich in hydrogen-producing bacteria. Hydrogen yield obtained was 2.2 mole/mole of sucrose by using mixed microbial culture with hydrogen conversion efficiency of 27.5% of seed inoculums was preheated 70-90°C for 15-20 minutes. *S. Venkata Mohan et al.* (2008 a) investigated influence of different pretreatment methods applied on anaerobic mixed inoculum for selectively enriching the hydrogen producing mixed culture using dairy wastewater as substrate. Different pretreatment methods: 2-bromoethane sulphonate sodium salt (0.2 g/l; 24 h), by integration of pH (pH 3; adjusted with ortho-phosphoric acid) and chemical pretreatment evidenced higher hydrogen production by anaerobic consortia. *C.Y.Lin and C.H.Lay* (2005) have shown that the production of fermentative biohydrogen by mixed micro flora was effected by the carbon/nitrogen ratio. When *Clostridium pasteurinum* was heated at 100°C HRT for 12h, the C/N ratio of 47, the hydrogen production (HP) rate was on peak with 4.8 mol-H₂/mol sucrose. Hydrogen production rate (HPR) and specific hydrogen production rate (SHPR) also increased. They also examined the VFA formation, and have concluded that C/N ratio is responsible for metabolic shift and gave the optimal biohydrogen production. *Chiu-You Lin et al* (2008) have examined the hydrogen production from starch using natural mixed culture. At the initial cultivation pH 6.5 they got maximum hydrogen and hydrogen production rate (HPR). At this pH, microbial growth was also high. They utilized the CSTR fermentor for enhancing the hydrogen yield (HY) by reducing hydraulic retention time (HRT). *Ching-Hsiung Wang et al.* (2007) have
reported that hydrogen was produced by anaerobic mixed microflora with phosphate-buffered medium containing starch or enzyme-treated starch hydrolyzate as the carbon substrate. The best condition (41°C, pH 5.2, 2.1% (v/v) enzyme dosage, 27 h reaction time) for starch hydrolysis with concentrated crude amylase was obtained using *Bacillus subtilis* ATCC 21332. Mixed culture was able to produce hydrogen at an optimal pH of 7.0. Direct starch fermentation attained a highest maximum H\(_2\) production rate (R\(_{\text{max}}\)), overall hydrogen production rate (overall), and hydrogen yield (Y\(_{\text{H}_2}\)) was 25.6 ml/h, 88 ml/h/l, and 5.28 mmol H\(_2\)/g starch (4.64 mmol H\(_2\)/g COD), respectively. The soluble metabolites consisted primarily acetate (HAc), ethanol (EtOH), butyrate (HBu), and 2, 3 butadiol.

*Wang Jian Long and Wan Wei (2008)* investigated effect of substrate concentration ranging from 0 to 300 g/L on fermentative hydrogen production by mixed cultures in batch tests using glucose as substrate at 35 and initial pH 7.0. During the fermentative hydrogen production, the maximal hydrogen production potential of 426.8 mL and maximal hydrogen production rate of 15.1 mL/h were obtained at the substrate concentration of 25 g/L.

**FEEDSTOCK:**

**Use of simple sugars**

Glucose and sucrose have primarily been used as a carbon sources in the studies where pure carbohydrates are used as feed.

Table 1. gives comparative yields and rates of bio-hydrogen production from pure carbohydrates by dark fermentations in batch mode (*Kapdan and Kargi, 2006*).

**Table 1. Hydrogen yield of different carbon sources using various types of bacteria:**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Carbon source</th>
<th>SHPR(^1)</th>
<th>VHPR(^2)</th>
<th>H(_2) yield</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. cloaca</em> IIT-BT 08</td>
<td>Glucose (1%)</td>
<td></td>
<td>447 mL/L h</td>
<td>2.2 mol/mol glucose</td>
</tr>
</tbody>
</table>
Batch and continuous hydrogen gas production from sucrose has been widely studied. *Enterobacter cloacae* ITT-BY 08 produced 6 mol H$_2$/mol sucrose which is the highest yield among the other tested carbon sources.

Yields and rates of bio-hydrogen production from pure carbohydrates by continuous dark fermentations are summarized in table 2.

