academicJournals

Vol. 12(35), pp. 5375-5388, 28 August, 2013
DOI: 10.5897/AJB2013.12890
ISSN 1684-5315 ©2013 Academic Journals
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African Journal of Biotechnology

Review

Bioprocess systems applied for the production of bio-ethanol from lignocellulosic biomass of cocoa pod husk (*Theobroma cacao L.*) and other agricultural residues: A review

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Accepted 31 July, 2013

Bioenergy is fast becoming one of the most dynamic and rapidly changing sector of the global energy economy. The associated accelerated growth in the production, supply, conversion and use of bioenergy especially in the liquid bio-ethanol sector present a new reality that is attracting interest from key stakeholders in developed and developing countries alike. Petroleum, in addition to being the main source of transportation energy, has also been the mainstay of the Nigeria economy up to date. This feedstock is, however, not sustainable since it is not renewable over the period of time over which we use them. Present technologies to produce bioethanol largely depend on food-based materials and this has caused significant stress on food prices and food security. The growing interest in the use of biomass-based materials like cocoa pod husk (CPH) for bio-ethanol production especially when accruing as wastes from the agricultural sector is generally a welcomed development. Biomass feedstock are considered to be the most abundant renewable resource in the world, not only as an alternative source of energy but also hold remarkable potentials to mitigate greenhouse gas emission and for the development of organic chemical industries. However, before lignocellulosic materials can be effectively utilized, there is need for some conversion processes. Currently, enzymatic saccharification and acid hydrolysis are the main conventional methods for breaking these complex materials into smaller units prior to fermentation. The inability of *Sacharomyces. cerevisiae* to fully utilize pentose sugars present in biomass have been pointed out as one of the bottlenecks for the commercialization of cellulosic ethanol production. To circumvent this limitation, gene cloning techniques are used to adapt yeast for the bioconversion processes.

Key words: Biomass, bio-ethanol, cocoa pod husk, greenhouse gas.

INTRODUCTION

The wake of contemporary utilization of limited fossil fuels and the unending catastrophes giving rise to massive destruction of the ecosystem has compel the world for desperate search for alternative sources of liquid transportation fuels to address vital, strategic, economic and environmental problems. There is growing evidence that the global conventional oil use is nearing the point where half of the accessible reserves have been depleted; pointing towards the real possibility that production will not be able to keep up with the demand in the near future (Kerr, 2005). Furthermore, there is also wide spread prediction that the world population will likely increase by

about 50% in the next 50 years with increase in the world demand for petroleum energy (Igbinadolor, 2012). If the current production and consumption rates of petroleum resources continue, global oil reserves will be exhausted in less than 65 years as predicted by the United Nation Conference on Trade and Development (UNCTAD) (Spore, 2006). In addition, the International Energy Agency (IEA) predicts that biofuels will provide 26% of total transportation fuel in 2050 (Spore, 2011). This situation is compounded by the tremendous growth in oil demand by non-oil producing countries (IEA, 2004; Yang and Lu, 2007). Nigeria is an oil-producing nation and she depends solely on this as its source of transportation fuel and revenue. This growing dependence on petroleum in Nigeria has strategically made her to be vulnerable to disruptions and price hikes that produce economic chaos. These aside, petroleum is the greatest contributor to emissions of carbon dioxide, which in turn has the greatest influence on global climate change (Igbinadolor, 2012). In view of the vital strategic, economic and environmental issues that continue to grow, petroleum consumption must by necessity be reduced (Lugar and Woolsey, 1999) if the world is to ultimately address the impending crises to which petroleum use will surely lead. Furthermore, any new fuel newly developed should be sustainable if the world is to dramatically cut greenhouse gas emissions. The overwhelming dominance of gasoline and diesel for transportation clearly shows our preference for liquid fuels. When the spectrum of sustainable resources and fuels that may be derived from them are examined, biomass clearly represent the only sustainable, low cost resources that can be converted into liquid transportation fuels on a large scale enough to have a meaningful impact on petroleum use in the near term and perhaps beyond (Wyman, 1996; Lynd, 1996).

Bio-ethanol produced from agricultural residues especially when accruing as waste biomass like cocoa (*Theobroma cacao L*) pod husk, shows many potentials advantages in comparison with sugar or starch-based stocks since the latter materials are also food for human and animals. However, the complex nature of this biomass necessitates the use of genetic techniques to produce engineered organisms that are able to transform this polymer into the desired product. The growing interest in the use of wastes residues for bio-ethanol production is driven by the need for sustainable sources of materials since biomass are diffusely distributed and the need for diversification of materials as starch and sugar feedstock use for first generation bio-ethanol are also food-based materials for humans and animals.

Several biofuel routes to production of liquid fuels have been pursued over the years (Yang and Wyman, 2007), which include: Gasification of biomass to syngas for conversion to synthetic diesel fuel, pyrolysis of biomass to oils, direct liquefaction, conversion of plant oils to biodiesel, release of sugars for fermentation to ethanol. For biomass-to-ethanol conversion to become a reality, biomass processors must prove their technology. This write-up, therefore, provide a perspective on biomass processing by highlighting the key elements required for commercializing lignocellulosic biomass conversion, with particular emphasis on some technological advances in bioethanol development.

THE IMPORTANCE OF BIO-ETHANOL PRODUCTION FROM BIOMASS

Currently, the problem of energy demand and the environment are critical to the development and advancement of human civilization. The importance of alternative bioenergy source has become even more necessary not only due to the continuous depletion of limited fossil fuel stock, but also for the safer use of the environment and therefore sustainable source of energy. Using waste biomass to produce energy will also help to increase the agricultural income for rural people in developing countries. Biomass appears to be attractive feedstock for the following reasons: (1) It is a renewable resource that could be sustainably developed in the future; (2) it appears to have formidably positive environmental properties resulting in no net release of carbon dioxide and (3) it appears to have significant economic potentials to increase energy security. Thus to promote more balanced development of bio-ethanol production, there is need for diversification of resources for its production, as this will lessen the pressure on a single raw material. In contrast to food-based materials, lignocellulosic material is glimpsed at as a promising choice as a second generation bioethanol fuel and are diffusely distributed. Plant biomass, particularly when accruing as a waste product, is an attractive feedstock for bioethanol production. Among the different lignocellulosic raw materials, cocoa pod husk is an abundant source of biomass very much available in Nigeria as agricultural waste residues in the farm (Figure 1) with vast quantities of sugars occurring as structural polysaccharide – cellulose and hemicellulose (Igbinadolor, 2012).

It is estimated that 0.8 to 1.0 million tones of cocoa pod husk (CPH) is generated annually in cocoa farms in Nigeria (Igbinadolor, 2012). CPH consisting of dry matter 84%, crude protein 10.16%, crude fibre 34.92%, ether extract 2.49%, potassium 3.64%, theobromine 0.32% and

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Abbreviations: UNCTAD, United Nation Conference on Trade and Development; **IEA,** International Energy Agency; **CPH,** cocoa pod husk; **DP,** degree of polymerization; **AFEX,** ammonia fiber/freeze explosion; **SSF,** simultaneous saccharification and fermentation; **SHF,** separate hydrolysis and fermentation; **HMF,** 5-hydroxymethyl-2-furaldehyde; **XR,** xylose reductase; **XDH,** xylitol dehydrogenase; **GHG,** greenhouse gas; **LHW,** liquid hot water.

