



Commercial feasibility of lignocellulose biodegradation: possibilities and challenges

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The main source of energy supply worldwide is generated from fossil fuels, which undoubtedly are finite and non-environmental friendly resources. Bioethanol generated from edible resources also has economic and environmental concerns. Despite the immense attention to find an alternative (inedible) source of energy in the last two decades, the total commercial production of 1st generation biofuels is limited and equivalent only to approximately 3% of the total road transport fuel consumption. Lignocellulosic waste represents the most abundant biomass on earth and could be a suitable candidate for producing valuable products including biofuels. However, cellulosic bioethanol has not been produced on a large scale due to the technical barriers involved that make the commercial production of cellulosic bioethanol not economically feasible. This review examines some of the current barriers to commercialization of the process.

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Introduction

One of the main contemporary issues facing society today is the development of sustainable energy from cheap, abundant and non-edible materials to enable its commercialization before the main energy sources are depleted. Throughout the twentieth century, fossil fuels (crude oil, coal and natural gas) were heavily exploited as the main source of energy. Currently, the main source (80–88%) of

energy supply worldwide is generated from fossil fuels, which undoubtedly are finite and exhaustible resources and their current rate of consumption cannot be sustained in the long term [1,2,3**].

The majority of fossil fuel consumption is used in the transportation sector (60–65%) which is expected to increase dramatically as the world population increases [2,4]. In 2008, there were about 700 million light duty vehicles, automobiles, light trucks and minivans being driven on roadways around the world. This number is expected to at least double (to 1.3 billion vehicles by 2030) or perhaps triple (over 2 billion vehicles) by 2050. Although short-term prices of oil have currently dropped, the projected gap between the universal crude oil demand and the total actual production is probably to increase rapidly in future years leading once again to skyrocketing prices of fossil fuels in the long term. Furthermore, the overconsumption of these finite resources has also significantly contributed to security concerns of the oil supply and environmental pollution worldwide [5]. For example, transport vehicles account for a substantial percentage of air pollution globally, from each gallon of gasoline being burnt or used by a vehicle, about 8.16 kg of carbon dioxide (CO₂) is emitted. The adverse effects of using fossil fuels as the main source of energy are well documented and include environmental pollution such as increasing global carbon dioxide (CO₂) emissions [6].

It is not only that fossil fuels are the main source of air pollution, open-field burning of lignocellulosic waste (LCW) is a globally common practice as it represents a cheap, fast and practical means of preparing the field for the next crop. Air emissions from the burning process of wood, crop residues and other lignocellulosic biomass are not only a threat to public health but also wasting our natural resources. The burning process reduces the local air quality, creating a variety of health concerns from the discharge of carcinogenic oxides (NO_x, SO₂ and CO_x) into the atmosphere leading to asthma or pulmonary morbidity in humans. It has been estimated that annual emissions from open-field burning of lignocellulosic biomass were approximately 0.37 Tg of SO₂, 2.8 Tg of NO_x, 1100 Tg of CO₂, 67 Tg of CO and 3.1 Tg of methane (CH₄) [2,4,7,8].

Reducing the accumulated, atmospheric CO₂ concentration can be achieved whether by reducing the use of fossil fuels or utilising LCW in a more environmentally friendly manner [7]. The pressure on society to find suitable,

renewable, less polluting and cheap sources of bioenergy and the desire to promote domestic rural economies are substantially increasing globally [4]. Therefore, there is an immense interest in the production of renewable biofuels from sustainable, cheap, plentiful and clean resources such as lignocellulosic biomass. A gradual shift from exhaustible fuels to sustainable biofuels will not only reduce the reliance on finite and expensive oil imports but will also help to maximise economic, environmental and social benefits [2,8].

Production of bioethanol from edible resources

Biofuels are derived from different sustainable feedstocks such as edible sugar or starch-based crops (corn in the USA and sugarcane in Brazil) and non-edible resources such as LCW which represent first and second generations of bioenergy substrates respectively [9]. Bioethanol produced from the 1st generation biofuels can be blended with current petroleum-based fuels either as a 10% (v/v) blend up to an 85% (v/v) blend, as well as being used as a pure hydrated ethanol [2,8,10]. The superior characteristics of edible bioethanol in the transportation sector also make it an excellent biofuel and suitable source for current and future advanced vehicles without major modifications [5,8].