**Table 2. Hydrogen yield of different reactors using mixed cultures:**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Carbon source</th>
<th>SHPR</th>
<th>VHPR</th>
<th>H$_2$ yield</th>
<th>Reactor (HRT)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Clostridia sp.</em></td>
<td>Glucose</td>
<td>14.2 mmol/g VSS h</td>
<td>359 mmol/Ld</td>
<td>1.7 mol/mol glucose</td>
<td>CSTR (6)</td>
</tr>
<tr>
<td>20g COD/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed culture</td>
<td>Glucose</td>
<td>191 mL/gVSS h</td>
<td>2.1 mol/mol</td>
<td></td>
<td>CSTR (6)</td>
</tr>
<tr>
<td></td>
<td>(7 g/L)</td>
<td></td>
<td>glucose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------------</td>
<td>---------</td>
<td>----------------------</td>
<td>------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Mixed culture</strong></td>
<td></td>
<td>Sucrose</td>
<td>105 mol/h</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(20g COD/L)</td>
<td>3.47 mol/mol sucrose</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CSTR (8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Mixed culture</strong></td>
<td></td>
<td>Sucrose</td>
<td>3.7 mmol/gVSS h</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(20 g COD/L)</td>
<td>470 mmol/Ld</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.6 mol/mol glucose</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SBR (4–12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Mixed culture</strong></td>
<td></td>
<td>Wheat starch</td>
<td>131 mL/Lh</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(10 g/L)</td>
<td>0.83 mol/mol starch d</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CSTR (12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Mixed culture</strong></td>
<td></td>
<td>Starch (6 kg starch/m³)</td>
<td>97.5 mL/gVSS h</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1497 L/m³d</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>1.29 L/g starch COD</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>CSTR (20)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

S.Jayalaxmi et al. (2007) have reported that hydrogen is being produced by the kitchen waste using heat treated anaerobically digested biogas plant slurry at various ambient mesophilic temperature from 70°C to 100°C. The rate of hydrogen production was 176.2 mL/kg TS/h. Agriculture wastes like coffee waste, guava waste, mango waste, litchi waste and papaya waste have the potential for fermentative hydrogen production and examined that maximum hydrogen yield and production rate was 1.06mmol H₂/g COD substrate and 0.51 mmol/H₂/l/l respectively (Yeong-Song Chuang et al. 2008). Biohydrogen has been produced from starch using mixed microflora at 35°C and at 5.5 pH with hydrogen yield of 1.1 mol-H₂/mol-hexose and hydrogen production rate (HPR) of 10.4 mmol-H₂/L/h (Chiu–Yue Lin et al, 2009).

Robbert Kleerbezem et al. (2009) have studied the conversion of xylose in anaerobic environments into volatile fatty acids and alcohols via fermentation by mixed microbial cultures. They observed that in case of xylose, a higher fraction of the carbon was converted into catabolic products (butyrate, acetate, and ethanol) and the biomass of xylose was approximately 20% lower than on glucose. This lower yield was likely related to the need of an extra ATP during xylose uptake. When submitted to a pulse of glucose, the population cultivated on xylose could instantaneously consume glucose.
Hallenbeck and Benemann (2002) have harnessed the endogenous metabolic machinery of algal cells to quantitatively convert stored carbohydrate (starch or glycogen in green algae and cyanobacteria, respectively) to hydrogen by a strictly dark reaction. Yongzhen Tao et al. (2007) used a two-step process to increase the hydrogen yield from dark-fermentation of sucrose. The maximum hydrogen production rate was >360 mLH₂/L h and the maximum hydrogen yield was 3.67 molH₂/mol sucrose. The total hydrogen yield from sucrose increased from the maximum of 3.67 molH₂/mol sucrose in dark-fermentation to 6.63 molH₂/mol sucrose, by using the two-step process. Sun- Kee Han et al.; (2004) studied hydrogen fermentation of food waste in a leaching-bed reactor by heat-shocked anaerobic sludge, and also investigated the effect of dilution rate (D) on the production of hydrogen and metabolites in hydrogen fermentation. It was feasible to produce hydrogen by anaerobic co-digestion of food waste (Kim et al.; 2004) and sewage sludge via anaerobic mesophilic and thermophilic acidogenesis (Shin et al.; 2004).

The conversion of food waste into hydrogen by thermophilic acidogenesis as a function of organic loading rate (OLR), hydraulic retention time (HRT) and pH in a CSTR has been studied by Hang-Sik Shin & Jong-Ho Youn (2005). Hydrogen production was increased as OLR increased up to 8 gVSL/1 d/1, but drastically decreased at 10 gVSL/1 d/1, hydrogen production was depended on concentration of total organic acids (TOA). Hydrogen-production microorganism was Thermoanaerobacterium thermosaccharolyticum that involved in acetate/butyrate fermentation. D.Zurawaski, et al.(2005) studied that biowaste digested sewage sludge produced hydrogen. Hydrogen was produced by the anaerobic degradation at thermophilic temperature 60⁰C in batch operation. They gave the heat pretreatment at 80⁰C, which increased the production of hydrogen. The highest hydrogen production was 221 m mol (H₂) g VSS by using biowaste corn on potato and produced hydrogen with yield of 60%, 50% respectively.