Figure 1. Cocoa Pod Husk bio-wastes residue generated during cocoa processing.

Table 1. Availability and composition of other agricultural waste residues.

Fibre source	Availability (10^3) tonnes	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Ash (%)	Reference
Corn stover	727	38 - 40	28	7 - 21	3.6 – 7	Reddy and Yang, 2004
Barley straw	195	31 - 45	27 – 38	14 - 19	2 – 7	Rowell, 1997
Pineapple leaf fibre	-	70 - 82	18	5 - 12	0.7 – 0.9	Majumdar and Chanda, 2001
Coir	100	36 - 43	0-15 – 0.25	41 - 45	2.7 – 10.2	Banerjee, 2002
Bagasse	100	32 - 48	19 – 24	23 - 32	1.5 – 5	Rowell, 1997
Banana fibre	-	60 - 65	6 – 8	5 - 10	4.7	Majumdar and Chanda, 2001
Wheat straw	568	33 - 38	26 – 32	17 - 19	6 - 8	Gressel and Zilberstein, 2003
Rice straw	579	28 - 36	23 – 28	12 - 14	14 - 20	Lim, 2001
Sorghum stalks	252	27	25	11	-	Gressel and Zilberstein, 2003

gross energy 20.32 MJ/kg (Barnes et al., 1998), represent one of the most important Nigerian agricultural residues. Other lignocellulosic materials that could be used as starting materials for the production of bioethanol are shown in Table 1.

COMPOSITION OF LIGNOCELLULOSIC BIOMASS

Lignocellulosic materials containing cellulose, hemicellulose and lignin are the most abundant renewable organic resources on earth (Aristidou and Penttila, 2000). The chemical composition of biomass varies among species (Table 2) and is inherent according to the particular needs of the plants, but biomass consist of 25% lignin and 75% carbohydrate polymers (cellulose and hemicellulose) (Aristidou and Penttila, 2000). The proportion of these components in a fibre depends on the age, source of the fibre and the extraction conditions used to obtain the fibre.

Table 2. Chemical compositions of common Lignocellulosic biomass.

Constituent	Hardwood (%)	Softwood (%)
Cellulose	40 to 50	40 to 50
Hemicellulose	25 to 35	25 to 35
Lignin	20 to 25	20 to 25
Pectin	1 to 2	1 to 2
Starch	Trace	Trace

Source: Miller (1999).

Cellulose

Cellulose is an organic compound with the formula $(C_6H_{10}O_5)_n$. It is a polysaccharide consisting of a linear chain of several (1,4) linked D-glucose unit (Crawford, 1981). It is the main structural component that provides strength and stability to the plant cell walls and the fibre (Reddy and Yang, 2005). The amount of cellulose in a fibre influences the properties, economics of fibre production and the utility of the fibre for various applications (Reddy and Yang, 2005). The cellulose in a plant consists of parts with a crystalline (organized) structure, and parts with a not well-organised, amorphous structure. The chains are bundled together and form so called cellulose fibrils or cellulose bundles (Figure 2). These cellulose fibrils are mostly independent and weakly bound through hydrogen bonding (Laureano-Perez et al., 2005). Cellulose is a high-molecular weight linear glucose polysaccharide with the elementary formula $\left[C_6H_{10}O_5\right]_n$. It has a degree of polymerization (DP) in the range of 200-2000 kDa (4000-8000 glucose molecules connected with β-1, 4 glycosidic bonds). Some animals, particularly ruminants and termites can digest cellulose with the help of symbiotic microorganisms that live in their guts (Reddy and Yang, 2005). Cellulose is not digestible by humans and is often referred to as “dietary fibres” or roughage, acting as hydrophilic bulking agent for faeces.

Cellulose is the structural component of the primary cell wall of green plants; many forms of algae. Some species of bacteria secrete it to form biofilm. The major combustible component of non-food energy crops is cellulose, with lignin second. Cellulose has no taste, is odourless, hydrophilic, insoluble in water and most organic solvent, it is chiral and biodegradable (Klemm et al., 2005). Cellulose is derived D-glucose units, which condensed through β(1→ 4) glycosidic bonds. This linkage motif contrasts with that of α(1→4) glycosidic bonds present in starch, glycogen and other carbohydrates. Cellulose is very strong and its links are broken by cellulase enzyme cleaving the molecule by the addition of water molecules (Hamelinck, 2005).

$$\left[C_6H_{10}O_5\right]_n + nH_2O \rightarrow nC_6H_{12}O_6$$

Cellulose is a straight chain polymer in which unlike starch, no coiling occurs and the molecule adopts an extended and rather stiff rod-like conformation. The multiple hydroxyl groups on the glucose residues from one chain form hydrogen bonds with oxygen molecules on another chain holding the chains firmly together side by side and forming microfibrils with high tensile strength (Figure 3) (http://www.en.wikipedia.org/wiki/cellulose). This strength is important in cell walls, where they are meshed into a carbohydrate matrix, conferring rigidity to plants cells. Compared to starch, cellulose is also much more crystalline. Whereas starch undergoes a crystalline to amorphous transition when heated beyond 60 to 70°C in water (as in cooking), cellulose requires a temperature of 320°C and pressure of 25 MPa to become amorphous in water (Deguchi et al., 2006). Many properties of cellulose depend on its degree of polymerization or chain length and the number of glucose units that make up one polymer molecule. Molecules with very small chain length resulting from the breakdown of cellulose are known as cellodextrins; in contrast to long chain cellulose, cellodextrins are typically soluble in water and organic solvents. Plants derived cellulose is usually contaminated with hemicellulose, lignin, pectin and other substances, while microbial cellulose is quite pure, has much higher water content and consists of long chain (Klem et al., 2005).

Hemicellulose

Hemicellulose in plants is slightly crosslinked and is composed of multiple polysaccharide polymers with a degree of polymerization and orientation less than that of cellulose (Reddy and Yang, 2005). Hemicellulose usually acts as filler between cellulose and lignin and consists of sugars including glucose, xylose, galactose, arabinose and mannose. Mechanically, hemicellulose contributes little to the stiffness and strength of fibers or individual cells (Thompson, 1993). Hemicellulose is more easily hydrolyzed into sugars than cellulose hence fibers con-taining a higher proportion of hemicellulose would be preferable for producing sugars, and eventually for fuels such as ethanol. Hemicellulose is also a low-molecular weight heteropolysaccharide (DP < 200, typically β-1,3 links), with a wide variation in both structure and compo-sition. Commonly occurring hemicelluloses are xylans, arabinoxylan, glucomannan, galacto-glucomann, and so on. In contrast to cellulose, which is crystalline strong, and resistant to hydrolysis, hemicellulose has a random, amorphous structure with little strength (Aristidou and Penttilà, 2000).