Bioethanol has already been produced commercially in Brazil and the US beginning in 1975 using different edible feedstocks (sugarcane and corn respectively), and despite the problems associated with these bioenergy resources, it is still among the most promising renewable biofuels mainly for the transportation sector [8]. Although the 1st generation of biofuels offers great potential commercially as an alternative, sustainable, less polluting source compared to fossil fuel, it has considerable economic, food security and environmental limitations. The first generation of biofuels appears unsustainable on the basis of food-based feedstocks like corn, sugarcane or wheat as raw material, as it raises major nutritional and ethical concerns. Therefore, the first generation of biofuels (from edible resource) has been considered as

socially flawed, as more than 800 million people suffer from hunger and starvation [11–13]. Moreover, the current production of 1st generation biofuels (less than 840 thousand barrels per day), although it has increased sevenfold since 2000, met only 2.3% of the total final transportation fuel demands [8]. Therefore, other cheap, plentiful and untapped feedstocks are urgently required for long term production [13].

Potential of LCW for biofuel production

Compared to fossil fuels, LCW are geographically evenly distributed globally and abundantly available. It was estimated that the annual production of these materials ranged between 10 and 50 billion tonnes worldwide, accounting for about half of the global biomass yield [3^{**},14,15]. Lignocellulosic material represents the most abundant biomass on earth and could be a suitable candidate for producing valuable products including biofuels. However, cellulosic bioethanol has not been produced on large scale due to the technical barriers involved such as commercial feasibility of lignocellulose biodegradation into fermentable sugars.

Wheat straw and rice straw are by far the most abundant agricultural wastes globally. The annual average production of wheat, rice and sugarcane straw worldwide is 354, 731 and 181 million tonnes, respectively (Table 1) [16]. Globally about 200 billion tonnes of plant biomass are produced annually and more than 90% of the total production of plant biomass is classified as LCW [17]. These waste materials are often available at very low cost and as a cheap substrate for commercial biofuel production. Previous studies suggest that LCW can be an ideal feedstock for biofuel production [8,16,18–21]. Different types of lignocellulosic residues could be used for biofuels production such as straws, crop residues, wood pellets, wood chips and agro-waste [22^{**},23^{*}].

Because of the low price, availability throughout the year and wide distribution geographically, LCW is considered not only the most feasible option for biofuel production but also for fossil fuel replacement since these raw materials do

Table 1

Composition and potential of edible and non-edible feedstocks for bioethanol production

Biofuel resources	Carbohydrates (%)			Residue:crop ratio	Dry matter (%)	Average of worldwide production (1997-2001 in million tonne)	Bioethanol production potential (L per tonne of dry biomass)	Reference
	Cellulose	Hemicellulose	Lignin (%)					
Wheat straw	32.9–50	24–35.5	8.9–17.3	1.3	90.1	354.34	280–290	[8,24–28]
Wheat	35.85		NA		89.1	594.01	340–400	
Rice straw	36.2–47	19–24	9.9–24	1.4	88.0	731–900	280	
Rice	87.5		NA		88.6	590.87	430–480	
Sugarcane straw	40–41.3	27–37.5	10–20	0.6	71.0	180.73	280	
Sugarcane	67		NA		26.0	1266.60	70–500	
Corn stalks	35–39.6	16.8–35	7–18.4	1.0	78.5	203.61	225.7–290	
Corn	73.7		0.6		86.2	603.30	360–460	

not compete with food crops and have the significant potential of bioethanol productivity compared to edible resources (Table 1). For instance the bioethanol production from wheat straw (inedible) is expected to be 290 L/1000 kg of dry biomass compared to edible bioethanol generated from wheat 340 L/1000 kg of dry biomass (Table 1) [8,24–28]. LCW has the potential capability to produce about 4.19×10^{11} litres (419 GL) of bioethanol annually [27]. An earlier estimate reported that the potential ethanol that could be derived from corn stover alone in the US was 15 billion litres per year.