Food processing industry produces highly concentrated carbohydrate-rich wastewaters. Steven W.Van Ginkel et al. (2005) have used wastewaters obtained from four different food-processing industries that had chemical oxygen demands of 9 g/L (apple processing), 21 g/L (potato processing), and 0.6 and 20 g/L (confectioners A and B). Hydrogen gas production was generally in the range of 5–11%. Overall hydrogen gas conversions were 0.7–0.9 L-H₂/L-wastewater for
the apple wastewater, 0.1 L/L for confectioner-A, 0.4–2.0 L/L for confectioner B, and 2.1–2.8 L/L for the potato wastewater. When nutrients were added to samples, there was a good correlation between hydrogen production and COD removal, with an average of 0.10±0.01 L-H₂/g-COD. Gas produced by a domestic wastewater sample (concentrated 25×) contained only 23±8% hydrogen. Shiue-Lin Li et al., (2006) have reported that the biochemical potential with biomass taken from a 3m³ pilot scale of an maximum hydrogen production was 60 mL H₂/ 80 mL mixed liquid in the serum bottle, and the maximum hydrogen production rate was 3.12 mL H₂/ g-VSS. The kitchen waste loading rate was 10 kg COD/m³/day. A small amount of methane also produced due to hydrogen reutilizing methanogenesis and corn starch loading rate is also 10 kg COD/m³/day. It gives a hydrogen producing rate of 1L H₂/L/day. Only 50% of carbohydrate was degraded. Moon et al. (2004) have shown the production of hydrogen by ethanol-acetate fermentation at pH of 5.0 ± 0.2 and HRT of 3 days. The yield of hydrogen was 100-200 ml g Glu⁻¹ with a hydrogen content of 25-40%. This fluctuation in the hydrogen yield was attributed to the formation of methanogens.

Georgia Antonopoulos et al. (2008) have investigated the potential of hydrogen production and subsequent methane production from raw cheese whey at 35 °C. Fermentative hydrogen production process from raw cheese whey was conducted in a continuous-type stirred tank bioreactor, operated at low hydraulic retention time (HRT; 24 h). At this HRT, the hydrogen production rate was 7.53 L of H₂/day, while the yield of hydrogen produced was 0.041 m³ of H₂/kg of chemical oxygen demand (COD) added or 2.49 L of H₂/L of cheese whey by a periodic anaerobic baffled reactor (PABR), a baffled-type bioreactor. Methane production rates were 105.9 L of biogas/day and 75.6 L of CH₄/day respectively (Binfei Xie et al., 2008). Biohydrogen production by two-phase anaerobic process from potatoes using heat shock has been studied. Hydrogen yield increased from 200.4 ml/g-TVS to 217.5 ml/g-TVS and maximum specific hydrogen productivity rate (SHPR) also increased from 703.4 ml/g-VSSd to 800.5ml/g-VSSd. The energy efficiency increased from 20% to 60% by hydrogen production process. Yi, Wang et al. (2008) studied inhibition of acetate addition on hydrogen production from sucrose-rich synthetic wastewater by mixed anaerobic culture and showed that the added acetate had a significant influence on both substrate degradation and hydrogen production during the fermentation process.
Jingwei Ma et al. (2008) investigated mesophilic hydrogen production from food waste made by rice. Experiments were conducted in serum bottles to determine the optimal operating conditions to maximize hydrogen production and a mixed microbial culture was involved in the fermentation process with hydrogen, propionate, acetate, butyrate, and CO₂ as major products. At the optimal condition of pH 5.0 and the waste containing 10g COD/L food waste, the maximum hydrogen yield was 113mL/g COD. Heguang Zhu et al. (2008 a) studied the co-production of methane and hydrogen by two stage anaerobic digestion process from potato waste. They used continuous stirred tank reactor (CSTR) having different volumes of 11 and 51 at temperature 4°C. The maximum hydrogen produced was 270ml/H₂ and average was 119 ml/H₂. In this process 64% total COD was removed. Total energy from potato waste was 2.14 kwh/kg TS and hydrogen gas 2.74 kwh/kg TS. Coproduction of hydrogen and methane from potato waste by anaerobic digestion at pH of 5.5 and hydrogen production rate of 270 ml/h. (Heguang Zhu et al.2008 b)

Chiu-Yue Lin et al. (2006) examined that natural cultures using xylose produced fermentative biohydrogen which was affected by its initial cultivation pH. They used sewage sludge and heat treated it at 100°C for 45 min. It produced maximum hydrogen at 6.5 pH.