Lignin

Lignin on the other hand is a complex aromatic heteropolymer consisting of phenylpropane units (P –coumaryl, Coniferyl and Sinapyl alcohol) synthesized from phenylpropanoid precussors (Adler, 1977). Lignin is divided into two classes namely “guaiacyl lignin” and guaiacyl-syringyl

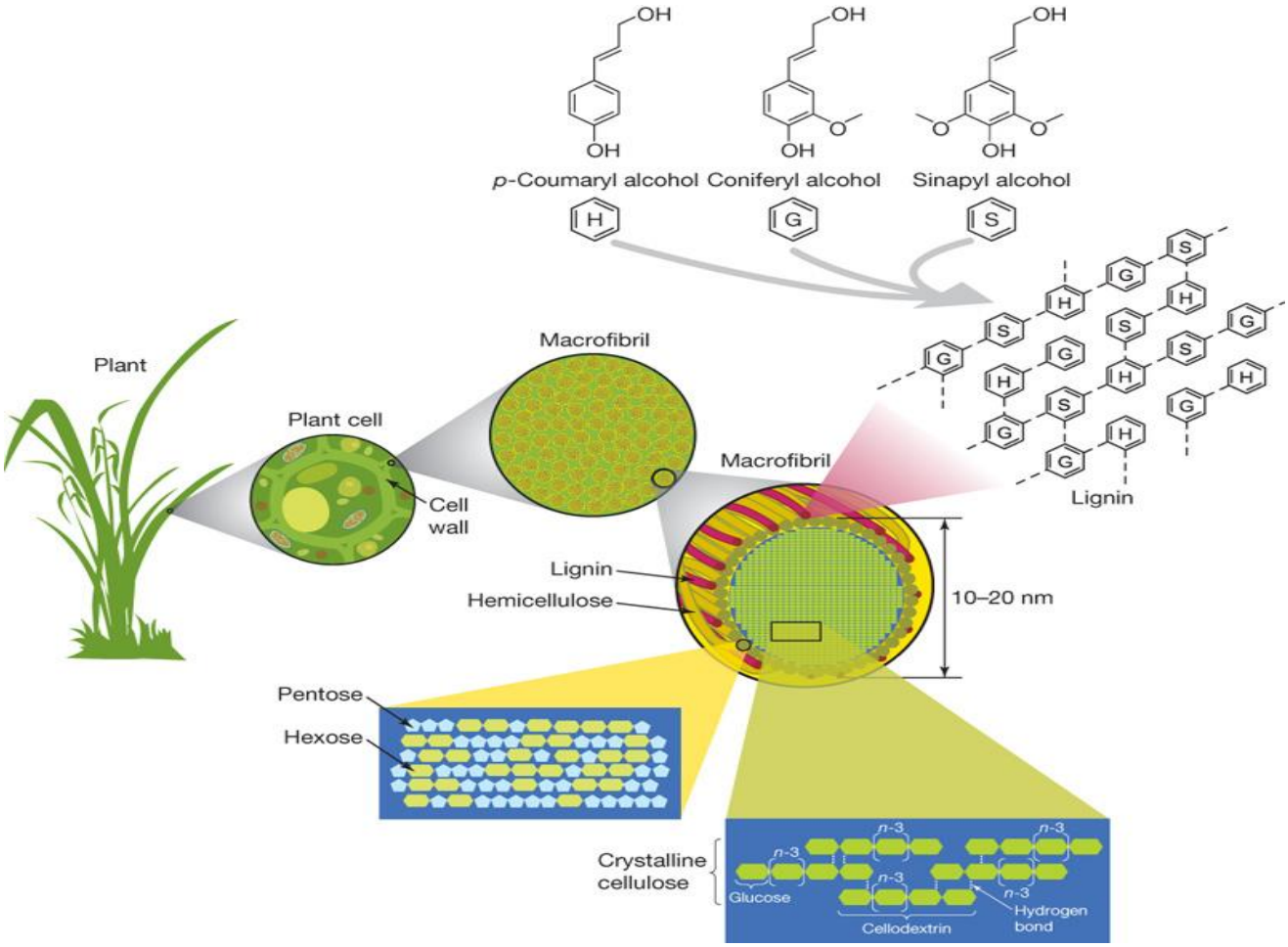

Figure 2. Structure of lignocellulose (adapted from Genomics of cellulosic biofuel) (http://www.nature.com/nature/journal/v454/n7206/full/nature07190.html).

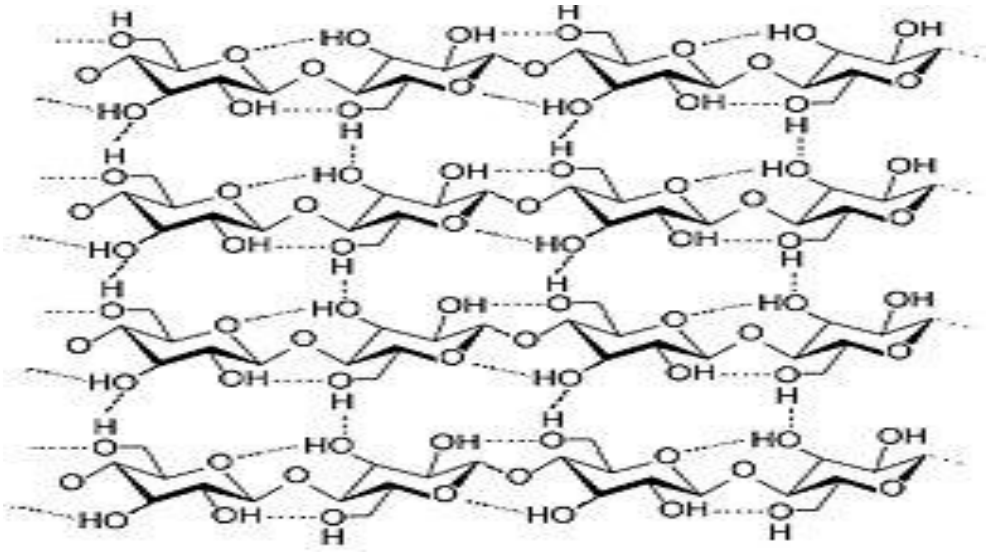

Figure 3. A strand of cellulose, showing the hydrogen bonds (dashed) within and between cellulose molecules (adapted from http://www.en.wikipedia.org/wiki/cellulose).

