

## A REVIEW OF BIOETHANOL PRODUCTION FROM PLANT-BASED WASTE BIOMASS BY YEAST FERMENTATION

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(Received: May 2016 / Revised: August 2016 / Accepted: December 2016)

### ABSTRACT

Commercialization of bioethanol has recently intensified due to its market stability, low cost, sustainability, alternative fuel energy composition, greener output and colossal fossil fuel depletion. Recently, because of greenhouse intensity worldwide, many researches are ongoing to reprocess the waste as well as turning down the environmental pollution. With this scenario, the invention of bioethanol was hailed as a great accomplishment to transform waste biomass to fuel energy and in turn reduce the massive usages of fossil fuels. In this study, our review enlightens various sources of plant-based waste feed stocks as the raw materials for bioethanol production because they do not adversely impact the human food chain. However, the cheapest and conventional fermentation method, yeast fermentation is also emphasized here notably for waste biomass-to-bioethanol conversion. Since the key fermenting agent, yeast is readily available in local and international markets, it is more cost-effective in comparison with other fermentation agents. Furthermore, yeast has genuine natural fermentation capability biologically and it produces zero chemical waste. This review also concerns a detailed overview of the biological conversion processes of lignocellulosic waste biomass-to-bioethanol, the diverse performance of different types of yeasts and yeast strains, plus bioreactor design, growth kinetics of yeast fermentation, environmental issues, integrated usages on modern engines and motor vehicles, as well as future process development planning with some novel co-products.

**Keywords:** Bioethanol; Conversion process; Lignocellulosic biomass; Plant-based waste biomass; Yeast fermentation

### 1. INTRODUCTION

In terms of organic chemistry, bioethanol (C<sub>2</sub>H<sub>5</sub>OH) or ethyl alcohol is an alcohol conformation that recently has emerged as a renewable bio-energy, biodegradable clear-colorless liquid, eco-friendly potential fuel to power automotive engines, as well as a potential petrol substitute for road transport vehicles (Hossain & Jalil, 2015b). Usually Bioethanol is synthesized from alcoholic fermentation of sucrose or simple sugars of diverse types of biomass, either from feedstock or non-feedstock sources (Gnansounou & Dauriat, 2005). Nowadays bioethanol production from cellulosic and lignocellulosic materials, especially wastes proffer an alternative solution to existing environmental, economic and energy problems being faced worldwide (Srivastava & Agrawal, 2014). Thus, a review of bioethanol production from plant-based waste biomass is currently needed to be researched extensively in order to decipher environmental and

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Permalink/DOI: <https://doi.org/10.14716/ijtech.v8i1.3948>

energy issues.

In the 1940s the US Army built the first industrial-scale fuel ethanol plant in Omaha, Nebraska to fuel army vehicles and for fuel-blending (Cheng, 2010). Alternative biofuel from lignocellulosic biomass production was in the pilot and demonstration phase as of 2004. According to Gnansounou and Dauriat (2005), Brazil consumed 12,500 million liters of bioethanol fuel and exported 2,500 million liters at an average price of 0.21 US\$ (21 cents)/liter. The US produced 12,900 million liters and the EU Member States produced 500 million liters. Asian countries such as China, Korea, Japan, India and others started to import bioethanol from Brazil in early 2005 and successful applications of bioethanol encouraged them to produce bioethanol, due to direct association with cost effect on raw materials, environment-friendly characteristics and fuel-blending purposes. In 2010, an Italian company Mossi & Ghisolfi constructed a high scaled-up bioethanol plant with 200,000 ton/year production capacity. Meanwhile, Japan, Korea, India, and Germany also generated both pilot and large scale bioethanol plants (Gnansounou & Dauriat, 2005; Franceschin et al., 2011). Bioethanol was chosen as a high demanded blending fuel by researchers. POET-DSM and Abengoa Bioenergy commercialized bioethanol up to 25 million gallons yearly (2014). In 2015, Dupont produced 30 million gallons yearly. The prominent raw material candidate of these bioethanol manufacturing companies was mainly plant-based biomass, such as corn, sweet sorghum, sugarcane bagasse, wheat and crop residue etc. and the feedstock cost was very reasonable such as 7.40 US\$/ton (including transportation cost). Crop residues costs were less than that even in India. Consequently, commercialization of this 2<sup>nd</sup> generation biofuel turned out to very promising and beneficial (Gnansounou & Dauriat, 2005; Devarapalli & Atiyeh, 2015).

The fermentation method is a very popular, traditional, well-established natural metabolic process for conversion of lignocellulosic biomass to bioethanol where an organism transforms complex carbohydrate into simple sugar and sugar into an alcohol or an acid. This fermentation process occurs on an experimental basis with yeast, bacteria or enzymes. In this research, yeast fermentation has been focused on, due to its effectiveness, efficiency and easily operational process. Yeast fermentation contains fewer setbacks than other fermenting vehicles (Hossain & Jalil, 2015b). To highlight the comparison between yeast fermentation and enzymatic fermentation Gnansounou stated that “High concentration of cellobiose and glucose inhibits the activity of cellulase enzymes and reduces the efficiency of the saccharification. One of the methods used to decrease this inhibition is to ferment the reduced sugars along their release. This is achieved by simultaneous saccharification and fermentation (SSF), in which fermentation used yeasts (*Saccharomyces cerevisiae*) (Pandley, 2009). This statement clarified that yeast fermentation is a more convenient and efficient approach than an enzymatic one. On the other hand, during bacterial fermentation, some prime factors, such as temperature, pH, and pressure of the media always require careful supervision, otherwise there is high possibility to easily infect the media. This approach is also proportionally associated with the high cost of building up sophisticated fermentation reactors (Gnansounou & Dauriat, 2005). In a nutshell, rather than using other fermentation processes, yeast fermentation appeared as more cost-effective and yield-efficient for bioethanol production with lower risk. Thus, yeast fermentation needs to be emphasized in this review work.