Chunmei Pan et al. (2009) studied treatment of wheat bran to produce hydrogen during the anaerobic degradation process with effects of the hydrogen-producing microbial sources, pretreatment condition, substrate concentration and inoculum concentration. The predominant hydrogen producing bacteria were identified as Clostridium sp. Conventional acid pretreatment of wheat bran was essential for adequate converting wheat bran into biohydrogen. Soluble saccharides in the pretreated wheat bran increased from 0.086 g/g total solid (TS) to 0.392 g/g-TS compared with the raw wheat bran. Maximum hydrogen yield of 128.2 mL/g total volatile solid (TVS) and hydrogen production rate of 2.50 mL/(g-TVS h) were obtained at an initial pH 5.0, 80 g/L of pretreated wheat bran.

Venkataramana Godhamshetty et al. (2009) attributed feasibility of biohydrogen production by dark fermentation at two temperatures (22°C and 37°C) in unbuffered batch reactors using heat treated compost as inocula and sucrose as substrate. Hydrogen gas production was quantified by two different pressure release methods-intermittent pressure release (IPR) and continuous
pressure release (CPR). High yield of hydrogen was 4.3 mol of hydrogen /mol of sucrose obtained under IPR condition at 22⁰C in unbuffered reactor. Biohydrogen was produced with ground wheat solution by dark fermentation using heat treated anaerobic sludge in a completely mixed fermentor operating in fed-batch mode. Feed wheat powder concentration was varied between 10 and 30 g L⁻¹. Maximum hydrogen production, hydrogen yield was 465 mL H₂ starch or 3.1 mol H₂ mol⁻¹ glucose and hydrogen production rate was 864 mL H₂ d⁻¹ of fed-batch operation with 20 g L⁻¹ feed concentration. VOLATILE fatty acids were produced in higher concentrations. (Fikret Kargi and M. Yunus Pamukoglu, 2009)

Yao Ting et al. (2006) have reported for the first time the efficient conversion of beer lees wastes into biohydrogen gas by microorganisms. Batch tests were carried out to analyze influences of several environmental factors on yield of hydrogen from beer lees wastes. The maximum yield of hydrogen was 68.6 ml H₂/g TVS. Hidayet et al. (2009) studied the maximum cumulative hydrogen formation via combined dark and light fermentation of ground wheat solution with optimum dark to light biomass ratio of 1/7. Yohei Akutsu et al.(2009a) have investigated the effect of heat –pretreated inocula on the fermentative hydrogen production of various type of substrates like starch, glycerol, oil and peptones. Significant amount of hydrogen production was observed from starch (20.5-174.4 ml H₂/g-COD starch) and glycerol (11.5-38.1 ml H₂/g -COD glycerol). Yohie Akatsu et al; (2009b) have investigated the effect of hydraulic retention time(HRT),pH and substrate concentration on the thermophilic hydrogen production of starch with an upflow anaerobic sludge bed reactor.

Use of waste materials:

Hydrogen production from wastes containing soluble and insoluble starch, cellulose, food industry wastes and wastewater has been demonstrated in CSTR experiments using mesophilic mixed microflora.

Many agricultural and food industry wastes contain starch and/or cellulose which are rich in terms of carbohydrate contents. Complex nature of these wastes may adversely affect the
biodegradability. Starch containing solid wastes is easier to process for carbohydrate and hydrogen gas formation. Starch can be hydrolyzed to glucose and maltose by acid or enzymatic hydrolysis followed by conversion of carbohydrates to organic acids and then to hydrogen gas. Cellulose containing agricultural wastes requires further pre-treatment. Agricultural wastes should be ground and then delignified by mechanical or chemical means before fermentation. Cellulose and hemicellulose content of such wastes can be hydrolyzed to carbohydrates which are further processed for organic acid and hydrogen gas production.

Yields and rates of bio-hydrogen production from different waste materials by dark fermentation are given in table 2.3. (Kapdan and Kargi, 2006)

Table 2.3: YIELD COEFFICIENT FOR DIFFERENT CARBON SOURCES AND ORGANISMS

<table>
<thead>
<tr>
<th>Organism</th>
<th>Carbon source</th>
<th>SHPR</th>
<th>VHPR</th>
<th>$Y_{PS}$ yield coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesophilic mixed culture</td>
<td>Food waste (3% VS)</td>
<td>0.7 mL/g VSS h</td>
<td></td>
<td>0.05 mol/mol hexose</td>
</tr>
<tr>
<td>E. aerogenes</td>
<td>Molasses (2% sucrose)</td>
<td>36 mmol/L culture h</td>
<td>138 mL/L h</td>
<td>1.5 mol/mol sucrose</td>
</tr>
<tr>
<td>Mixed culture</td>
<td>Rice winery WW (36 g COD/L)</td>
<td>389 mL/g VSS h</td>
<td>159 mL/L h</td>
<td>2.14 mol/mol hexose</td>
</tr>
<tr>
<td>Mixed culture</td>
<td>Biosolid</td>
<td></td>
<td></td>
<td>1.2 mg/g COD</td>
</tr>
<tr>
<td>Mixed culture</td>
<td>Filtrate</td>
<td></td>
<td></td>
<td>15 mg/g COD</td>
</tr>
<tr>
<td>C. butyricu+ E. aerogenes</td>
<td>Sweet potato starch residue (0.5%)</td>
<td></td>
<td></td>
<td>2.4 mol/mol glucose</td>
</tr>
</tbody>
</table>
**Mixed culture** | Domestic WW | 0.01 L/L WW  
--- | --- | ---  
**Mixed culture** | Potato Ind. WW (21 g COD/L) | 2.8 L/L WW