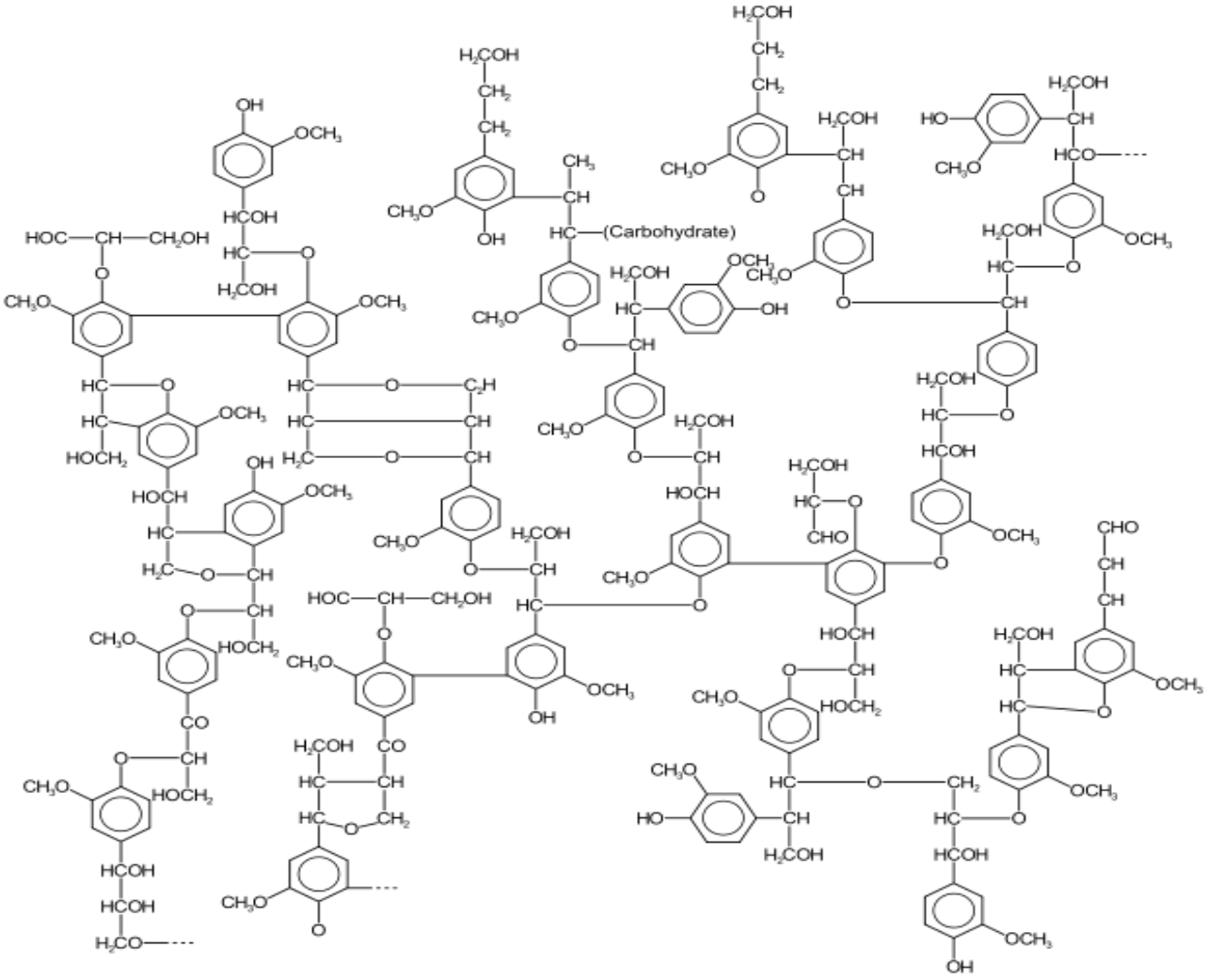

Figure 4. Lignin structure (Adapted from htpp:// www.en.wikilpedia.org/wiki/lignin).

lignin" differing in the substituents of the phenylpropanoid skeleton. Guaiacyl-lignin has a methoxy group in the 3 carbon position, whereas guaiacyl-syringyl lignins have a methoxy group in both the 3-carbon and 5-carbon positions (Figure 4). Softwood and hardwood lignin belong to the second category respectively. Softwoods generally contain more lignin than hardwoods (Saka, 1991) Lignin are cross-linked to each other with a variety of different chemical bonds and acts as glue between individual cells and between the fibrils forming the cell wall (Mohanty, 2000).

Lignin is first formed between neighboring cells in a 'middle lamella' binding them tightly into a tissue, and then spreads into the cell wall penetrating the hemicelluloses and bonding the cellulose fibrils (Majumdar and Chanda, 2001). Lignin degradation is primarily an aerobic process and in an anaerobic environment, lignin can persist for very long periods (Van Soest, 1994). Because lignin is the most recalcitrant component of the plant cell wall, its presence lowers the bioavailability of cellulose and hemicellulose for enzymatic penetration and activity (Haug, 1993). With the advent of modern genetics and engineering tools, the cost of producing sugars from these recalcitrant, lignocellulosic fractions and converting them into products like ethanol has been significantly reduced.

PRETREATMENT OF LIGNOCELLULOSIC BIOMASS AND SACCHARIFICATION

To ensure successful biological conversion of lignocellulosic materials, the interaction between lignin and the polysaccharide components of the cell wall must be reduced through pre-treatment, a process that is considered to be one of the most important steps in the process (Wyman et al., 2005). The purpose of the pretreatment is to alter or disorganize the crystalline structure of macro and microfibrils of lignocellulose in order to release the polymer chains of cellulose and hemicellulose,

and modify the pores in the material to allow enzymes to penetrate into the fibre to render it amenable to enzymatic hydrolysis (Galbe and Zacchi, 2002). Native lingocellulosic biomass is extremely recalcitrant to enzymatic digestion.

The nature of lignocellulosic biomass like cocoa pod husk makes the pretreatment a crucial step due to the physical and chemical barriers caused by the close association of the main components; cellulose, hemicellulose and lignin. During the pretreatment step, the enzyme accessibility to cellulose is enhanced; therefore, the efficiency of cellulases to release fermentable sugars is increased (Almeida et al., 2007). Pretreatment, however, increases the available area in several ways (Zeng et al., 2007).

The pretreatment methods can be divided into physical and chemical methods, and combinations of these two are commonly used (Mosier et al., 2005). The type of feedstck strongly affects the choice of pretreatment method. The hemicellulose is, for instance, acetylated to a high degree in xylan-rich materials (Olofsson et al., 2008). Since acetate is liberated during hydrolysis, the pretreatment of these materials is to some extent autocatalytic and requires less added acid and milder process conditions. However, the liberated acetate adds to the toxicity of the hemicellulose hydrolysates.

Physical pretreatment

Ammonia fiber/freeze explosion (AFEX)

AFEX pretreatment is regarded as an attractive method for pretreatment of agricultural residues, yielding highly digestible cellulose (Dale and Moreira, 1982; Holtzapple et al., 1991). AFEX depolymerizes the lignin, removes the hemicellulose and decrystallizes the cellulose (Iyer et al., 1996; Sharma et al., 2002). The moderate temperature and pH also minimize formation of sugar degradation products. However, the method suffers from high costs of ammonia and ammonia recovery (Holtzapple et al., 1991). In this context, the lime method, based on calcium (or sodium) hydroxide (MacDonald et al., 1983; Chang and Holtzapple, 1997; Sharma et al., 2002) comes under mention. Alkali pretreatments are run at lower temperatures for long residence times, and as for the AFEX method, a delignification of the biomass is obtained.