Biological yeasts are multicellular or eukaryotic microorganisms classified under the fungus kingdom. Various types of yeast strains are available in the market worldwide. Usually yeasts are used in traditional fermentation processes from ancient times to produce different types of alcohol. Various species of *Saccharomyces* were used in yeast fermentation processes, since they were known to be very effective for conversion of complex sugars to ethanol and other substances. Biologists claim that among the many types of yeast, *Saccharomyces cerevisiae* was the most efficient in various experiments (Hossain & Jalil, 2015b; Borglum, 2010; Cheng

et al., 2007). According to Rattanapan et al. (2011), thin-shell silk cocoons, a residual from the silk industry, were used as raw material with *Saccharomyces cerevisiae* fermentation for bioethanol production. Under continuous fermentation in a packed-bed reactor, a maximum bioethanol productivity of 19.0 g/(L h, liters/hour) with an bioethanol concentration of 52.8 g/L was observed at a 0.36/h dilution rate.

The main purpose of this research is: (i) to propose biofuel substitutes for fossil fuels that could diminish the combined ill-effects of air, soil and water pollution and global warming. Due to impending exhaustion of fossil fuels, our world desperately requires biofuel replacement for oil in the future; (ii) to convert biodegradable lignocellulosic wastes in a productive way. This process would be beneficial for stakeholders ranging from farmers to industrialists. Barren lands could possibly be cultivated with suitable non-feedstock energy crops; (iii) to enhance the conventional fossil fuel composition with bioethanol additives since bioethanol performs as octane enhancer in unleaded gasoline in place of the methyl tertio butyl ether (MTBE) (for volatility and flammability purposes) and oxygenated compound for cleaning combustion of the gasoline and improving the air quality; (iv) use as an alternative fuel for reducing CO<sub>2</sub> emission[s] and limiting the risk of climate change: use as renewable energy sources to partly substitute oil and to increase security of supply (Gnansounou & Dauriat, 2005); (v) to promulgate well-practiced and cheap processing methods, yeast fermentation for bioethanol generation worldwide, which only requires elementary experimental tools and methods; (vi) to draw an integrated design plant view of bioethanol with several value-added by-products. These bio-ethanol fuel production costs can be offset for use by a flourishing biomass market. The economic viability of working lands supports a positive incentive to help in preserving farms and forests from the accelerating threat of urban and suburban sprawl (Hossain & Jalil, 2015b).

## **2. POTENTIAL CANDIDATES FOR VARIOUS TYPES OF PLANT-BASED WASTE BIOMASS**

### **2.1. Forest and Industrial Residue Waste Biomass**

Among the prominent candidates for lignocellulosic biomass in bioethanol production, biomass experts prefer sugarcane waste biomass, such as sugarcane bagasse and sugarcane molasses, in the categories of forest and industrial residue waste biomass, due to their ready availability worldwide. In the initial stages, bioethanol production was commercially pioneered alongside sugar production and refinery industries in United States. Based on an assumption related to an ethanol production plant attached to a sugar refinery, Gnansounou and Dauriat (2005) envisaged that the production of 125 million liters of bioethanol would be associated with 250,000 tons of concentrated sugar syrup, which could have economic implications for the animal feed industry with a drop in the price of syrup.

Oil Palm Tree (*Elaeis guineensis jacq.*) waste biomass is another highly potential candidate for bioethanol production with fermentation of both forest and industrial residue biomass, especially in Asia and Africa. Usually oil palm trunks, Fresh Fruit Bunch (FFB), empty fruit bunch, oil palm kernel, oil palm shells, oil palm fronds and other unused parts are scavenged as forest waste biomass for bioethanol production.

Palm Oil Mill Effluent (POME) is considered as industrial residue waste biomass. Lignocellulose is a major constituent of POME and consists of lignin, hemicelluloses and cellulose which result in a high biotechnological impact, due to their high energy content. Environmentalists projected that if they are utilized appropriately, lignocellulose can be a good substrate for the growth of micro-organisms, which give off products of high value and high potential sources of bioethanol (Kabbashi, 2007).

The concept of oil palm waste is being intensively researched and experiments are being undertaken to optimize the potential for bioethanol production, especially in Southeast Asian countries, such as Malaysia, Indonesia, Thailand etc. For example, oil palm trunks are able to generate 58.43% (w/w) bioethanol by *S. cerevisiae* fermentation with some added nutrients (Hossain & Jalil, 2015b). Statistics indicate that in Malaysia and Indonesia, roughly 3 hm<sup>3</sup> of bioethanol can be fabricated using the sap of the logged oil palm trunk (Yamada et al., 2010). Nevertheless, oil palm empty fruit bunch produced the highest concentration of bioethanol of 13.8% (w/w) by 15 mg/ml of glucose under experimental conditions (Cheng et al., 2007).

Regarding industrial waste, POME could produce substantial quantities of sugar to manufacture bioethanol by fermentation from oil palm mill and coconut oil mill effluents, as well as mill effluent from sesame, sunflower, safflower, olive, mustard and others. The highly organic matter and solids from cell walls, organelles, short fibers and carbohydrates from hemicellulose can be easily transfigured to simple sugars, nitrogenous compounds from proteins to amino acids, free organic acids and minor organic and mineral constituents. Along with the economic advantages, oil palm industries brought forward the waste generation issue with its significant environmental implications. The process of oil extraction in oil mills usually generates a highly polluting effluent Palm Oil Mill Effluent (POME) and in Malaysia, it is estimated that 50 million tons of POME and 40 million tons of palm oil biomass are given off from palm oil industries annually. The oil mill industries simply dump the effluent wastes into the environment, rivers or oceans, which threaten the environment with severe pollution (Kanmani et al, 2015). Apart from those facts, the oxygen depleting capabilities of POME in water bodies are also very terrifying.

