

# Chapter 2

## 2.0 LITERATURE REVIEW

### 2.1 BIOFUELS

Biofuels can be defined as solid, liquid or gas fuel derived from biological material. Biofuels are considered as an alternative to the depleting fossil fuels and also advantageous to reduce the green house gas emissions. Timothy *et al.*, (2008) reported that substituting of biofuels for gasoline reduced greenhouse gases because biofuels sequestered carbon through the growth of the feedstock. These analyses have failed to count the carbon emissions that occur as farmers worldwide respond to higher prices and convert forest and grassland to new cropland to replace the grain (or cropland) diverted to biofuels. By using a worldwide agricultural model to estimate emissions from land-use change, we found that corn-based ethanol, instead of producing a 20% savings, nearly doubles greenhouse emissions over 30 years and increases greenhouse gases for 167 years. Biofuels from switchgrass, if grown on U.S. corn lands, increase emissions by 50%. This result raises concerns about large biofuel mandates and highlights the value of using waste products. The term biofuel usually encompass the range of fuels derived from biomass including biodiesel, bioethanol, biohydrogen or biogas. Ethanol (ethyl alcohol,  $C_2H_5OH$ ) is an alcohol made by fermenting and distilling simple sugars. Ethanol is produced by the fermentation of monosaccharides such as glucose and fructose. The monosaccharides are typically formed through the hydrolysis of any number of biomass options. Ethanol can be manufactured from biomass via the fermentation of sugar derived from grain starches of many crops including wheat wastes and sugar, biomass via the utilisation of the lignocellulosic fraction of crops or petroleum and natural gas. Ethanol is used in the manufacture of alcoholic beverages and it is denatured (made unfit for human consumption) when used for fuel and a variety of other manufactures

such as methylated spirits. The biomass source for ethanol production varies depending primarily on local economics and resources. This can be considered an advantage in that regional development of fuel production facilities can optimize for the economics and resources of their own particular surroundings. The two largest producers of Ethanol, Brazil and the United States use biomass sources that are high in carbohydrate content. Other cellulose feed sources are possible as well, such as grass or woodchips.

## **2.2 ETHANOL**

Fuel ethanol is generally blended in gasoline to reduce emissions, increase octane, and extend gasoline stocks. Recent high oil and gasoline prices have led to increased interest in alternatives to petroleum fuels for transportation. Further, concerns over climate change have raised interesting developing fuels with lower fuel-cycle greenhouse-gas emissions. Ethanol is substitutes for transportation fuel when oil crisis arise. It considered as one of alternative way to overcome this problem. Fuel ethanol still taking place at a research institute but in Brazil it already produced on a large scale for the market. From research ethanol performs well as a fuel in cars it is also friendly to environment. Another reason to be mentioned the world demand for fuel in this era is predicted to be more than the capacity of global oil production (Sanchez, 2008). Ethanol from renewable resources has been of interest in recent decades as an alternative fuel or oxygenate additive to the current fossil fuels. Ethanol made from cellulosic biomass is called bioethanol. In the coming years it is believed that cellulosic biomass will be the largest source of bioethanol. A major challenge is developing biocatalysts capable of fermenting lignocellulosic biomass for efficient industrial application. Lignocellulosic materials are cheap renewable resources, available in large quantities (Sanchez, 2008). Rice straw is one of the abundant lignocellulosic waste materials in the world. Cellulose, the major fraction of lignocellulosic

biomass, can be hydrolyzed to glucose by cellulase enzymes. This hydrolysis can be affected by porosity of lignocellulosic biomass, cellulose fiber crystallinity and lignin and hemicellulose content. A pretreatment process is essential in order to remove lignin and hemicellulose, reduce cellulose crystallinity and increase the porosity of the materials. The broad category of biomass for the production of ethanol includes agricultural crops and residues and wood. Biomass resources are abundant and have multiple application potential. Among the various competing processes, bioethanol from lignocellulosic biomass appears to have near-term economic potential. The crops residues such sugarcane bagasse where has are high content of sugar also easy to get in our place will be used as our raw materials, it also used to derive desired economic and environmental benefits and thus they could be important resource bases for bioethanol production (Sanchez, 2008)