Steam explosion

Steam explosion is an intensively studied pretreatment method (Mosier et al., 2005). The effects of uncatalyzed steam explosion – and liquid hot water pretreatments – on the biomass are primarily attributed to the removal of hemicelluloses. By adding an acid catalyst, the hydrolysis can be further improved (Brownell and Saddler, 1984). Dilute acid pretreatments using H_2SO_4 (Nguyen et al., 1998; Soderstrom et al., 2003; Sassner et al., 2008) or SO_2 (Clark and Mackie 1987; Clark et al., 1989; Stenberg et al., 1998; Soderstrom, 2002; Ohgren et al., 2005) are the most investigated pretreatment methods because of their effectiveness and inexpensiveness. These methods have been applied in pilot plants and, hence, are close to commercialization (Ropars et al., 1992; Schell and Duff, 1996). Acid catalyzed treatment improves the hemicellulose removal (Brownell and Saddler, 1984) gives a partial hydrolysis of cellulose (Clark and Mackie, 1987; Clark et al., 1989; Nguyen et al., 1998) and alters the lignin structure (Wong et al., 1988; Donaldson et al., 1988; Ramos et al., 1999). The main drawbacks are related to the process equipment requirements (Galbe and Zacchi, 2002; Mosier et al., 2005) and inhibitor formation (Palmqvist, 2000). So far, successful pretreatments with alkali, AFEX and liquid hot water have been limited to agricultural residues and herbaceous crops (Holtzapple et al., 1991; Van Walsum et al., 1996; Kim et al., 2000; Varga et al., 2002), whereas acid catalysed steam pretreatments have generated high sugar yields from these materials as well as from softwood feedstocks (Nguyen et al., 1998; Soderstrom et al., 2002). Typical values for acid catalyzed steam explosion pretreatment of softwood are in the range 2 to 4 (Soderstrom et al., 2002; 2003).

Optimal pretreatment conditions in a simultaneous saccharification and fermentation (SSF) process do not necessarily differ much from those of separate hydrolysis and fermentation (SHF) processes utilizing lignocellulosic biomass. However, several compounds present in pretreatment hydrolysates, which inhibit enzymatic hydrolysis are converted by the fermenting organisms. This is a probable explanation behind the higher reported ethanol yields in SSF compared to SHF (Tengborg et al., 2001; Soderstrom et al., 2005). Inhibitor formation from the pretreatment may therefore be tolerated to a higher extent in an SSF process. Inhibitory compounds can be put into three major groups; furaldehydes, weak acids, and phenolics. The two most common furaldehydes, 5-hydroxymethyl-2-furaldehyde (HMF) and furfural (2-furaldehyde) are formed at severe conditions from hexoses and pentoses, respectively (Ulbricht et al., 1984; Palmqvist and Hahn-Hagerdal, 2000). Weak acids from lignocellulosic materials, such as acetic, formic and levulinic acid are mainly formed by de-acetylation of hemicellulose or HMF breakdown (Ulbricht et al., 1984). Phenolic compounds are formed chiefly during lignin breakdown, and are to be found in numerous variants, depending on the type of lignin (Perez et al., 2002).

Chemical treatment

Liquid hot water (LHW)

Liquid hot water pretreatment is one of the oldest methods applied for pretreatment of cellulosic materials. Autohydrolysis plays an important role in this process, where no chemical is added. It results in dissolution of

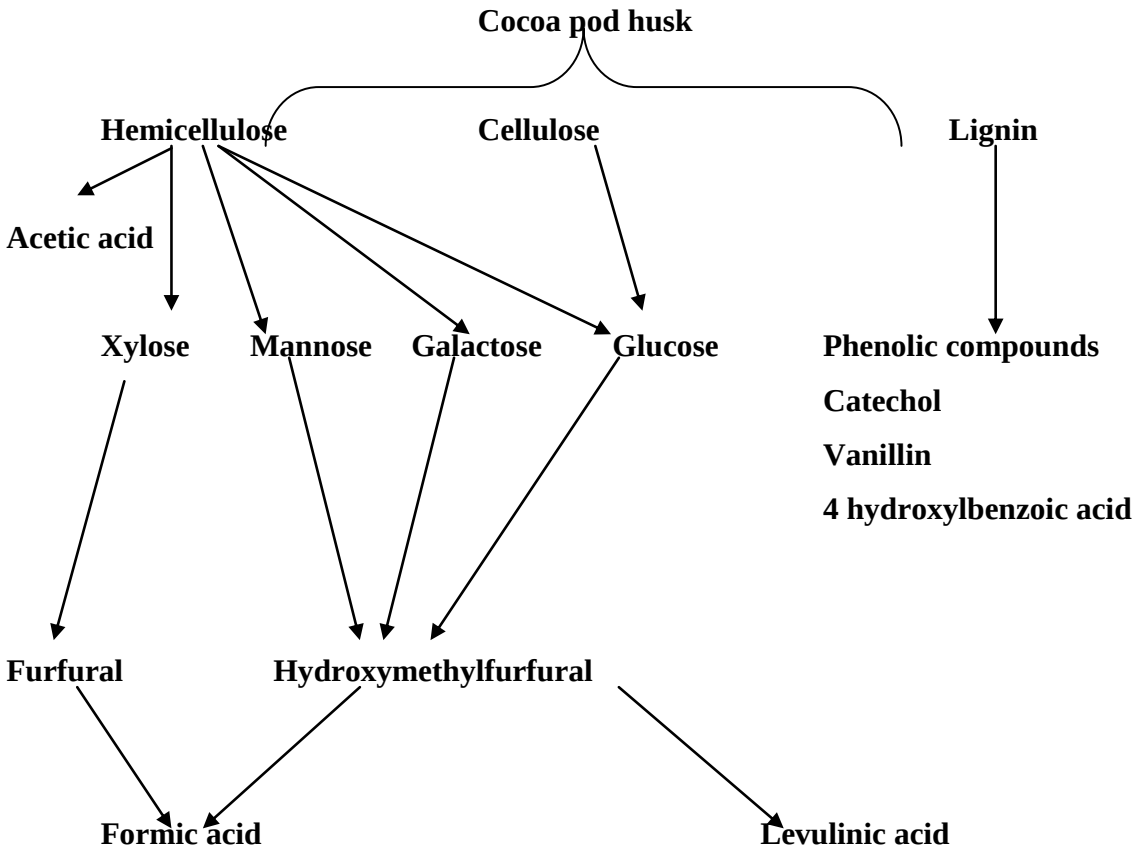

Figure 5. Reactions occurring during the hydrolysis of lignocellulosic materials. Source: Igbinadolor, 2012.

hemicelluloses mostly as liquid-soluble oligosaccharides and separates them from insoluble cellulosic fractions. The pH, processing temperature and time should be controlled in LHW pretreatment in order to optimize the enzymatic digestibility of lignocellulosic materials (Wyman, 1996; Mosier et al., 2005). LHW pretreatment of corn fibre at 60°C and a pH above 4.0 dissolved 50% of the fibre in 20 min (Mosier et al., 2005). It has also been established that LHW pretreatment of particle size-reduced cocoa pod husk achieved considerable dissolution of the fibre at 130°C at a pH of 5.0 for 1 h (Igbinadolor, 2012). LHW causes ultrastructural changes and formation of micron-sized pores that enlarge accessible and susceptible surface area and make the cellulose more accessible to hydrolytic enzymes (Zeng et al., 2007).

Hydrolysis

The goal of this process is to generate fermentable monomeric sugars from cellulose and hemicellulose content of lignocellulosic biomass. This process can be accompanied by two different processes, which are acid hydrolysis and enzymatic hydrolysis. The main degradation product during hydrolysis of cocoa pod husk is schematically represented in Figure 5 (Igbinadolor, 2012).