## 2.2. Agricultural Waste Biomass

All over the world, especially in Asia and Africa, rice straw is one of the most popular and copious lignocellulosic feedstock. About 667.6 million tons of biomass is post-harvested annually in Asia. The major practice to diminish or eliminate this massive amount of post-harvest residue is allocated partially for domestic animal food consumption and other residue is disposed of with open field burning in what represents an extensively hazardous situation for eco-life. But according to environmentalists, rice residue is very easily fermentable to produce bioethanol, instead of wasting it. Based on some experimental results, without any nutritional supplementation, rice straw yielded bioethanol around 0.45~0.5 g/g in rice straw hydrolysates (Khan & Dwivedi, 2013; Sarker et al., 2012). Along with rice straw, rice husk is also considered as potential source for bioethanol production, using yeast fermentation. The maximum bioethanol production from rice husk can be  $3.20 \pm 0.36$  g/l with an ethanol yield of (0.27 g/g) total sugar (Srivastava & Agrawal, 2014). Besides rice straw, other residues, such as wheat straw, corn straw, cereal straw can be prominent candidates as well for producing bioethanol with a fermentation method. Bioethanol production from agro-waste biomass is shown in Table 1 (Khan & Dwivedi, 2013).

Table 1 Amount of agro-waste biomass available for bioethanol production (Million Tons)(Khan & Dwivedi, 2013)

Agro-waste	Africa	Asia	Europe	America	Oceania
Rice Straw	20.90	667.60	3.90	37.20	1.70
Wheat Straw	5.34	145.20	132.59	62.64	8.57
Corn Straw	0.00	33.90	28.61	140.86	0.24
Bagasse	11.73	74.88	0.01	87.62	6.49

Coconut waste biomass has been recognized as an other remarkable source. Everywhere coconut trees are widely planted for use as by-products of coconut water, coconut milk, coconut

oil, etc. After treating the inner part of coconut, mature coconut fiber, green coconut shell, and mature coconut shell can be noteworthy alternatives as substrates for bioethanol production. Usually coconut residues carry a high amount of sugar and with an ordinary fermentation process, it is possible to turn out high amounts of bioethanol. The maximum bioethanolic yield of coconut waste was 90.09% and productivity was 0.21 g/(L.h), derived only from green coconut shell by *Saccharomyces cerevisiae* (yeast) fermentation. Recently, commercial bioethanol experiments from coconut waste are being conducted in the Northeast region of Brazil (Goncalves et al., 2015).

Sweet Sorghum juice and bagasse is one of the promising potential candidates for bioethanol generation with fermentation processes undertaken on a large scale. In India, sweet sorghum juice and bagasse are being processed commercially, using yeast fermentation for bioethanol production. Each ton of sweet sorghum generates approximately 640 kg sorghum juice and 360 kg of bagasse. After sugar extraction, 30-35% of the lignocellulosic residue was left over. It is being utilized now as the raw material to produce bioethanol. Based on an industrial production output, sweet sorghum bagasse and juice obtained a bioethanol yield of 157 L/tons and 121 L/tons, respectively (Gnansounou & Dauriat, 2005).

### **2.3. Municipal Plant-based Waste Biomass**

Regarding environmental cleanliness and public health safety, in many areas, the R&D sector is currently concerned about recycling and utilizing waste from municipal drainage. Korea already initiated a bioethanol production project, utilizing municipal waste and sludge from a local industrial complex (Park et al., 2010). Meanwhile, Sweden started bioethanol generation processed by fermentation from starch plants obtained from slurries and streams (Linde et al., 2008). Apart from industrial waste, bioethanol can be produced even from kitchen waste by fermentation process. Based on experimental research, without adding any nutrients, a high bioethanol working rate, 24.0 g/L.h resulted from using a flocculating yeast strain KF-7 in a continuous bioethanol fermentation process at a dilution rate of 0.8/h. Through this process, 1 kg of kitchen waste brought forth 30.9 g bioethanol and 65.2 L biogas (Gnansounou & Dauriat, 2005). Household and food waste biomass, such as vegetable and fruit peels also yielded bioethanol by yeast fermentation, since fruit skins, such as orange peels, vegetable peels, banana peels, etc. are enriched with high levels of starch, cellulose and hemi-cellulose. Only banana pulp and banana skin are additional ingredients used to produce 346.5 L/t to 388.7 L/t bioethanol (Velasquez & Ruiz, 2010).

## **3. CONVERSION FROM PLANT-BASED WASTE BIOMASS TO BIOETHANOL**

### **3.1. Structure of Plant-based Waste Biomass Raw Materials**

The recent volatility of crude oil and the expected price increases, associated with the urge of pollution reduction, biofuels processed under yeast fermentation have created a new interest in the biofuel production. Consequently, biofuels, such as bioethanol have turned out to be an outstanding scientific concept in this 3<sup>rd</sup>-generation biofuel invention era. Growing fuel utilization and consumption are the core reasons for the rise of CO<sub>2</sub> emissions in the Earth's atmosphere, what threaten our existing world by the 'Green House Effect'. To solve this issue, in the light of the Kyoto GHG reduction targets, many countries were motivated to utilize waste biomass for biofuel manufacturing as an alternative and environmentally-friendly fuel. Thus, a few decades ago, the technological advancements for bioethanol generation have been initialized in many areas all over the world (Hossain & Jalil, 2015a).