### **2.3 Ethanol from lignocelluloses biomass**

It is evident the importance of lignocelluloses biomass as a feedstock for ethanol production. Lignocelluloses complex is the most abundant biopolymer in the Earth. It is considered that lignocelluloses biomass comprises about 50% of world biomass and its annual production was estimated in 10–50 billion ton Claassen *et al.*, 1999). In general, prospective lignocellulosic materials for fuel ethanol- production can be divided into six main groups: crop residues (cane bagasse, corn stover, wheat straw, rice straw, rice hulls, barley straw, sweet sorghum bagasse, olive stones and pulp), hardwood (aspen, poplar), softwood (pine, spruce), cellulose wastes (newsprint, waste office paper, recycled paper sludge), herbaceous biomass (alfalfa hay, switchgrass, reed canary grass, coastal bermudagrass, thimothy grass), and municipal solid wastes (MSW). The composition of most of these materials can be found elsewhere (Sun and Cheng, 2002). Numerous studies for developing large-scale production of ethanol from

lignocellulosics have been carried out in the world. However, the main limiting factor is the higher degree of complexity inherent to the processing of this feedstock. This is related to the nature and composition of lignocellulosic biomass. Two of the main polymers of the biomass should be broken down into fermentable sugars in order to be converted into ethanol or other valuable products. But this degradation process is complicated, energy-consuming and non-completely developed.

#### **2.4 Pretreatment of lignocelluloses biomass**

The main processing challenge in the ethanol production from lignocellulosic biomass is the feedstock pretreatment. The lignocellulosic complex is made up of a matrix of cellulose and lignin bound by hemicellulose chains. During the pretreatment, this matrix should be broken in order to reduce the crystallinity degree of the cellulose and increase the fraction of amorphous cellulose, the most suitable form for enzymatic attack. Additionally, main part of hemicellulose should be hydrolyzed and lignin should be released or even degraded. The fact that the cellulose hydrolysis is affected by the porosity (accessible surface area) of lignocellulosic materials should be also considered. The yield of cellulose hydrolysis is less than 20% of the theoretical when pretreatment is not carried out, whereas the yield after pretreatment often exceeds 90% of theoretical (Lynd *et al.*, 1996). Therefore, the aim of the pretreatment is the removal of lignin and hemicellulose, the reduction of crystalline cellulose and the increase in the porosity of the materials. Additionally, the pretreatment should improve the formation of sugars or the ability to form them during the succeeding enzymatic hydrolysis, and avoid the formation of inhibitors for subsequent hydrolysis and fermentation processes. For the pretreatment of lignocellulosics, several physical, physical–chemical, chemical and biological processes have been proposed and

developed (Sun and Cheng, 2002). The main pretreatment methods reported in the literature are shown in Table 5.

#### **2.4.1 Physical methods**

Waste materials can be comminuted by a combination of chipping, grinding and milling to reduce cellulose crystallinity. This reduction facilitates the access of cellulase to the biomass surface increasing the conversion of cellulose. The energy requirements of mechanical comminution of lignocellulosic materials depend on the final particle size and biomass characteristics. Although mechanical pretreatment methods increase cellulose reactivity towards enzymatic hydrolysis, they are unattractive due to their high energy and capital costs (Ghosh and Ghose, 2003). Pyrolysis has also been tested as a physical method for pretreatment of lignocellulosic biomass since cellulose rapidly decomposes when is treated at high temperatures.

#### **2.4.2 Physico–chemical methods**

Physico–chemical pretreatment methods are considerably more effective than physical. The steam explosion is the most studied method of this type. During this process, the use of saturated steam at high pressure causes autohydrolysis reactions in which part of the hemicellulose and lignin are converted into soluble oligomers. The factors affecting steam explosion pretreatment are residence time, temperature, chip size and moisture content. To consider the combined action of both temperature and time over the performance of steam explosion pretreatment, the so-called severity index has been defined including a correction term when this process is carried out under acidic conditions (Shahbazi *et al.*, 2005, Söderström *et al.*, 2003). In some cases (e.g. herbaceous waste), the use of very small particles is not desirable considering the economy of the process (Ballesteros *et al.*, 2002). This method is recognized as one of the most cost-effective for hardwood (poplar, oak, birch, maple) and agricultural residues, but is less efficient for softwood

(pine, cedar). Shahbazi *et al.*, (2005) proposed a fractionation procedure for softwood based on steam explosion and alkaline delignification in order to produce ethanol and related co-products. Söderström *et al.*, (2003) mentioned a two-step steam pretreatment of softwood by dilute-acid impregnation that includes a partial hydrolysis of cellulose during the second step. According to these authors, this variant of pretreatment is a promising method for increasing the overall yield during ethanol production.