Acid hydrolysis

This process utilizes mineral acids such as hydrochloric acid, sulfuric acid, nitric acid among others, which are widely employed for the biomass hydrolysis. Acid hydrolysis is a well-established process (Parisi, 1989; Joshi et al., 2011), which gives good yields within a short reaction time. It has, however, several drawbacks, such as for example the requirement of costly corrosive-resistant construction materials (Nguyen, 1993). Furthermore, acid hydrolysis gives rise to inhibitory compounds which might inhibit the ethanolic fermentation (Olsson and Hahn-Hagerdal, 1996; Larsson et al., 1999). Therefore, enzymatic hydrolysis offers advantages. Lignocellulosic materials must be pretreated prior to enzymatic hydrolysis in order to make the cellulose macromolecules accessible for the cellulolytic enzymes. There are several advantages and disadvantages of dilute-acid and enzymatic hydrolyses, which are listed in Table 3. Enzymatic hydrolysis is carried out under mild conditions, whereas acid hydrolysis requires high temperature and low pH, which results in corrosive conditions. While it is possible to obtain cellulose hydrolysis of close to 100% by enzymatic hydrolysis (Ogier et al., 1999), it is difficult to achieve such high yield with the acid hydrolyses. Furthermore,

Table 3. Comparison between dilute-acid and enzymatic hydrolyses.

Comparing variable	Dilute-acid hydrolysis	Enzymatic hydrolysis
Mild hydrolysis conditions	-	+
High yields of hydrolysis	-	+
Product inhibition during hydrolysis	-	+
Formation of inhibitory by-products	+	-
Low cost of catalyst	+	-
Short time of hydrolysis	+	-

Source: Taherzadeh and Karimi (2007) as modified by Igbinadolor (2012).

several inhibitory compounds are formed during acid hydrolysis, whereas this problem is not so severe for enzymatic hydrolysis (Wyman 1996; Lee et al., 1999; Taherzadeh 1999).

Lime pretreatment and detoxification

The toxicity of lignocellulosic hydrolysate can be removed by optimized over liming with lime. Since the least expensive alkali is lime, available either as quicklime (CaO) or slaked lime ($Ca(OH)_2$), pretreatment with this chemical provides a low-cost alternative for lignin removal at higher pH values (Kim and Lee, 2005; Chang et al., 1998). This process results in the removal of all lignin and part of hemicellulose. It also increases the reactivity of cellulose in further hydrolysis steps during enzymatic step through opening up of the structure and reducing the non-productive cellulase adsorption (Hamelinck et al., 2005). Effective removal of lignin minimizes adsorption of enzyme onto lignin and thus allows for effective interactions with cellulose (Aswathy et al., 2010). Pretreatment with sodium hydroxide causes swelling of the fibres and increases the digestibility of cellulose from 14 to 55% while decreasing the lignin content from 25 to 20% (Kumar et al., 2009). However, lime treatment has been less effective on woody biomass than for many herbaceous plants or agricultural residues at the same process conditions because of the generally higher lignin content of wood (Yang and Wyman, 2007). Alkali pretreatment process has been shown to decrease sugar degradation and is more effective on agricultural residues as compared to woody materials (Kumar et al., 2009). Between NaOH and $Ca(OH)_2$, pretreatment with calcium hydroxide is preferable because it is less expensive, more safer as compared to NaOH and it can easily be recovered from the hydrolysate by reaction with carbon dioxide (Mosier et al., 2005).

Enzymatic hydrolysis

Enzymatic hydrolysis of cellulose to glucose is carried out by cellulase enzymes that are highly specific catalysts. The hydrolysis is performed under mild conditions of pH 4.5 to 5.0 and temperature of 40 to 50°C. Therefore, one may expect low corrosion problems, low utility consumption and low toxicity of the hydrolysates as the main advantages of this process (Taherzadeh and Karimi, 2007). Enzymatic hydrolysis of cellulose consists of the cellulase adsorption unto the surface of the cellulose, the biodegradation of cellulose to sugars and desorption of the cellulose.

Important factors in enzymatic hydrolysis

Substrate concentration and quality, applied pretreatment method, cellulose activity, and hydrolysis conditions such as temperature, pH, and mixing are the main factors in enzymatic hydrolysis of lignocellulosic materials. The optimum temperature and pH are functions of the raw material, the enzyme source, and hydrolysis duration. The optimum temperatures and pH of different cellulases are usually reported to be in the range of 40 to 50 °C and pH 4 to 5 (Olsson and Halm-Hagerdal, 1996).

Addition of surfactants during hydrolysis can modify the cellulose surface properties, block lignin and enhance enzymatic saccharification of cellulose (Tu et al., 2009). An important effect of surfactant addition in a process for lignocellulose conversion is the possibility to lower the enzyme loading. A number of surfactants have been examined for their ability to improve enzymatic hydrolysis. Non-ionic surfactants were found to be the most effective. Fatty acid esters of sobitan polyethozylates (Tween-20 and Tween-80), and polyethylene glycol, are among the most effective surfactants reported for enzymatic hydrolysis (Alkasrawi et al., 2003; Kim et al., 2006; Borjesson et al., 2007). Addition of polyethylene glycol to lingocellulose substrates increased the enzymatic conversion from 42 to 78% in 16 h (Borjesson et al., 2007). One reason for this effect might be the adsorption of surfactants to lignin, which prevents unproductive binding of enzymes to lignin and results in higher productivity of the enzymes (Eriksson et al., 2002) and consequently improve the efficiency of cellulose hydrolysis (Igbinadolor, 2012; Yasmashita et al., 2010). However, the surfactant should be selected carefully, since it may have negative impact on the fermentation of the hydrolysate. For instance, addition of 2.5 g/l Tween-20 helped to reduce enzyme loading by 50%, while retaining cellulose conversion (Eriksson et al., 2002). However, this surfactant is an inhibitor to *D. clausenii* even at low concentration of