Lignocellulose is known as the principal constituent of plant-based waste biomass that is usually composed of polysaccharides (cellulose & hemi-cellulose), lignin, phenolic polymers and proteins. Cellulose represents the main component of lignocelluloses which is a glucan

polysaccharide containing large reservoirs of output/input energy ratio and provides real potential for conversion into biofuels. They are simply bountiful in nature such as agricultural and forestry residues (bagasses, grass, woody biomass, corn stover etc), industrial waste (poplar, oil mill effluent etc), municipal plant based waste biomass (fruit skin, vegetable peel etc) and they do not interrupt human food chain. The conversion from lignocellulosic biomass to ethanol has involved some pretreatments which are followed by polysaccharide hydrolysis to simple sugars by yeast fermentation (Srivastava & Agrawal, 2014; Gnansounou & Dauriat, 2005; Murphy & McKarty, 2012).

### 3.2. Biomass Pre-treatment

Plant-based waste biomass can be varied as two types of biomass, either a juicy, wet biomass or a dry biomass. Leafy and trunk-based agricultural or forest biomass, grass, municipal waste such as kitchen waste, various types of raw plants, cactus, etc., are classified as wet biomass. Rice straw, rice husk, coconut shells, etc., are the examples of dry biomass. Different types of biomass demand different experimental procedures and pre-treatments. Compared to dry biomass, wet biomass requires simpler handling steps. Generally speaking, wet biomass is squeezed, sap is collected and filtered, and then heated up to get the desired concentration for a proper fermentation process. On the other hand, for dry biomass, delignification was pursued as the basic pre-treatment process, which was carried out using basic chemical compounds, such as sodium hydroxide (NaOH) and sodium chlorite (NaClO<sub>2</sub>). Additionally, the NaOH treatment is a highly effective lignin removal method, due to its strong alkalinity level, resulting from lignin. Lignin (Latin: *Lignum*: wood) is a strong organic polymer, which structurally forms wood or bark or algae. Subsequent fungal treatment is also applied in some cases to acquire the highest conversion of lignocelluloses to sugars (Hossain & Jalil, 2015b; Srivastava & Agrawal, 2014; Abo-State et al., 2014).

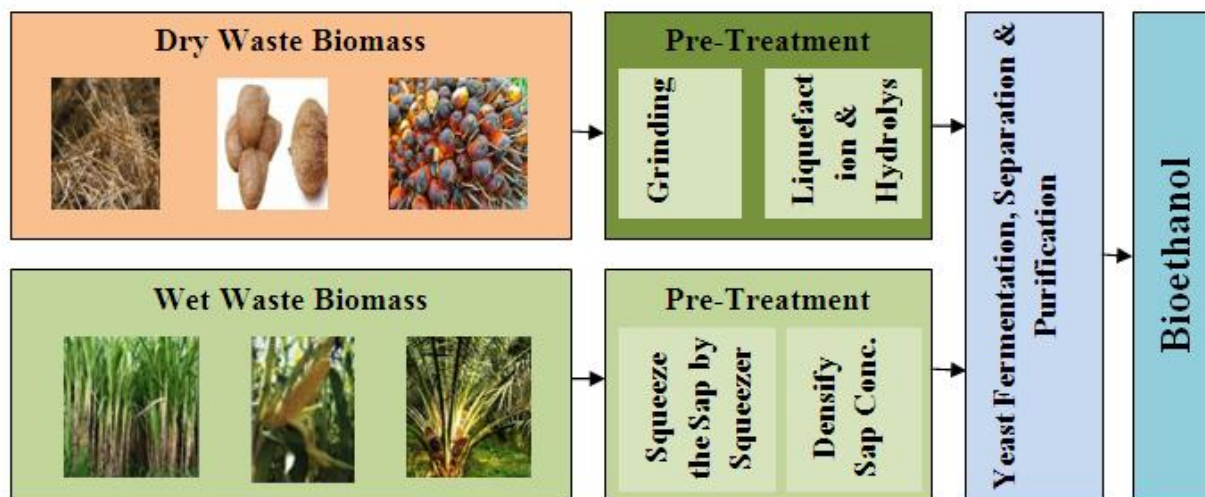


Figure 1 Bioethanol production from first generation biomass (Devarapalli & Atiyeh, 2015)

### 3.2. Yeasts Involved in the Fermentation Process

There are manifold fermentation approaches that are being practiced by laboratories and industries nowadays to manufacture bioethanol and maximize the yield, such as separate hydrolysis and fermentation (SSF), simultaneous saccharification and fermentation (SSCF), consolidated bio-processing (CBP), syngas fermentation (SF), solid-state fermentation etc. (Devarapalli & Atiyeh, 2015). In this research, yeast fermentation was the topic focused on, where yeast acts as the major vehicle to run the whole fermentation process. Yeast is eukaryotic micro-organism involved as a conversion vehicle to produce bioethanol from lignocellulosic waste biomass. A large variety of yeasts and yeast strains are utilized for bioethanol production,

such as *Saccharomyces cerevisiae*, *Endomicopsis burtonii*, *Scwanniomycetes castelli* etc. Among these, *S. cerevisiae* is very well known, available and inexpensively available in the market worldwide. *S. cerevisiae* use was successfully employed to produce bioethanol from Oil Palm Trunk (OPT) sap with very high yield. Nevertheless, some additional nutrients, such as Alanine amino acid ( $C_3H_7NO_2$ ), Epsom salt ( $MgSO_4$ ), Vitamin B<sub>12</sub> and some other types of nutrients multiplied the production efficiency of bioethanol on a yield basis (Hossain & Jalil, 2015b; Khan & Dwivedi, 2013). A simplistic view of the bioethanolic yield of some yeasts and yeast strains for batch fermentation in the laboratory are represented in Table 2.