One of the most promising methods is the pretreatment with Liquid Hot Water (LHW) or thermohydrolysis. Laser *et al.*, (2002) mention that under optimal conditions, this method is comparable to dilute acid pretreatment but without addition of acids or production of neutralization wastes. In addition, this technology presents elevated recovery rates of pentoses and does not generate inhibitors (Ogier *et al.*, 1999). Nevertheless, solid load is much less than for steam explosion, which is usually greater than 50%. Negro *et al.*, (2003) compared steam explosion and LHW pretreatments for poplar biomass and showed best results for the latter at 210 °C during 4 min. Other physical–chemical method is the Ammonia Fiber Explosion (AFEX) process whose fundament is similar to steam explosion. As AFEX method and steam explosion, CO<sub>2</sub> explosion uses the same principle but the yields are relatively low (Sun and Cheng, 2002).

### **2.4.3 Chemical methods**

Chemical pretreatments employ different chemical agents as ozone, acids, alkalis, peroxide and organic solvents. Inorganic acids as H<sub>2</sub>SO<sub>4</sub> and HCl have been preferably used for biomass pretreatment. Hydrolysis with dilute sulfuric acid has been successfully developed given that high reaction rates can be achieved improving significantly the subsequent process of cellulose hydrolysis. In contrast, the costs of dilute acid pretreatment are higher than the corresponding one of steam explosion or AFEX process (Sun and Cheng, 2002). Schell *et al.*, (2003) studied the

dilute-acid pretreatment of corn stover at pilot plant level using high solid loads and obtained a xylose yield of 77% at 190 °C. This pretreatment method was evaluated through a kinetic model that allowed the prediction of process conditions in order to maximize the yield. Similar kinetic studies were carried out for cane bagasse pretreated with nitric acid (Rodríguez-Chong *et al.*, 2004) or without acid addition (Jacobsen and Wyman, 2002). Dilute acid pretreatment also can be accomplished in a two-stage way. For this, a first depolymerization stage of hemicellulose at 140 °C during 15 min is carried out in order to avoid the formation of furan compounds and carboxylic acids, followed by a second stage at 190 °C during 10 min to make cellulose more accessible to enzymatic hydrolysis (Saha *et al.*, 2005a, Saha *et al.*, 2005b). These authors point out that the realization of dilute-acid pretreatment at low temperatures (121 °C) allows avoiding the degradation of sugars to furfural and hydroxymethylfurfural (HMF), but the sugars yields are lower.

Dilute acid pretreatment along with steam explosion are the most widely studied methods. The National Renewable Energy Laboratory (NREL) of the US Department of Energy, which currently is developing ethanol production technologies from biomass, has preferred the dilute acid pretreatment for the design of its process alternatives (Aden *et al.*, 2002, Woolley *et al.*, 1999b and Lynd, 1996) pointed out that the main advantage of this process related to steam explosion was the higher recovery of sugars derived from hemicellulose. For hardwood, this recovery was about 80% for dilute acid pretreatment, and did not exceed 65% for steam explosion. Ogier *et al.*, (1999 ) stated of that the methods appearing as the most efficient were dilute-acid pretreatment, steam explosion with catalyst addition and LHW. These methods were also chosen by Hamelinck *et al.*, (2005) as the more perspective in short-, mid- and long-term evaluations.



Concentrated acids also have been used for pretreatment. A fuel ethanol production process from cane bagasse involving the pretreatment with concentrated sulfuric acid has been patented (Farone and Cuzens, 1996.). This technology implies the retrofitting of sugar mills in order to produce ethanol and improve energetic indexes of this kind of processes (Cuzens and Miller, 1997). An alternative approach was tested by Teixeira *et al.*, (1999a) and Teixeira *et al.*, (1999b), which employed a silo type system by introducing the feedstock (bagasse or hybrid poplar) in plastic bags to which a peracetic acid solution was added. Cellulose conversion of pretreated material reached 93.1% during 120 h using 21 wt% acid concentrations or during 24 h using 60 wt% acid concentrations. This system required low energy since the process has been carried out at room temperature.

Alkaline pretreatment is based on the effects of the addition of dilute bases on the biomass: increase of internal surface by swelling, decrease of polymerization degree and crystallinity, destruction of links between lignin and other polymers and breakdown of lignin. The effectiveness of this method depends on the lignin content of the biomass (Sun and Cheng, 2002) In general, the utilization of bases as sodium hydroxide or solvents such as ethanol or methanol (organosolv process) allows the dissolution of lignin, but their costs are so high that these methods are not competitive for large scale plants (Lynd *et al.*,2005).