*Gustavo Davila et al (2008)* emphasized that biologically produced hydrogen is a valuable gas, since its utilization via combustion or fuel cells produces pure water. Heterotrophic fermentations for biohydrogen production are driven by a wide variety of microorganisms such as strict anaerobes, facultative anaerobes and aerobes kept under anoxic conditions. Substrates such as simple sugars, starch, cellulose, as well as diverse organic waste materials can be used for biohydrogen production. Various bioreactor types have been used and operated under batch and continuous conditions; substantial increases in hydrogen yields have been achieved through optimum design of the bioreactor and fermentation condition. *Kapdan and Kargi (2006)* have reported significant advantages of biological hydrogen production over chemical methods. Major biological processes utilized for hydrogen gas production are bio-photolysis of water by algae, dark and photo-fermentation of organic materials, usually carbohydrates by bacteria. Carbohydrate rich, nitrogen deficient solid wastes such as cellulose and starch containing agricultural and food industry wastes and some food industry wastewaters such as cheese whey, olive mill and baker’s yeast industry wastewaters can be used for hydrogen production by using suitable bio-process technologies. *D. Das and T.N. Veziroglu, (2001)* have categorised hydrogen as the fuel of the future mainly due to its high conversion efficiency, recyclability and nonpolluting nature, mostly controlled by either photosynthetic or fermentative organisms. Genetic manipulation of cyanobacteria (hydrogenase negative gene) improves the hydrogen generation. About 28% of energy can be recovered in the form of hydrogen using sucrose as substrate. Fermentative hydrogen production processes have an edge over the other biological processes. *K.L.Kovacs et al. (2000)* have investigated that is the fundamental and principal difficulty of the future energy supply was the formation of fossils fuel being much slower than the rate of their exploitation. Therefore the reserves which can be recovered in an energetically feasible manner are shrinking parallel with an increasing world-wide energy demand. Among the
alternative energy carriers, hydrogen was preferred because it was easy to transport and store and it burns to environmentally friendly water vapors when utilized.

*S. Venkat et al. (2007)* investigated biohydrogen production from dairy wastewater in conjunction with wastewater treatment by anaerobic mixed inoculums. *Peilin Yang et al. (2007)* have reported hydrogen production from simulated cheese processing wastewater via anaerobic sequencing fermentation using mixed microbial communities under mesophilic condition. Hydrogen yield of 8 and 10 mM/g COD fed were achieved at food to microorganism (F/M) ratios of 1.0 and 1.5 respectively in batch experiments. pH of the bioreactor was in a range of 4.0–5.0 by addition of carbonate in the feed material. Maximum hydrogen yields were between 1.8 and 2.3 mM/gCOD fed for the loading rates (LRs) tested with a hydraulic retention time (HRT) of 24 h. Growth kinetics of hydrogen producing bacteria using three different substrates, namely sucrose, non-fat dry milk (NFDM), and food waste in dark fermentation and hydrogen production potential has been studied by *Wen-Hsing Chen et al. (2006)* Hydrogen production rate increased with an increasing substrate concentration Maximum hydrogen yields from sucrose, NFDM, and food waste were 234, 119, and 101 mL/g COD, respectively. Low pH (pH < 4) inhibited hydrogen production and resulted in lower carbohydrate fermentation at high substrate concentration. Three strains of *Lactobadllus* (L. helveticas ATCC 15009 and CRL 581, and *L casei* LC 3) were paired with three strains of *Propionibacterium* (P. freudenreichii AP8, P. freudenreichii GP6 and P. acidipropionici CRL 756) and grown in individual and mixed cultures in a complex medium. *(Perez et al.1995)* Bacterial growth, carbohydrate consumption, and production of acids, was determined and compared after mono and binary culture to produce hydrogen.

*S. Venkata Mohan et al (2008b)* investigated biohydrogen production from simultaneous wastewater treatment by anaerobic sequencing batch biofilm reactor (AnSBBR) using distillery wastewater as substrate at two operating pH values. Enriched anaerobic mixed consortia, sequentially pretreated with repeated heat-shock (100°C; 2 h) and acid (pH 3, 24 h) methods, was used as parent inoculums to startup the bioreactor at ambient temperatures (28 ± 2°C) with detention time of 24 h in periodic discontinuous batch mode. Batch experiments were conducted
to convert starch in wastewater into hydrogen at 55.8°C at various wastewater pH (4.0–9.0) and starch concentrations (9.2–36.6 g/l).