Technical obstacles involved in the biodegradation of lignocellulosic biomass

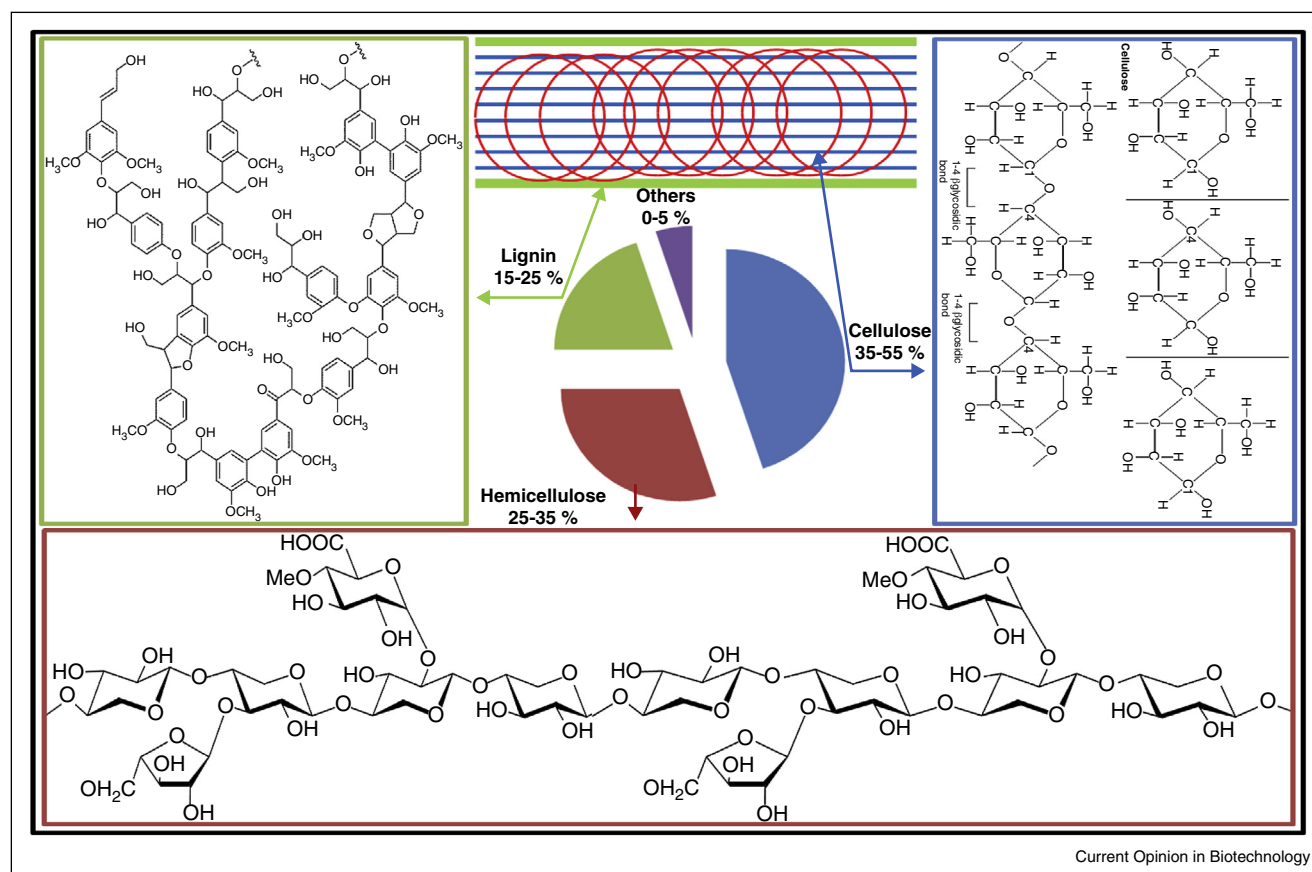
Despite the fact that cellulosic biofuels offer great promise as cheap, sustainable feedstock supplies, conflict-free with food production, environmentally friendly and above all able to reduce the world's dependence on expensive fossil fuels, producing cellulosic bioethanol from LCW presently is not commercially feasible [10]. Cellulosic biofuels cannot yet be produced economically on a large scale because there are a number of technical barriers which make the operational and production costs ineffective commercially

[9,29]. A major current limitation of the cellulosic bioethanol at the commercial scale is the complete biodegradation of lignocellulosic biopolymers components into reducing sugars [4,10,11,30]. The three main components of LCW are cellulose, hemicellulose and lignin (Figure 1). Lignocellulosic biomass is mainly composed of structural carbohydrates in biopolymer forms (such as cellulose and hemicellulose) and non-carbohydrates polymer (such as lignin) [31,32]. These three components of lignocellulosic biomass (containing 55–75% carbohydrates), create a heterogeneous complex of carbohydrate polymers (cellulose and hemicellulose), and lignin (which imparts further strength), to form a tightly packed, tough and complex, water-insoluble structure that is resistant to depolymerisation by microbial and chemical attack [33–35].

Pre-treatment of lignocellulosic biomass

Cellulose is coated or sheathed by hemicellulose which acts as a blocking seal limiting the access of cellulase and hemicellulase enzymes to the cellulose–hemicellulose complex [36]. In addition, the polysaccharide biopolymers (cellulose–hemicellulose complexes) are encapsulated with lignin which forms a physical barrier that

Figure 1



General structure of lignocellulosic biomass, average of each component (cellulose, hemicellulose and lignin) and the chemical structure of cellulose, hemicellulose and lignin [28].

increases resistance to chemical and microbial attack and hinders polysaccharide polymer hydrolysis into reducing sugars [33,34,37]. Therefore, a pre-treatment step is required to enhance the digestibility of lignocellulosic material effectively. There are many biological, chemical, physio-chemical and physical processes used singly or in combination for the pre-treatment of lignocellulosic biomass. The effects of different pre-treatments on the physical and chemical composition of lignocellulose are

well-reviewed (see Table 2) but no pre-treatment process has yet proven to be generally applicable [34,38].