#### **2.4.4 Biological methods**

Biological pretreatment has low energy requirements and mild environmental conditions. However, most of these processes are too slow limiting its application at industrial level. Many white-rot fungi degrade the lignin and, for this reason, they have been utilized for ligninases production and lignocellulose degradation. Lee (1997) reported the main microorganisms producing lignin-degrading enzymes and indicates the fermentation processes for producing

them by both submerged culture and solid-state fermentation. In fact, the fungus has been proposed in the patent of Zhang (2006) for degrading the lignin in a biomass-to-ethanol process scheme involving the separate fermentation of pentoses and hexoses. Kang *et al.*, (2004) highlighted the viability of producing cellulases and hemicellulases by solid-state fermentation. According to preliminary evaluations of the NREL, the cost of cellulases produced *in situ* by submerged culture was US\$0.38/100,000 FPU (Filter Paper Units, a way for measuring cellulase activity), (Tengerdy and Szakacs, 2003). Thus, cellulase costs comprise of 20% of ethanol production costs assuming them in US\$1.5/gallon. On the other hand, commercial cellulase cost (US\$16/100,000 FPU) was prohibitive for this process. In contrast, these authors indicated that the cost of producing cellulases by solid-state fermentation of corn stover could reach US\$0.15/100,000 FPU that would correspond to US\$0.118/gal EtOH, i.e. near 8% of total costs.

One of the main problems during the pretreatment and hydrolysis of biomass is the variability in the content of lignin and hemicellulose. This variability depends on factors as the type of plant from which the biomass is obtained, crop age, method of harvesting, etc. This makes that no one of the pretreatment methods could be applied in a generic way for many different feedstocks (Claassen *et al.*, 1999). The future trends for improving the pretreatment of lignocellulosic feedstocks also include the production of genetically modified plant materials with higher carbohydrate content or modified plant structure to facilitate pretreatment in milder conditions or using hemicellulases. It is estimated that the use of these new materials along with improved conversion technologies, could reduce the ethanol cost from lignocellulosic biomass in US\$0.11/L in the next ten years (Wooley *et al.*, 1999a)

## **2.5 Detoxification of lignocellulosic hydrolyzates**

During pretreatment and hydrolysis of lignocellulosic biomass, a great amount of compounds that can seriously inhibit the subsequent fermentation are formed in addition to fermentable sugars. Inhibitory substances are generated as a result of the hydrolysis of the extractive components, organic and sugar acids esterified to hemicellulose (acetic, formic, glucuronic, galacturonic), and solubilized phenolic derivatives. In the same way, inhibitors are produced from the degradation products of soluble sugars (furfural, HMF) and lignin (cinnamaldehyde, *p*-hydroxybenzaldehyde, syringaldehyde), and as a consequence of corrosion (metal ions) (Lynd, 1996, Palmqvist and Hahn-Hägerdal, 2000b). For this reason and depending on the type of employed pretreatment and hydrolysis, detoxification of the streams that would require for fermentation. Detoxification methods can be physical, chemical or biological. As pointed out by Palmqvist and Hahn-Hägerdal (2000a) these methods cannot be directly compared because they vary in the neutralization degree of the inhibitors. In addition, the fermenting microorganisms have different tolerances to the inhibitors.

## **2.6. Ethanol Background**

### **2.6.1 Processes of ethanol production**

Raw materials containing sugars or materials which can be transformed into sugars can be used as fermentation substrates. The fermentable raw materials can be grouped as directly fermentable sugary materials, starchy, lignocellulosic materials and urban/industrial wastes. Direct fermentation of sugarcane, sugar beet and sweet sorghum to produce ethanol has also been reported (Bryan, 1990). Sugar containing materials require the least costly pretreatment, where starchy, lignocellulosic materials and urban/industrial wastes needed costly pretreatment to convert into fermentable substrates (Sun and Cheng., 2002). Sugar containing materials which

can be transformed into glucose, can be used as fermentation substrates under anaerobic conditions, glucose is converted to ethanol and carbon dioxide by glycolysis. The phosphorylation of carbohydrates is carried out through the metabolic pathway and the end products are two moles of ethanol and carbon dioxide (Ingram, 1998). Although fungi, bacteria, and yeast microorganisms can be used for fermentation, specific yeast (*S. cerevisiae* also known as Bakers' yeast, since it is commonly used in the baking industry) is frequently used to ferment glucose to ethanol. Theoretically, 100 g of glucose could produce 51.4 gram of ethanol and 48.8 g of carbon dioxide. However, in practice, the microorganisms use some of the glucose for growth and the actual yield is less than 100% (Badger, 2002). Ethanol production from grain involves milling of grain, hydrolysis of starch to release fermentable sugar, followed by inoculation with yeast. Chemically starch is a polymer of glucose (Peterson, 1995). Yeast cannot use starch directly for ethanol production. Therefore, grain starch has to be wholly broken down to glucose by combination of two enzymes, viz., amylase and amyloglucosidase, before it is fermented by yeast to produce ethanol. Alcohol produced from fermented broth and remaining spillages is processed to produce Distiller's Dried Grain and Soluble (DDGS), which is an excellent ingredient for animal feed (Sheorain, 2000). Ethanol can be produced by four main types of industrial operations: batch, continuous, fed-batch and semi-continuous. In batch fermentation, substrate and yeast culture are charged into the bioreactor together with nutrients. Most of the ethanol produced today is done by the batch operation since the investment costs are low and are not required much control and can be accomplished with unskilled labour. Complete sterilization and management of feedstock are easier than in the other processes. The other advantage of batch operation is the greater flexibility that can be achieved by using a bioreactor for various product specifications. In the continuous process, feed, which contains substrate, culture medium and other required nutrients, is pumped continuously into an agitated vessel