Table 2 Performances of various types of yeast fermentation (Khan & Dwivedi, 2013)

Yeasts and Yeast Strains	Bioethanolic Yields (g/L)
<i>Saccharomyces cerevisiae</i> (recombinant)	0.91
<i>Schizosaccharomyces pombe</i> (recombinant)	0.42
<i>C.shehatae</i> CBS 4705	0.48
<i>C.shehatae</i> CSIR-Y492	0.29
<i>P.tannopilus</i> RL 171	0.28
<i>P.stipitis</i> CBS 5776	0.45

Recombinant yeast is genetically engineered and modified to analyze the genetic interactions of all double-deletion mutants through synergetic genetic array analysis. Usually due to post-genetically modification, yeasts bring out a higher ethanolic yield than the usual yield. Recently, many researches proved that at a lab scale, recombinant yeast exhibited revolutionary bioethanolic yield and higher capabilities of fermenting pentose sugars. To the best of our knowledge, yet recombinant yeast has not been established in industrial usage for bioethanol production because of its very high instrumental cost. Current researches are ongoing on optimization of bioethanol production, using recombinant yeast (Park et al, 2010). According to Table 2, among different types of yeast and yeast strains, *S. cerevisiae* (recombinant) is the most prudent vehicle for fermentation as it results in more than twice the bioethanolic yield, compared to other yeasts and strains. Recombinant *Saccharomyces cerevisiae* is preferred for higher productivity than usual *S. cerevisiae* available in local markets. To illustrate the bioethanolic yield difference between these two *S. cerevisiae* yeast types, it should be mentioned that usual *S. cerevisiae* showed a bioethanolic yield of 0.41 g/l (without nutrients) and 0.49 g/l (with nutrients), which is much lower than the recombinant *S. cerevisiae* bioethanolic yield 0.91 g/L (Hossain & Jalil, 2015b).

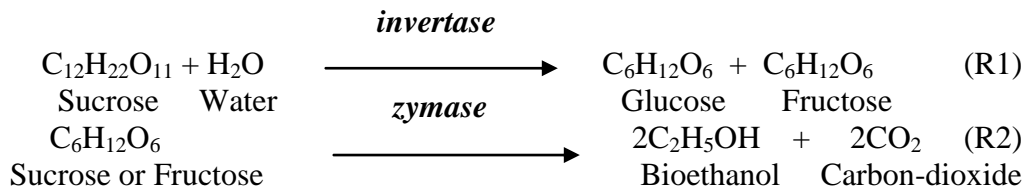
### 3.3. Enzymatic Hydrolyses of Polysaccharides and Fermentation of Simple Sugars

According to Gnansounou and Dauriat (2005), “Bioethanol can be produced from a large variety of carbohydrates (mono-, di-, and polysaccharides)... Polysaccharides are often organised in chains of bonded monosaccharides, which result from dehydration syntheses.” Polysaccharides and disaccharides are usually broken down to monosaccharides and later monosaccharides are converted to bioethanol and CO<sub>2</sub>. Monosaccharides (glyceraldehydes, xylose, ribose, sucrose, glucose) consist of single sugars bound together with a general formula of  $(CH_2O)_n$ , where n= 3-7. The most common monosaccharides in plant-based waste biomass are pentoses (n=5, xylose) and hexoses (n=6, glucose & fructose) (Gnansounou & Dauriat, 2005).

Yeast fermentation is a well-established natural metabolic process where industrial yeast strains turn complex carbohydrates into single sugars and sugar into an alcohol or an acid. Usually, two reactions perform as basic ingredients in converting cellulose into bioethanol by enzymatic hydrolysis and fermentation process. Enzymatic hydrolysis is subjected through catalytic decomposition of chemical compound by reaction with water. Hydrolysis alters complex

polysaccharides (sugar) into simple sugar as a middle metabolic reaction here. Usually, the whole process is held and maintained by an anaerobic condition (Hossain & Jalil, 2015b).

The most available disaccharides are glucose and fructose and they bring out bioethanol. Fermentation of sucrose is usually handled by commercial yeast, such as *Saccharomyces cerevisiae*. The series of bio-catalyzed reactions are composed of enzymatic hydrolysis of sucrose followed by fermentation of simple sugars. Firstly, *invertase* (yeast containing enzyme) catalyzes the hydrolyses of sucrose to form it into glucose and fructose. Secondly, another yeast containing enzyme *zymase* metamorphoses the glucose and the fructose into ethanol and CO<sub>2</sub> (Hossain & Jalil, 2015b). Both of the enzymatic reactions are stated as reactions and are noted below as Reactions (R1) and (R2).



Theoretically, 1 ton of hexose (glucose or fructose) yields 511 kg of bioethanol. Practical efficiency of yeast fermentation is about 92% of this yield (Gnansounou & Dauriat, 2005).

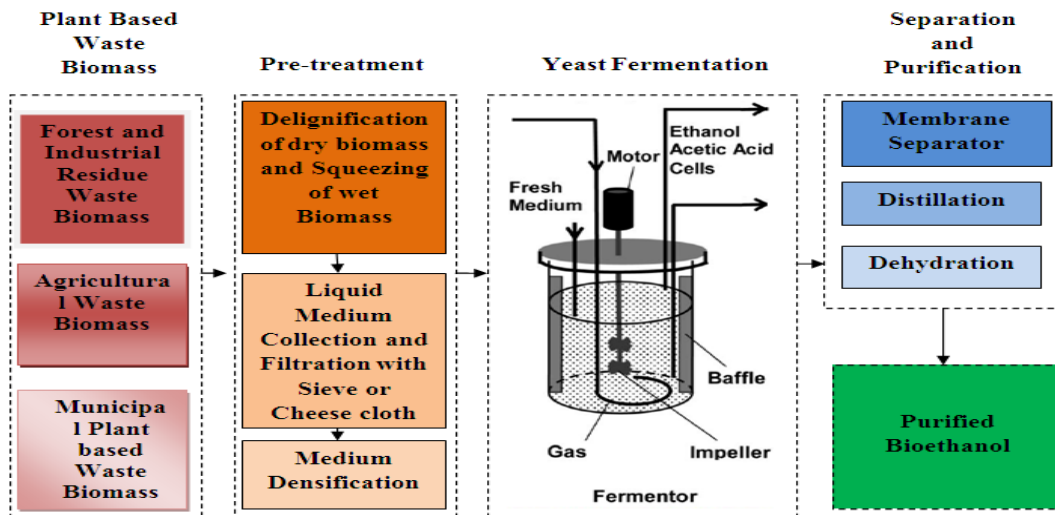


Figure 2 Bioethanol fermentation conversion process from plant-based waste biomass (Devarapalli & Atiyeh, 2015)