*Tong Zhang et al. (2003)* reported the maximum hydrogen yield of 92 ml/g at wastewater pH 6.0 and the maximum specific hydrogen production rate of 365 ml/g-VSS/d at wastewater pH 7.0. The best performance in terms of H₂ yield (0.23 L H₂/g carbohydrate) and H₂ production rate (2.54 m mol H₂/L reactor, h) was obtained at HRT 37.6 h and 20.5 h, respectively in UFBR (*Marcello Camilli and Paola M. Pedroni* 2005).

*Dae-Yeol Cheong and Conly L.Hansen* (2007) have treated biological sludge from an animal wastewater treatment plant to enrich hydrogen-producing mixed bacteria and effects on hydrogen yield were investigated during anaerobic fermentation at 55°C. Enrichment of hydrogen-producing bacteria was conducted at pH adjustment of inocula to 3 and 5 with and without additional heat treatment (NHT and HT). An enriched mixed bacteria were cultivated at initial pHs of 5, 6, and 7 with synthetic organic wastewater containing different levels of nitrogen (2.0 and 0.8 g/l as total nitrogen) under static batch conditions. The dominating intermediate metabolites were acetate, n-butyrate, and ethanol. Yields of produced hydrogen were significantly dependent upon levels of n-butyrate. Production of fermentative biohydrogen using the preheated anaerobic sewage sludge micro flora at 100°C for 45 min, which inhibited the methane productivity has been reported by *C.Y.Lin and C.H.Lay* (2004) They got 3.43mol H₂ (mol sec⁻¹) using acidogenic mixture formulations.

*Chiu-Yeu-Lin and Rang-Chong Chang* (2004) explored the fermentative hydrogen production at the ambient temperature (15-34°C) without pH control using glucose as the substrate. They used a chemostat type anaerobic reactor. The experimental results show that without temperature control the anaerobic sewage sludge micro flora could be acclimated to produce hydrogen. Performance characteristics of the hydrogen-producing fermentor were stable.

*Dawei Liu et al.* (2008) investigated hydrogen production from household solid waste (HSW) via dark fermentation by using an extreme-thermophilic mixed culture. The highest hydrogen production yield was 257 ± 25 mL/gVS added, at the optimum pH of 7.0. The hydrogen yield was 36 ± 25 mL/gVS added, which was almost seven times lower than the yield of 254 ±13
mL/gVS added, which was achieved at lower acetate concentration (5–25 mM). Thermophilic micro flora was seeded into an anaerobic sequencing batch reactor for hydrogen production from palm oil mill effluent supplemented with nitrogen, phosphorus and iron sources for biostimulants and hydrogen production yield from 1.6±0.1 to 2.24±0.03 mol H₂ mol⁻¹ hexose and hydrogen production rate from 4.4±0.38 to 6.1±0.03 lH₂⁻¹. Nutrient supplementation strategy increased the bacterial diversity in the reactor and promoted in particular the growth of hydrogen-producing bacteria, e.g. *Thermoanaerobacterium thermosaccharolyticum*, as assessed by denaturing gradient gel electrophoresis (*Sompong O-Thong et al. 2007*).

*Dawei Liu et al. (2006)* investigated biohydrogen production from household solid waste. Yield of 43mL H₂/g volatile solid (VS) at optimum pH 5-5.5 but below pH 5 there was a significant decreases in hydrogen production. *Shu-Yii Wu et al. (2005)* have used ethylene-vinyl acetate (EVA) copolymer to immobilize acclimated sewage sludge for hydrogen production under anaerobic conditions. Using sucrose as the sole carbon substrate, the resulting immobilized cells achieved an optimal H₂ production rate (vH₂) of 488 mlH₂/gVSS and the best substrate-based yield (YH₂/sucrose) of 1.74 molH₂/mol sucrose. Operation at a temperature of 40 °C resulted in the most efficient H₂ production. They showed that a Monod type model is able to describe the dependence of specific hydrogen production rate on sucrose concentration. Sewage sludge was acclimated to establish hydrogen producing enrichment cultures for converting sucrose (20 g COD/l) into hydrogen in an upflow anaerobic sludge blanket (UASB) reactor and operating hydraulic retention times (HRTs) were 24 to 4 h. Hydrogen productivity was HRT dependent and nearly constant at the HRT of 8 to 20 h. However, it drastically decreased at an HRT of 4 or 24 h. Butyrate and acetate were the main fermentation volatile fatty acids. (*Feng-Yung Chang and Chiu-Yue Lin 2004*).