where the microorganisms are active. The product, which is taken from the top of the bioreactor, contains ethanol, cells, and residual sugar. The fed-batch operation, which may be regarded as a combination of the batch and continuous operations, is very popular in the ethanol industry. In this operation, the feed solution, which contains substrate, yeast culture and the required minerals and vitamins, are fed at constant intervals while effluent is removed discontinuously. The main advantage of the fed-batch system is that intermittent feeding of the substrate prevents inhibition and catabolite repression. If the substrate has an inhibitory effect, intermittent addition improves the productivity of the fermentation by maintaining a low substrate concentration. It is essential to keep the culture volume constant in continuous operation, whereas there is volume variation in the fed-batch processes. In semi-continuous processes, a portion of the culture is withdrawn at intervals and fresh medium is added to the system. In the continuous processes it is essential to maintain a constant culture volume, whereas there is a volume variation in semi-continuous processes. This method has some of the advantages of the continuous and batch operations. There is no need for a separate inoculum vessel, except at the initial startup. Another advantage of this operation is that not much control is required. However, there is a high risk of contamination and mutation due to long cultivation periods and periodic handling. Furthermore, since larger reactor volumes are needed, slightly higher investment and costly.

### **2.6.2 Ethanol as fuel**

The majority of the energy used today is obtained from the fossil fuels. Due to the continuing increases in the cost of fossil fuels, demands for clean energy have also been increasing. Therefore, alternative fuels sources are sought. Some of the most important fuels are biogas, natural gas, vegetable oil and its esters alcohols and hydrogen. Ethanol which is one of the renewable energy sources and is obtained from biomass has been tested intensively in the

internal combustion engines. Some properties of ethanol with comparison to gasoline are given in Table 2.1. Currently, ethanol for fuel market is produced from sugar or starch at competitive prices. However, this raw material base, which also has to be used for animal feed and human needs, will not be sufficient to meet the increasing demand for fuel ethanol and the reduction of greenhouse gases resulting from use of sugar or starch based ethanol is not as high as desirable (Farrell, 2006) .

Due to the high evaporation heat, high octane number and high flammability temperature, ethanol has positive evaporation pressure enable to storage and transportation safely. Since the oxygen contain has positive effect on environment. In spite of its positive effect when used in gasoline engine as alternative fuel, it is necessary to make some modification on the engine. The fuel system requires more fuel. The vehicle takes less distance with ethanol fuel than gasoline. Because of the first cold starting problem of the pure ethanol, the blend called E85 has a widespread usage as alternative fuel. This fuel consists of 15 vol% unleaded gasoline and 85 vol% ethanol. However, the other blend consisting of 90% gasoline and 10% ethanol called as gasohol. In addition, the flame of the ethanol is colorless in the natural burning processes and this is another advantage of ethanol (Das, 1996).

Ethanol is made from farm-produced raw products which are usually in surplus. Sugarcane is the primary grain used in ethanol production, and it supplies most of the raw material needed. Ethanol production is the third largest user of sugarcane, behind domestic livestock feed and export uses. The conclusions of the report verify that the federal ethanol program is cost effective. The increase in production and price would raise gross farm income. The increase in farm expenditures and employment opportunities in the ethanol industry is projected to increase annually in relationship to additional ethanol production and use. As the domestic ethanol

industry continues to grow, it is witnessing a surge in the construction of farmer-owned ethanol production facilities. Farmers are realizing the added benefits to the ethanol industry through ownership of manufacturing plants. Ethanol's importance to agriculture is evident like added markets for farmers, stimulating rural economies by increasing sugarcane prices and rural income (Farrell, 2006).