Several factors are involved in the fermentation process, such as temperature, pH, medium components, reducing agent, etc., that control the productivity of bioethanol as well as impact on cost analysis. The fermentation temperature exerts a significant effect on cell growth, medium solubility and enzyme activity. Another efficient factor is the pH level. Proper maintenance of pH leads to stability and metabolic enzyme functioning in the process. Medium components can be incorporated, such as various types of nutrients, vitamins, minerals as cofactors or coenzymes. For instance, Alanine amino acid (C<sub>3</sub>H<sub>5</sub>NO<sub>2</sub>) and Epsom salt (MgSO<sub>4</sub>) have an effect on enzymes and improve the bioethanol production amount a few times higher than medium without nutrients. Reducing agents are also deemed as significant factors for effective yeast fermentation process, since they are artificial electron carriers, which alter NADP/NAD ratio by controlling the oxidation-reduction reaction. For example, solven to



genesis enhances the NADH levels in cells and direct electron flow to the bioethanol production. After the fermentation process, a membrane separator was induced to separate bioethanol and other components of the whole medium. Then distillation and dehydration processes were set off to purify bioethanol for fuel purposes (Devarapalli & Atiyeh, 2015; Hossain & Jalil, 2015b).

### 3.4. Bioreactor Design for Yeast Fermentation

Bioreactors are provided a controlled environment for enhancing cell bioethanol acetic acid cell growth, substrate transmutation and monitoring the work rate of the biological process as well as to optimize the total output. Continuous stirred tank reactors (CSTR), bubble columns, packed columns, air-lift, trickle beds, hollow fiber reactors with biofilm formation, batch, and fed-batch reactors could be operated for massive bioethanol generation with or without cell recycling. The implication of a microsparger in the CSTR could elevate the mass transfer a few times higher. Bioreactors for bioethanol supply from plant-based waste biomass should allocate the gas-liquid mass transfer to balance the cells' kinetic requirements by not inhibiting metabolic activity, but instead to perpetuate biocatalyst viability and high concentration, minimize maintenance and operation cost, which should be easily scaled up. Additionally, bioethanolic yield and the required reactor size usually pivot on high cell concentration and mass transfer rate in the reactor. According to Devarapalli and Atiyeh (2015), the rate of mass transfer ( $dn/dt$ ) is given below as shown in Equation 1.

$$\frac{1}{V} \cdot \frac{dn}{dt} = -k_L a \cdot (C_I - C_L) \quad (1)$$

where,  $dn/dt$  is the rate of mass transfer ( $mmol/h$ );  $k_L a$  is the overall mass transfer coefficient ( $h^{-1}$ ),  $C_I$  is the concentration of the gas in the gas liquid interface ( $mmol/L$ ),  $C_L$  is the concentration of the gas in the bulk liquid ( $mmol/L$ ) and  $V$  is the working volume of the reactor (L).  $k_L a$  can be increased by higher agitation speed or the gas flow rate in the reactor that would reduce bubble size and increase interfacial area of mass transfer (Devarapalli & Atiyeh, 2015).

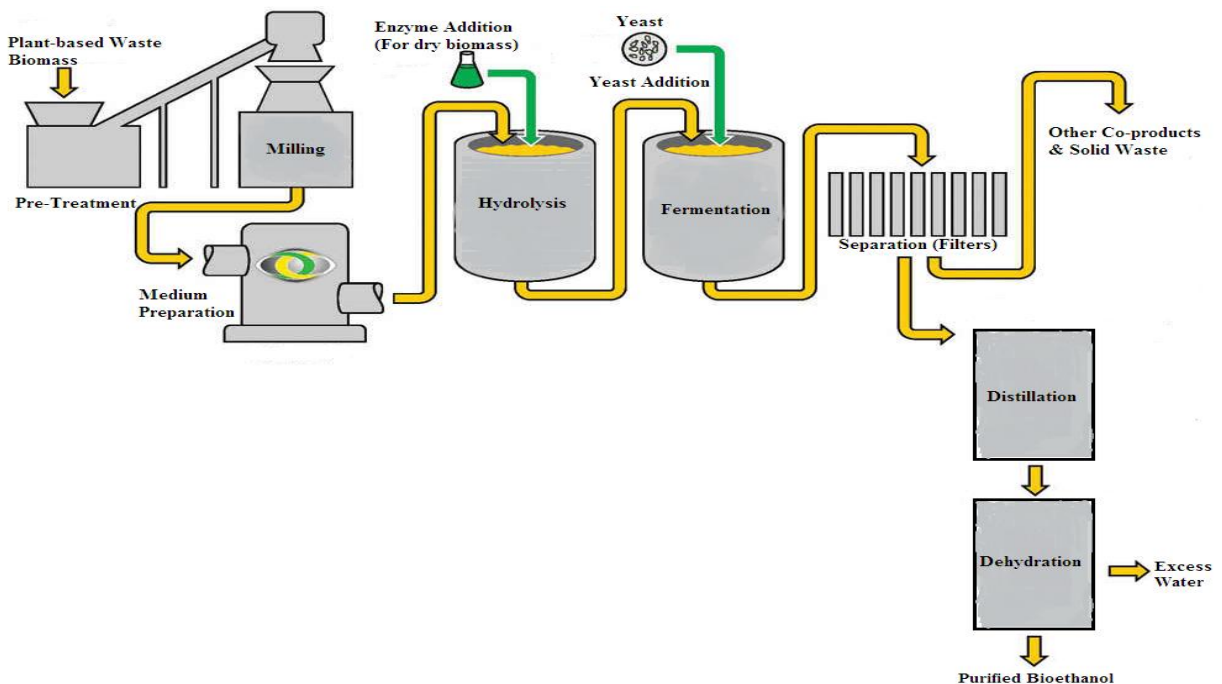


Figure 3 Simplified modeling/method of bioethanol production from plant-based waste biomass by yeast fermentation (Hossain & Jalil, 2015b)