*C.C. Wang et al.(2003)* have proved that waste biosolids collected from sewage works, containing a vast amount of polysaccharides and proteins, prohibits effective bio-hydrogen production. This is in contrary to the common assumption, that the solids phase in waste activated biosolids presents extra nutrients for anaerobes. Using filtrate after removal of solids from biosolids produces more hydrogen than using the whole biosolid. Long-term operation for biohydrogen production with an efficient carrier-induced granular sludge bed (CIGSB)
bioreactor had encountered problems with poor biomass retention at a low hydraulic retention (HRT) as well as poor mass-transfer efficiency at a high HRT or under a prolonged operation period and calcium ion was found to enhance mechanical strength of the granular sludge (Kuo-Shing Lee et al., 2004). Addition of 5.4–27.2 mg/l of Ca\(^{2+}\) increases \(\text{H}_2\) production rate (up to 5.1 l \(\text{H}_2/\text{h}/\text{l}\)) and liquid reflux (LR) strategy enhanced the \(\text{H}_2\) production rate by 2.2-fold at an optimal liquid upflow velocity of 1.09 m/h, which also gave a maximal biomass concentration of ca. 22 g VSS/l.98. Olive mill wastewater (OMW) containing 36.02 g carbon, 5.26 g hydrogen, and 0.96 g nitrogen in 100 g suspended solid was used as a sole substrate for the production of hydrogen gas by \textit{Rhodobacter sphaeroides} O.U.001 in 400 ml glass, column-photobioreactors (Ela Eroglu et al., 2004). A maximum hydrogen production potential (HPP) of 13.9 l\(\text{H}_2/\text{l}\) OMW was obtained at 2% OMW During the biological hydrogen production process, chemical oxygen demand (COD) of the diluted wastewater decreased from 1100 to 720 mg/l; biochemical oxygen demand (BOD) decreased from 475 to 200 mg/l, and the total recoverable phenol content (ortho- and meta-substitutions) decreased from 2.32 to 0.93 mg/l.

\textit{Dong–Yeol Lee et al. (2009)} investigated effect of FeSO\(_4\) on continuous hydrogen production in a membrane bioreactor using anaerobic mixed microflora under mesophilic condition. \(\text{H}_2\) production of 41.6l/day was obtained at 10.9 mg FeSO\(_4\)/l, which was 1.59 times higher than that at 2.7 mg FeSO\(_4\)/l. At constant concentration of FeSO\(_4\), an increase in butyric acid together with decreases in lactic acid promoted a reduction of the number of protons and release of hydrogen. The effects of inorganic ions (Cl\(^-\), K\(^+\), Na\(^+\) and Mg\(^{2+}\)) and glucose concentrations on hydrogen production using sewage sludge microbes in the batch experiment was studied by the heat mixed microorganisms at pH 5.5 and 35\(^\circ\)C. Cumulative hydrogen production increased with time for each ion and concentration to a maximum after 5-6 days. Cumulative hydrogen production for a given time was highest in the absence of each ion except for Na\(^+\). Glucose concentration affects hydrogen and volatile fatty acid (VFA) production and optimal loading concentration of glucose is about 20 g/l for maximum hydrogen production (J. Wongtanet and B. Prapagde, 2008).

\textit{Moon et al. (2004)} have reported hydrogen production by ethanol–acetate fermentation at pH of 5.0 ± 0.2 and HRT of 3 days. The yield of hydrogen was 100–200 ml g\(^{-1}\) Glucose with a hydrogen content of 25–40%. This fluctuation in the hydrogen yield was attributed to the
formation of methanogens. **Gustavo Davilla-Vazquez et al. (2009)** have shown an enhancement of volumetric hydrogen production late by increasing organic loading rate using cheese whey and mild microbial community. **Siyi Luo et al. (2009)** have shown that organic municipal solid waste (MSW) was effectively converted to heat and fuel gas by lab-scale shredder.

**Xian-Jun Zheng and Han-Qing Yu (2005)** have attributed the inhibitory effects of butyrate addition on hydrogen production from glucose by using anaerobic mixed cultures and results showed that addition of butyrate at 4.18 and 6.27 g/l slightly inhibited hydrogen production, and addition of butyrate at 8.36–12.54 g/l imposed a moderate inhibitory effect on hydrogen production. **Nanqi Ren et al., (2006)** studied a pilot-scale of biohydrogen production in a continuous flow anaerobic fermentation reactor (with an available volume of 1.48 m³) for over 200 days. The hydrogen bio-producing reactor (HBR) system was operated under the organic loading rates (OLR) of 3.11–85.57 kg COD/m³ reactor/d (COD: chemical oxygen demand) with molasses as the substrate. The hydrogen yield reached 26.13 mol/kgCOD removed within OLR range of 35–55 kg COD/m³ reactor/d. Hydrogen yield was affected by the presence of ethanol and acetate in the liquid phase.