### **2.6.3 Ethanol and environments**

Directly related to fossil energy consumptions the question of greenhouse gas emissions. Proponents of ethanol argue that over the entire fuel cycle it has the potential to reduce greenhouse gas emissions from automobiles relative to gasoline, therefore reducing the risk of possible global warming. Because ethanol contains carbon, combustion of the fuel necessarily results in emissions of carbon dioxide (CO<sub>2</sub>), the primary greenhouse gas. Further, greenhouse gases are emitted through the production and use of nitrogen-based fertilizers, as well as the operation of farm equipment and vehicles to transport feedstock and finished products. However, since photosynthesis (the process by which plants convert light into chemical energy) requires absorption of CO<sub>2</sub>, the growth cycle of the feedstock crop can serve to some extent as a "sink" to absorb some fuel-cycle greenhouse emissions. Other studies that conclude higher fuel-cycle energy consumption for ethanol production also conclude higher greenhouse gas emissions for the fuel (Brent, 2006).

### **2.6.4 Rambutan waste as a source of bioethanol**

The Rambutan is a tropical tree which belongs to the Sapindaceae family and also the fruit of this tree. Rambutan is widely distributed throughout Indonesia, Malaysia and Southeast Asia and is identified in some countries, by different names. In Malaysia, another type of rambutan is sold called wild Rambutan. Although the common color of Rambutan is red, the fruit of this

particular type is yellowish. They typically taste sweet though some are sour as well as sweet. It is frequently used for eating and some other useful activities. Mohamed et al (1994) have reported antimicrobial activity of some tropical fruit Wastes (Guava, star fruit, Banana, papaya, rambutan and rambai. Rambutan fruit is rich in fat, calcium, iron, protein, nitrogen, ash, Sodium, zinc, Magnesium, Manganese, Potassium, Phosphorus, PH, Vitamin C, Vitamin A, Thiamin, Riboflavin, Fiber. Nutrient contents of rambutan are shown below in Table 2.1

Table 2.1 : The nutrient composition of Rambutan

Fat	.68%
Protein	.91%
Nitrogen	.14%
Ash	.33%
Calcium	9.58 mg/100g
Iron	.34 mg/100g
Magnesium	12.3 mg/100g
Manganese	1.06 mg/100g
Potassium	84.1 mg/100g
Sodium	20.8 mg/100g
Zinc	.17 mg/100g
Phosphorus	16.6 mg/100g
Ph	4.66
Vitamin A	<40 IU/100g
Vitamin C	59.4 mg/100g
SUGAR PROFILE	52 %
Fructose	2.9 %
Glucose	2.9 %
Sucrose	11.4 %
Maltose	<.1 %
Lactose	<.1%
TOTAL SUGARS	17.2 %
Riboflavin	0.050 mg/100g
Thiamin	<0.010 mg/100g
Fiber	.05%



### 2.6.5. Banana waste as a source of bioethanol

Considering the nutrition aspect, it is the world's leading fruit crop, and in terms of economical value it is the number five agricultural crop in world trade. There are nearly 100 banana producing countries. According to the FAO's statistics database FAOSTAT (2004). Banana fruit and its associated residual biomass are amilaceous and lignocellulosic compounds can be used for ethanol production, therefore they must be initially hydrolyzed to be converted into glucose which can be used as a feedstock to produce ethanol by fermentation and distillation (Hassain *et al.*, 2011) Banana fruits are used to produce ethanol and the hanging cluster also support to produce bioethanol. The banana fruit is exposed to acid hydrolysis peel and the banana pulp are submitted to acid hydrolysis, availing of amilaceous material. The hanging cluster support or the banana skin is submitted to enzymatic hydrolysis, taking advantage of lignocellulosic material. During the acid hydrolysis diluted  $H_2SO_4$  is used for reducing the pH of the mixture which is shaken and heated by steam up to  $100^\circ C$ . After 6 hours, about 95% of the starch chains are transformed into glucose (Bohorquez and Herrera, 2005). The syrup obtained must be neutralized until reaching the pH for fermentation; this is achieved by adding NaOH. The mixture is filtered in order to separate the residues that can be used as fuel or fertilizer. On the other hand, the lignocellulosic material is shattered and crushed before being passed through the delignification process. NaOH is used in delignification process increasing the pH. The lignin is a by-product that can be sold as an agglutinative agent or food for the animal industry. During enzymatic hydrolysis organic enzyme is used as an agent for obtaining glucose and diluted  $H_2SO_4$  is employed for reducing the pH of the mixture which is shaken and heated until  $50^\circ C$ . After 10 hours of treatment, approximately 70% of cellulosic material is transformed into glucose. The mixture is also neutralized and filtered before being prepared for fermentation. The syrup can be used as a raw material to produce ethanol. During the fermentation process about