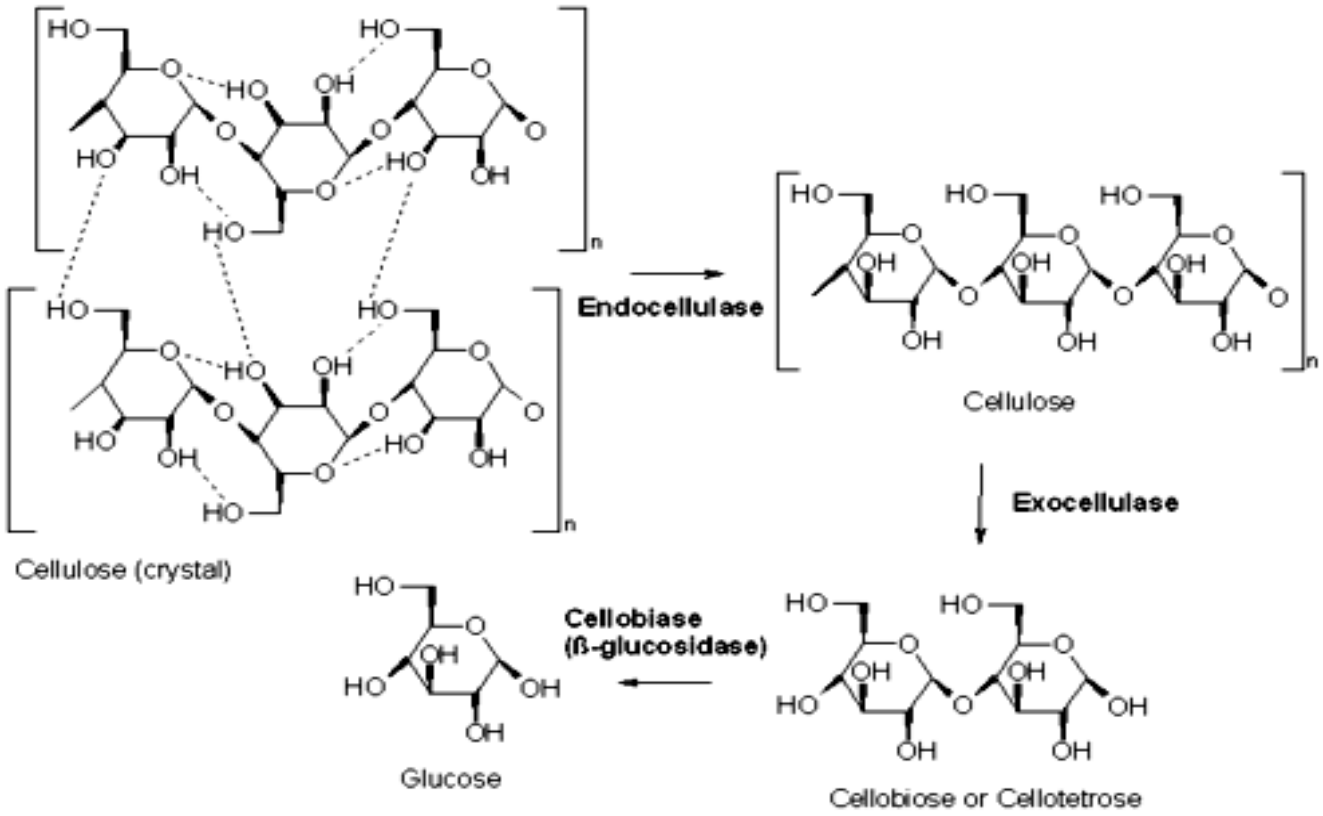

Figure 6. The 3 types of reaction catalysed by cellulases. Breakage of the non-covalent interactions present in the crystalline structure of cellulose (endo-cellulase) 2. Hydrolysis of the individual cellulose fibres to break into smaller sugars (exocellulases) 3. Hydrolysis of disaccharides and tetrasaccharides into glucose (beta-glucosidase) (adapted from http://www.en.wikilpedia.org/wiki/cellulose).

1.0 g/l (Wu and Ju 1998).

The recycling of cellulose enzymes is one potential strategy for reducing the cost of the enzymatic hydrolysis during the bioconversion of lignocelluloses to ethanol (Tu et al., 2007). However, presence of solid residuals (mainly lignin) and dissolution of the enzymes in the hydrolysates make the enzymes difficult to separate. Immobilization is an alternative approach that can be used to retain the enzymes in the reactor, but steric hindrance, freedom of movement and gradual reduction of the cellulases activity must be considered. In this regard, it should be kept in mind that endoglucanase and exoglucanase should diffuse into lignocelluloses and be adsorbed to the surface of the particles in order to initiate hydrolysis and convert the cellulose to cellobiose. However, cellobiose is in the aqueous phase, where it is converted to glucose by β-glucosidase. Therefore, immobilization of β-glucosidase might theoretically be possible and effective (Tu et al., 2006). It is also possible to co-immobilize β-glucosidase and a fermenting microorganism in order to improve the overall conversion of cellulose to ethanol (Lee and Woodward, 1983). One of the major problems in immobilization is to separate the immobilized support from the residual solid of the reactor. One possible solution could be immobilization of the enzymes in magnetic particles, such as magnetic agarose composite microspheres (Qiu and Li, 2000; 2001), or magnetic chitosan microspheres (Feng et al., 2006).

Enzymes used in hydrolysis

Cellulases: Enzymes specialized in breaking up the β-1-4-glycosidic bonds of glucan are collectively called cellulases. Reese et al. (1950) presented a model of enzymatic cellulose hydrolysis based on multiple enzymes (C_1 and C_X). The C_1 enzyme was assumed to produce shorter polyanhydro-glucose chains, while the solubilization was attributed to the C_X enzyme. The cellulases are divided into three sub-categories, representing three types of activity: an endo-1,4-β-glucosidase (EC 3.2.1.4) (endoglucanases), an exo-1,4-β-glucanase (EC 3.2.1.91) exoglucanases (cellobiohydrolases) and β-glucosidases (EC 3.2.1.21). Endoglucanases significantly reduce the degree of polymerization of the substrate by randomly attacking the interior parts, mainly in the amorphous regions of cellulose to create free ends. Exoglucanases (or cellobiohydrolases), on the other hand, incrementally shorten the glucan molecules by binding to the glucan ends and relea-

sing mainly cellobiose units. Finally, β-glucosidases split the disaccharide cellobiose into two units of glucose as represented in Figure 6.

Synergism between these two types of enzymes is attributed to the *endo-exo* form of cooperativity and has been studied extensively between cellulases in the degradation of cellulose by *Trichoderma reesei* (Bothast and Saha, 1997). β-Glucosidases hydrolyze cellobiose and in some cases cellooligosaccharides to glucose. This type of enzyme is generally responsible for kinetic regulation of the whole cellulolytic process and is a rate-limiting factor during enzymatic hydrolysis of cellulose, as both endoglucanase and cellobiohydrolase activities are often inhibited by cellobiose (Bothast and Saha, 1997). Thus, β-glucosidase, not only produces glucose from cellobiose but also reduces cellobiose inhibition, allowing the cellulolytic enzymes to function more efficiently. However, like β-glucanases, most β-glucosidases are subject to end-product (glucose) inhibition (Saha et al., 1995). Several types of microorganisms can produce cellulase systems including aerobic filamentous fungi, aerobic actinomycetes, anaerobic hyperthermophilic bacteria and anaerobic fungi (Lynd et al., 2002). Intensive research on the aerobic filamentous fungi *Trichoderma reesei* during the past decades has resulted in an efficient cellulase-producing organism, which is currently dominating the Industrial cellulase production (Esterbauer et al., 1991; Lynd et al., 2002).

Fermentation

Fermentation is the enzymatic oxidation of compounds by the action of microorganisms. The ability to ferment pentoses along with hexoses is not wide spread among organisms. *Saccharomyces cerevisiae* is the most favored organism for ethanol production from hexoses due to their high ethanol tolerance, being able to out-compete other yeasts and greater resistance to contamination and inhibitors generated from biomass (Jeffries, 2006). The most promising yeasts that have the ability to use both pentose and hexose sugars are *Pichia stipitis*, *Candida shehatae* and *Pachysolen tannophilus* (Parekh et al., 1986), however these organisms have low ethanol tolerance and highly sensitive to inhibitors generated from lignocellulosic biomass. Based on this, researchers are now focusing on developing recombinant yeasts, which can greatly improve the ethanol production yield by metabolizing all forms of sugars and reduce the cost of operation.