**M. Krupp and R. Widmann (2009)** studied biological hydrogen production via the fermentation of biogenous wastes by hydrogen producing bacteria in 30-L working volume reactors. pH and other parameters were observed to find boundary conditions for a stable continuous process with a minimum of online control measurements. Heat treated sludge of a wastewater treatment plant was used as inoculum. The highest achieve hydrogen yield were found at an optimum of 14-15 h retention time. The increase of an organic loading rate effected the hydrogen yield only slightly, hence for this reactor configuration with OLR upto 14 kg VS/m³d and optimal retention time was around 15 h.

**J.A. Menéndez et, al. (2009)** describes a new method for producing hydrogen-rich gases, chiefly syngas (H₂ + CO), from different biomass sources, including biosolids and biogas. The method is based in a microwave-assisted thermal treatment at high temperature and the objective is the energetic valorization of the biomass residues. In the case of using biosolids as feedstock, the treatment consists of the pyrolysis of the residue and the auto-gasification of the resulting char with the steam and CO₂ released during the process itself. This microwave-assisted method
minimizes the production of oils and solids and maximizes the production of syngas, up to 80 volume %. In the case of biogas, the microwave-assisted thermal treatment favours that the reforming of CH$_4$ with CO$_2$ (i.e. dry reforming) takes place.
2. PROPOSED OBJECTIVE:

The project aims at developing a bioprocess technology for the production of hydrogen via anaerobic fermentation of mixed microbial culture obtained from natural sources utilizing domestic solid waste as feed. The major steps to achieve this could be enumerated as follows:

- Selection of a suitable natural source for obtaining mixed microflora.
- Development of an enrichment methodology to enhance the growth of hydrogen producers and suppress the growth of hydrogen consumers i.e. methanogens in the mixed culture.
- Optimization of process parameters under batch culture conditions and establishment of an enriched culture producing hydrogen, which can be used as a source of inoculum.
- Optimization of process pH and hydraulic retention time (HRT) for continuous operation to maximize the yield of hydrogen obtained.
- Development of yield enhancement strategies to attain high hydrogen yields.
3. PROPOSED METHODOLOGY:

3.1 SAMPLING:

Domestic waste will be collected from the nearby area. The collected sample will be segregated into biodegradable and non-biodegradable portion. The total sample will be weighed and separate weights of the biodegradable and non-biodegradable portions would be reported as their respective weight percent.

3.2 CULTURE MEDIA:

Initial culture media would contain: 10 gm Glucose, 84 mg MgCl$_2$.7H$_2$O, 136 mg KH$_2$PO$_4$, 236 mg K$_2$HPO$_4$, 340 mg Yeast extract, 840 mg NH$_4$Cl in 1000 ml reactor volume (Lin C.Y. and Lay C.H. 2005) and neutral pH. This medium would be inoculated with mixed culture using cow dung as the parent source. Methane inhibition would be tried. After few repeated inoculation the carbon source would be replaced by biodegradable portion of DSW (Domestic Solid Waste).

3.3 ANALYSIS OF UNTREATED AND TREATED WASTE:

The various physical and chemical parameters will be analyzed before and after the treatment of domestic waste taken for the study.

3.3.1 PHYSICAL PARAMETERS:

3.3.1a pH

pH will determine using pH meter.

3.3.1b PROXIMATE ANALYSIS

In proximate analysis parameters analyzed would be –TS (Total Solid), VSS (Volatile suspended solid), TDS (Total dissolved solid), TSS (Total Suspended Solid). Standard methods would be formed for analysis of these parameter.
3.3.1 ULTIMATE ANALYSIS

In ultimate analysis the waste samples would be analyzed for elemental composition- C (Carbon), N (nitrogen), H (Hydrogen) using an elemental analyzer.

3.3.2 CHEMICAL PARAMETERS:

All chemical parameters would be analyzed as according to the standard methods. Some of the parameters to be analyzed are given in Table 3.1 standard methods.

TABLE 3.1

<table>
<thead>
<tr>
<th>TEST</th>
<th>METHOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar</td>
<td>DNS Method</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>Anthrone sulphuric acid test</td>
</tr>
<tr>
<td>Lipids</td>
<td>Weight percent of total lipids</td>
</tr>
<tr>
<td>Starch</td>
<td>Weight percent starch content</td>
</tr>
<tr>
<td>COD</td>
<td>Open reflux method</td>
</tr>
<tr>
<td>VFA conc.</td>
<td>Gas Chromatography(FID)</td>
</tr>
<tr>
<td>Gas Composition</td>
<td>Gas Chromatography(TCD)</td>
</tr>
</tbody>
</table>
REFERENCES:


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