2% of the syrup is used for yeast growing in aerobic conditions. The remaining syrup is used to produce ethanol. When the yeast is submitted to anaerobic conditions, its metabolic route is

Table 2.2: The nutrient composition of banana

Energy	371KJ (89 Kcal)
Carbohydrates	22.84 g
Sugars	12.23 g
Dietary fiber	2.6 g
Fat	0.33 g
Protein	1.09g
Vitamin A equiv	3 $\mu$ g (0%)
Thiamine ( Vit. B <sub>1</sub> )	0.031 mg ( 2% )
Riboflavin (Vit. B <sub>2</sub> )	0.073 mg ( 5% )
Niacin ( Vit. B <sub>3</sub> )	0.665 mg ( 4% )
Pantothenic acid ( B <sub>5</sub> )	0.334 mg ( 7% )
Vitamin B <sub>6</sub>	0.367 mg ( 28% )
Folate ( Vit. B <sub>9</sub> )	20 $\mu$ g ( 5% )
Vitamin C	8.7 mg ( 15% )
Calcium	5 mg ( 1% )
Iron	0.26 mg ( 2% )
Magnesium	27 mg ( 7% )
Phosphorus	22 mg ( 3% )
Potassium	358 mg ( 8% )
Zinc	0.15 mg ( 1% )

deviated to produce ethanol and CO<sub>2</sub> (Correa and Levaza, 2006). (Table 2.2) showing the nutrient value of banana. The theoretical fermentation reaction yield is 51%, however it reaches

about 91% of this theoretical conversion. Furthermore, during the fermentation process other compounds such as aldehydes, heavy alcohols, fatty acids, and residual biomass are produced. Ethanol at 96% w/w is produced in the distillation process. Normally two distillation columns are used and some by-products such as aldehydes and heavy alcohols are recovered. Stillage that is a mixture of water and other by-products is separated, and then about 70% of this liquid mixture is sent again to the fermentation process for increasing the process efficiency. Finally, the stillage is carried to the stillage treatment plant; the solids are separated and sent to the composting plant, where they are mixed with ashes and residual biomass to obtain an organic fertilizer. At the end of the process the product is dehydrated using molecular sieves to produce anhydrous ethanol at 99.8% (w/w).

#### **2.6.6. Pineapple waste as a source of bioethanol**

Because of current interest in the economic conversion of renewable resources into alcohol, residues of a number of crops were evaluated as substrates for alcohol production (Han & Cho 1973; Bu'Lock, 1979). Pineapple waste was one. The pineapple is the most famous and economically important member of the Pineapple family, it is the only bromeliad with edible fruit. The family is almost exclusively from the New World, with over 2000 species besides the pineapple native to the tropical Americas. One additional species is from Africa. The pineapple, however, can now be found in all tropical regions. Portuguese explorers carried A million tons of pineapple is produced every year and 40 to 80% is discarded as waste, being composed of peel, cores and pomace (Stuckey and Hamza, 1982; Tewari *et al.*, 1987). Pineapple fruit quality is at its best only if the fruit matures on the plant. They do not become sweeter if harvested earlier since there are no starch reserves to be converted to sugar. The sugar content must come from the rest of the plant.

Pineapples are 15% sugar along with malic and citric acids and a lot of cellulosic material. As a waste pineapple stuff having high biochemical oxygen demand (BOD) and chemical oxygen demand (COD) values, cause a serious pollution problem if not disposed of properly (Burbank & Kumagai, 1965). In areas near where it is grown, a pineapple wine is fermented. It does not store well so it is rarely seen outside of the tropics. Pineapple waste, consisting mainly of cellulose and starch, was suggested as a substrate for production of valuable fermentation and nonfermentation products (Tewari *et al.*, 1987).

In the past, pineapple waste from canneries has been utilized as the substrate for bromelin, vinegar, wine, food/feed yeast and organic acids (Dev and Ingle, 1982). The cultivation of pineapples has become an exact science. For best fruit quality the plant has specific environmental requirements such as mineral rich soil that is moist but well drained, low humidity, full sunlight, and temperatures that do not get below 32F or above 90°F. This environment can be found in many tropical countries, and the pineapple industry is significant in Thailand, the Philippines, United States (Hawaii), Mexico, Ivory Coast, South Africa, Malaysia, Kenya, Taiwan, Australia, and other countries. Interesting enough, prior to 1950 Hawaii produced 70% of the world's pineapples. Increasing production costs and foreign competition has reduced Hawaii's market share to less than 30%. Still, Hawaii produces 500,000 tons of pineapples each year. This study evaluates the potential of pineapple waste as a substrate for ethanol fermentation. (Table 2.3) the nutrient value of pineapple ([en.wikipedia.org/wiki/Pineapple](http://en.wikipedia.org/wiki/Pineapple))