### 3.5. Growth Kinetics for Yeast Fermentation

The rates of hexose consumption, bioethanol production and yeast population growth are correlated by a kinetic model. New yeast cells occur catalytically from the substrate with a specific growth rate during yeast growth in fermentation medium. This process can be expressed by the kinetic formula shown below in Equations 2 and/or 3.

$$r_x = \mu X \quad (2)$$

$$\frac{dX}{dt} = \mu X \quad (3)$$

where  $r_x$  or  $dX/dt$  is the rate of cell growth,  $\mu$  is the specific growth rate ( $\text{h}^{-1}$ ),  $X$  is cell concentration ( $\text{gL}^{-1}$ ) and  $t$  is time (h).

According to the Monod Equation, the relationship between the limiting substrate concentration,  $S$  and  $\mu$  as shown in Equation 4.

$$\mu = \frac{\mu_{\max} S}{K_s + S} \quad (4)$$

where  $S$  is limiting substrate concentration ( $\text{gL}^{-1}$ ),  $\mu_{\max}$  is the maximum specific growth rate ( $\text{h}^{-1}$ ),  $K_s$  is the saturation constant ( $\text{gL}^{-1}$ ).

According to Lineweaver-Burk method,  $K_s$  and  $\mu_{\max}$  can be predicted by taking reciprocals of both sides of equality sign as shown in Equation 5.

$$\frac{1}{\mu} = \frac{K_s}{\mu_{\max}} S + \frac{1}{\mu_{\max}} \quad (5)$$

Here  $1/\mu$  versus  $1/S$  will allow  $K_s$  to be evaluated while the intercept is  $1/\mu_{\max}$  (Mohamad et al., 2013).

## 4. ENVIRONMENTAL ISSUES AND MODERN USAGES

### 4.1. End of Fuel Monopolization and Greener Output

Bioethanol is widely available in USA and Brazil by blending ethanol with gasoline to oxygenate the fuel mixture, resulting in cleaner combustibility and lower pollution emissions. The most common blends are 10% ethanol and 90% petrol (E10) as well as 20% ethanol and 80% petrol (E20). Prior to biofuel applications, fossil fuels (gasoline and diesel) were the main source of fuel used for motors and engines. Following biofuel implementation, fossil fuel monopolization has been lowered. Bioethanol is obtained from a wide range of biomass, which could represent potential self-reliance in matters of energy demand, thus ending the hegemony of OPEC (Organization of Petroleum Exporting Countries). In brief, pilot scale and large scale bioethanol fabrication plants for fuel production purposes could reduce foreign fuel and fossil fuel dependence and shrink trade deficits (Ibeto et al., 2011).

As bioethanol is not composed of hydrocarbons, it can bring forth lower-grade greenhouse gas emissions upon combustion and be less harmful to the atmosphere. Bioethanol, unlike gasoline, is an oxygenated fuel, which contains 35% oxygen and it reduces air pollution, including particulate and  $\text{NO}_x$  emissions from combustion. Bioethanol use contributes to climate change mitigation and a decline in greenhouse gas emissions (GHG) (Lang et al., 2001). Since  $\text{CO}_2$  emissions are processed concurrently in our existing eco-system by plants and forests, when bioethanol is burnt, the pollution outcome generally appears as a 'zero' theoretical net contribution. Based on some research data, cane-based bioethanol reduced GHG emissions by 86-90% in proportion with negligible land use change (Isaias et al., 2004). Nowadays,

bioethanol reproduction by cellulosic yeast fermentation has been well-industrialized in many parts of the world, such as Brazil, USA, Sweden, Japan, Germany, China as well as in some African countries eg. Nigeria (Ibeto et al., 2011).

#### 4.2. Use of Bioethanol in Gasoline and Diesel-powered Motor Vehicles

Vehicle engines are able to run in moderation on up to 85% ethanol and 15% petrol blends (E85). Bioethanol can be adapted to existing automobile designs with no or minimal reorientation to the engines, although ignition systems may require modification. In many countries particularly in Brazil, bioethanol is being employed as an additive or even a substitute to conventional fuel. As it is plant-based fuel, the CO<sub>2</sub> emitted by bioethanol-based engines is recaptured in the nutrient cycle. Bioethanol is suitable for use in mixed fuel in the gasoline engines due to its higher relative octane number (RON). On the other hand, with diesel engines, because of low cetane number (LCN) and high heat of vaporization, diesel ignition may be affected. Bioethanol eliminates free water from engines that can plug fuel lines in cold climates (Lang et al., 2001).