Among the pentose fermenting organisms, *P. stipitis* has been shown to have most promise for industrial applications (Agbogbo et al., 2006). For example, the hemicellulosic hydrolysates of *Prosopis juliflora* (18.24 g sugar/L broth) when fermented with *P. stipitis* produced 7.13 g/L ethanol (Gupta et al., 2009). Detoxified xylose rich hydrolysate of *Lantana camara* when fermented with *P. stipitis* 3498 at pH 5 and 30°C for 36 h resulted 0.33 g alcohol/g lignocelluloses used (Kuhad et al., 2010). In yet another example, the detoxified water hyacinth hemicellulose acid hydrolysate (rich in pentose sugars) fermented with *P. stipitis* NCIM-3497 at pH 6-0 and 30°C resulted 0.425 g ethanol/g lignocellulose. *Candida tropicalis* is also capable of fermenting xylose (pentoses) under oxygen limited conditions in presence of increasing concentrations of polyethylene glycol (Hagerdal et al., 1985). Genetically engineered strains of *Escherichia coli, S. cerevisiae,* and *Zymomonas mobilis* have been developed to ferment xylose (Kim et al., 2005).

GENETIC ENGINEERING PROSPECT FOR BIOETHANOL PRODUCTION FROM BIOMASS

Since no naturally-occurring organism can satisfy all the specifications needed for lignocellulosic fermentation, genetic engineering techniques have been utilized with the aim of constructing organisms with most desirable properties for bioprocesses (Igbinadolor, 2012; Aristidou and Pentilla, 2000). Metabolic engineering was introduced by Bailey in 1991 as a subdiscipline of engineering and pertains to "the improvement of cellular activities by manipulation of enzymatic, transport, and regulatory functions of the cell with the use of recombinant DNA technology" (Bailey et al., 1990; Bailey 1991). Metabolic engineering, that is, the intentional redirection of metabolic fluxes, has played an exceptional role in improving strains of organisms for all industrial applications. In contrast to classical methods of genetic strain improvement such as selection, mutagenesis, mating, and hybridization (Panchal 1990; Attfield and Bell 2003), metabolic engineering has conferred several advantages: (i) Extending existing pathway to produce novel product; (ii) shifting metabolic flux towards synthesis of desired end product; (iii) accelerating rate determining step; (iv) the directed modification of strains without the accumulation of unfavorable mutations.; (v) the introduction of genes from foreign organisms to equip desired organisms with novel traits. The latter is particularly crucial for industrial biotechnology to provide pathways that extend the spectrum of usable industrial media (for example, lignocellulosic biomass) and/or to produce compounds not naturally formed. Since the first introduction of metabolic engineering (Bailey, 1991), there have been tremendous enhancements of its toolbox and have greatly enhanced the potential for using yeast in biotechnological production processes.

Metabolic engineering via application of recombinant DNA technology to direct the production of bioethanol from lignocellulosic biomass is an emerging field. The genes for pentose utilization encoded by xylose reductase (XR) and xylitol dehydrogenase (XDH) from *P. stipitis* CBS 6054 have been successfully cloned into *S. cerevisiae* yeasts (Igbinadolor, 2012). This has resulted in high level expression of both pentose degrading genes (YHR104w and YLR070c) in starter cultures of *S. cerevisiae* yeast leading to increased production of ethanol from cocoa pod husk hydrolysate (Igbinadolor, 2012).

To accomplish this, the coding sequences of XR and XDH from *P. stipitis* CBS 6054 were first cloned into the expression vectors pGAPZA and pVT100-U, respectively, and then transformed into the starter cultures of *S. cerevisiae* yeasts. Also, the genes for alcohol degydrogenase II and pyruvate decarboxylase from *Z. mobilis* have been successfully inserted into *E*. *coli* resulting to increased production of ethanol (Ingram et al., 1987).

ETHANOL AND ENVIRONMENT

Ethanol represents closed carbon dioxide cycle because after burning of ethanol, the released carbon dioxide is recycled back into plant material because plants used CO_2 to synthesize cellulose during photosynthesis cycle (Wyman, 1999; Chandel et al., 2007). Ethanol production process only uses energy from renewable energy sources; no net carbon dioxide is added to the atmosphere, making ethanol an environmentally beneficial energy source. In addition, the toxicity of the exhaust emissions from ethanol is lower than that of petroleum sources (Wyman and Hinman, 1990). Ethanol derived from biomass is the only liquid transportation fuel that does not contribute to the green house gas effect (Foody, 1988). As energy demand increases the global supply of fossil fuels cause harm to human health and contributes to the green house gas (GHG) emission, (Hahn-Hagerdal et al., 2006) alarmed to the society by seeing the security of oil supply and the negative impact of the fossil fuel on the environment particularly on GHG emissions. The reduction of GHG pollution is the main advantage of utilizing biomass conversion into ethanol (Yang and Wyman, 2008). Ethanol contains 35% oxygen that helps complete combustion of fuel and thus reduces particulate emission that pose health hazard to living beings. A study conducted on the ethanol blended diesel (E10 and E30) combustion at different loads found that addition of ethanol to diesel fuel simultaneously decreases cetane number high heating value, aromatics fractions and kinematic viscosity of ethanol blended diesel fuels and changes distillation temperatures. These factors leads to the complete burning of ethanol and less emissions. With its ability to reduce ozone precursors by 20 to 30% bio-ethanol can play a significant role in reducing the harmful gasses in metro cities world wide. Ethanol blended diesel (E-15) causes the 41% reduction in particulate matter and 5% Nox emission (Chandel et al., 2006).

CONCLUSION AND FUTURE PROSPECTS

There is increasing demand for transportation fuels across the globe. This demand is abnormally affecting developing countries in particular. The high demand in transportation fuel may be mitigated by ethanol in the scenario of shrinking economic and energy resources. In spite of laboratory based bioethanol success stories, the production of fuel ethanol at plant scale still remains a challenging issue. A positive solution to this issue could bring economic advantage not only for fuel and power industry, but also benefit the environmental rehabilitation. Lignocellulosic biomass is in abundance, do not compete with food materials and are renewable. Hence, bioethanol production from biomass holds tremendous potentials in terms of meeting energy needs and providing environmental benefits. Apart from bioethanol, a wide range of chemicals and value added products can be produced from lignocellulosic biomass. The improvement in pretreatment processes, improvement in the efficacy hydrolysis through robust enzyme system, acid hydrolysis, development of good fermentation process through genetic modification of organisms and removal of toxic by product through liming is an efficient technology process for the generation of ethanol from lignocellulose. Further more, the production of bioethanol from cellulosic biomass will enhance sustainability and its continual use will affect positively the stability of the ecosystems and global climate as well as global oil reserves.

Conclusively, the adaptation of this technology in developing nations like Nigeria is a far sighted vision that will make a mile stone in the history of Nigeria transformation agenda. Ethanol has many desirable features as a petroleum substitute and could obviously help in making a smoother transition from a monolithic petroleum based energy source to a diverse bio-based chemical economy.

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