Table 2.3 : Nutrient composition of pineapple

Energy	202KJ (48 Kcal)
Carbohydrates	12.63 g
Sugars	12.23 g
Dietary fiber	1.4 g
Fat	0.12 g
Protein	0.54 g
Thiamine ( Vit. B <sub>1</sub> )	0.079 mg ( 6% )
Riboflavin (Vit. B <sub>2</sub> )	0.031 mg ( 2% )
Niacin ( Vit. B <sub>3</sub> )	0.489 mg ( 3% )
Pantothenic acid ( B <sub>5</sub> )	0.205 mg ( 4% )
Vitamin B <sub>6</sub>	0.110 mg ( 8% )
Folate ( Vit. B <sub>9</sub> )	15 µg ( 4% )
Vitamin C	36.2 mg ( 60% )
Calcium	13 mg ( 1% )
Iron	0.28 mg ( 2% )
Magnesium	12 mg ( 3% )
Manganese	80.9mg ( 45% )
Phosphorus	8 mg ( 1% )
Potassium	115 mg ( 2% )
Zinc	0.10 mg ( 1% )

### 2.6.7. Mango waste as a source of bioethanol

Mango is the most important fruit crop. It contains good amount of sugar (16-18% w/v) and many numbers of organic acids and also a good antioxidant, carotene (as Vitamin A, 4,800 IU).

Some of the varieties are very rich in sugar. Sucrose, glucose and fructose are the principle

carbohydrates in ripe mango with small amount of cellulose, hemicellulose and pectin. The green tender fruits are rich in starch, during ripening the starch which is present is hydrolyzed into reducing sugars (Sastri, 1962). The unripe fruit contains citric acid, malic acid, oxalic acid and succinic and other organic acids. In ripen fruits the main acid source is malic acid (Bulen *et al.*, 1952). Waste mangoes include undersized, those with spotty or marked peel, or those that have suffered mechanical damage. Mangoes are grown for export are typically harvested and transported to a packing shed for grading. The grading rejects then require disposal. They have already been collected and brought to a centralized location, the waste mangoes are low-cost, concentrated biomass as feedstock. Such mangoes can be used as raw material in the generation of biomasses with increased added value, thus allowing farmers to diversify their harvests and receive higher income. In this context attempts for utilization of the surplus of mango fruits which are unattractive and of small size several applications have been studied, in which one of the possibility is ethanol production. Production of Ethanol from Mango can be done by fermentation. (Reddy *et al.*, 2007) have highlighted the scope of ethanol production from the surplus and non-attractive mango fruit. According to the report the mango juice contains 18-20% total soluble solids (TSS) and 5-18.5% of reducing sugars. Finally 8.5-10% (w/v) of ethanol was obtained from the fermentations which were conducted without adding any nutrients. The fermentation was completed within 72 h in all variety juices. Fermentation process optimized and pH 5.0, 30°C temperature, 3% (v/v) inoculum density and 3 days incubation was found be good for maximal ethanol production from mango juice. (Table 2.4) showing the nutrient value of mango fruit (Wikipedia 2009b).

Table 2.4 : Nutrient composition of mango

Energy	272KJ (65 Kcal)
Carbohydrates	17.00 g
Sugars	14.8 g
Dietary fiber	1.8 g
Fat	0.27 g
Protein	0.51 g
Vitamin A equiv	38 $\mu$ g (4%)
Thiamine ( Vit. B <sub>1</sub> )	0.058 mg ( 4% )
Riboflavin (Vit. B <sub>2</sub> )	0.057 mg ( 4% )
Niacin ( Vit. B <sub>3</sub> )	0.584 mg ( 4% )
Pantothenic acid ( B <sub>5</sub> )	0.160 mg ( 3% )
Vitamin B <sub>6</sub>	0.134 mg ( 10% )
Folate ( Vit. B <sub>9</sub> )	14 $\mu$ g ( 4% )
Vitamin C	27.7 mg ( 46% )
Calcium	10 mg ( 1% )
Iron	0.13 mg ( 1% )
Magnesium	9 mg ( 2% )
Phosphorus	11 mg ( 3% )
Potassium	156 mg ( 3% )
Zinc	0.04 mg ( 0% )
-beta-carotene	445 $\mu$ g ( 4% )