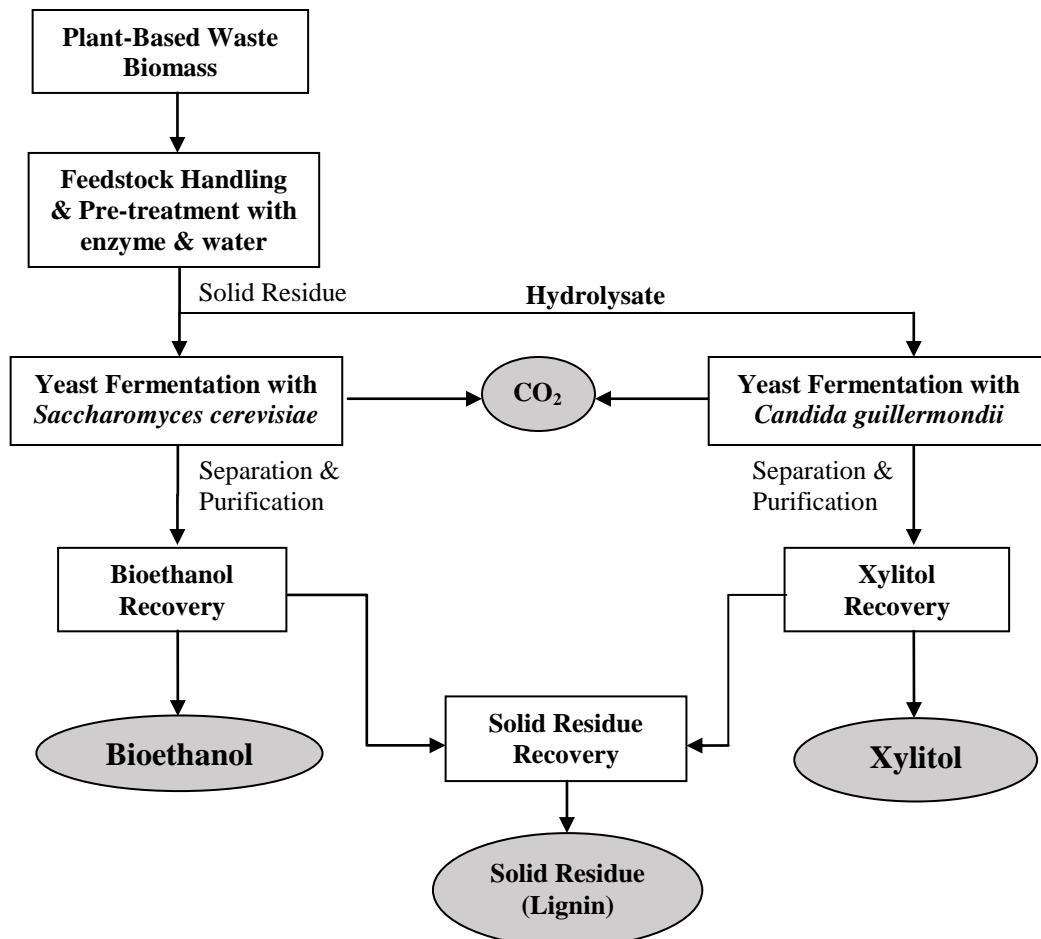


Figure 4 Bioethanol production with co-product xylitol from plant based waste biomass (Franceschin et al, 2011; Hossain & Jalil, 2015b)

### 5. VALUE-ADDED CO-PRODUCTS IN BIOETHANOL PRODUCTION

During the yeast fermentation process, lignocelluloses are turned into simple sugars by yeast enzymes. The most common sugars are hexoses, such as glucose, fructose that are easily converted to bioethanol, albeit in a range of 23-32% (w/w) lignocelluloses, pentoses are drained off as unproductive waste. If this huge fraction remains unused, a significant loss of potential

revenue would be projected. Therefore, an integrated bioethanol production system should be incorporated. New plans could be implemented by producing value-added co-products, where pentoses could be transformed that could save substantial waste disposal costs. Since the pentoses are left unfermented, utilization of pentoses associated with hexoses is increasingly becoming a topic of interest in the development of an economically viable bioethanol generation platform. Biomass experts forecast that the conversion of pentoses will lead to valuable co-products, such as xylitol, an alternative sweetener fermented by *Candida guilliermondii* or *Candida tropicalis*. Franceschin et al. (2011) mentioned that maximum xylitol yield from rice straw was 51.5 g/L and xylitol yield from sago trunk was 20.938 g/L. To carry out an integrated plant system for all types of plant-based waste biomass, xylitol could easily be obtained as co-product even from hardwood or maize.

Usually xylitol is similar to sucrose in sweetness, but xylitol is anti-carcinogenic and metabolized by an insulin-independent pathway that could be a core source for clinical anti-diabetic food production. Moreover, xylitol has already been commercialized as a popular sweetener in various foods, such as chewing gum, candy, baked goods, sweets, soft drinks and ice creams. Recently, the xylitol market is rising with high demand worldwide, due to increasingly health-conscious consumers and the fast growth of chewing gum sales (Franceschin et al., 2011; Mohamad et al., 2013). An integrated plant design for classical production of bioethanol from cellulose with an alternative use of hemicelluloses for xylitol production is outlined in Figure 4.

## 6. CONCLUSION

Bioethanol production from plant-based waste biomass by yeast fermentation is projected as successful and realistic approach for novel biofuel innovation and optimization by biomass experts all over the world. Significant numbers of commercial industries successfully scaled up their bioethanol generation projects from plant-based feedstocks and initialized a novel source of alternative fuel production and utilization in the market. Additionally, modern motor vehicles run perfectly on bioethanol blends without any engine modification that has led to a cleaner environment and energy-savings. Along with bioethanol industrial plants, integration of xylitol production will be the predominant advantage with economical rewards. This research could encourage the biofuel R&D sector worldwide to convert their forestry biomass and agricultural residues to bio-energy. Nevertheless, for further research works, it is strongly recommended to focus on economical feasibility and optimization conditions, such as pH, temperature, incubation time, additional nutrients, and electricity generation for lights in industrial applications. Moreover, it is also suggested to figure out the most efficient yeast or yeast strain for scaling up the yield from bioethanol production based on fermentation methods.

## 7. ACKNOWLEDGEMENT

The Brunei Darussalam Government Scholarship from the Ministry of Foreign Affairs and Trade (MOFAT), Brunei Darussalam awarded to Nazia Hossain is gratefully acknowledged.

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