Conversion of lignocellulosic biomass into reducing sugars

Coupled with optimising an efficient pre-treatment method, LCW must then undergo (chemically or biologically) hydrolysis, the saccharification of lignocellulosic material into fermentable sugars [39]. The conversion

Table 2

Different pre-treatment methods of lignocellulosic waste

Pre-treatment	Energy source	Effect	Advantages and disadvantages	References
Physical	Mechanical (milling, grinding or chipping) Wet, dry, vibratory and compression milling Comminution (ball mill, colloid, hammer, compression) Irradiation (electron beam, gamma-ray, microwave) Electric (pulsed electrical field) and sonication	Disrupt plant cells, increase pore sizes Decrease particle size and cellulose crystallinity Soften and partially depolymerize lignin Partially alter the structure of cellulose and hemicelluloses	No toxic waste and inhibitors from this processes Simple equipment and simple process but high power consumption Partial destruction of cellulose, hemicellulose and incomplete disruption of the lignin Cost depends on the initial and final particle sizes	[24,27,28]
Chemical	Organosolvent using different solvents Acid and alkaline hydrolysis Oxidation using oxidising agents	Extract lignin and improve hydrolysis Decrease the crystallinity of cellulose Digests lignin matrix Makes cellulose and hemicellulose more accessible Disrupt cell wall components	Fast and ideal for biomass with low-lignin content High in delignification and saccharification Economic only when solvents are recycled Expensive and explosive chemicals and solvents Generation of inhibitory compounds Water consumption Energy cost is very expensive (cost about \$60 per tonne dry biomass) Simple, has a short process time and low enzyme loading is required Energy and chemical consumption Requires specific costly equipment The recovery of ammonia is necessary to be economically feasible Consume 8.8 tonne water per tonne of dry biomass Expensive and can pollute the environment Produces undesired inhibitor compounds such as furfural, carboxylic acid (LHW) Less efficient for biomass with high lignin content	
Physio-chemical	Steam explosion or auto hydrolysis Ammonia fibre explosion (AFEX) Steam explosion or using hot water (liquid hot water) CO ₂ explosion	Makes biomass more accessible to cellulase attack Improve the efficiency of downstream processing High recovery of xylose (45–65%)	Environmentally friendly and low energy consumption Very slow rate of degradation and delignification Delignification ratio dependent on the microbial strains The microbes utilise sugars from cellulose and hemicellulose Lower amount of inhibitory compounds Cellulolytic activity of the microorganism should be low to reduce the sugar loss Using ligninolytic enzymes increase the delignification efficiency	
Biological	Microbe (bacteria, fungi and actinomycetes) White-rot fungi (Basidiomycetes or Ascomycetes) Solid state delignification or submerged state Enzymatic delignification processes using ligninolytic enzymes	Selective in lignin and lignin–hemicellulose biodegradation Disturbing the three components and alter or remove of hemicellulose and lignin Disrupt lignin polymers Facilitate the bioconversion process Increased number of pores Cellulose and hemicellulose are expected to remain intact		

of LCW into fermentable sugars is, however, considered the major technological and economical bottleneck in the production of biofuels from sustainable feedstocks commercially [15]. There are many factors that have been identified that affect the conversion of polysaccharides into monomeric sugars. These factors include the porosity of the LCW (accessible surface area), cellulose crystallinity, and lignin and hemicellulose content. All these factors affect the complete efficiency of the conversion process [40]. The conversion of lignocellulosic substrates into fermentable sugars can be carried out chemically or biologically [34,38]. For example, chemical hydrolysis can be carried out either using dilute acid hydrolysis (i.e. <1% H₂SO₄, 215 °C, 3 min with 50–70% glucose yield) or concentrated acid (i.e. 30–70% H₂SO₄, 40 °C, a few hours, >80% glucose yield). Biological hydrolyses (bioconversion) may be more advantageous and could be enhanced by novel microbial isolates (monocultures or consortia) or their metabolites. Despite the fact that, under the same conditions, the yield of the fermentable sugars achieved by enzymatic hydrolysis can be quantitatively better than that obtained by acid-catalysed hydrolysis [19,21], the high doses and cost of enzymes required for the bioconversion of lignocellulosic biomass represent the major impediments in the enzymatic hydrolysis of these feedstocks [21]. Each polymer requires a cocktail of different enzymes working together in synergy to break down the cellulose and hemicellulose biopolymers into simple sugar monomers. Lytic Polysaccharide Monooxygenases (LPMOs) are oxidases that may be important in biomass conversion due to their auxiliary activities (Figure 2A1) [41].

Enzymatic degradation of lignocellulosic biomass

Enzymatic hydrolysis or biodegradation of LCW can be performed by using microbial isolates or their enzymes, for example cellulases and hemicellulases operating at 50 °C for several days resulting in 75–95% glucose yield [42]. The main enzymes involved in the saccharification of cellulose and hemicellulose into fermentable sugars are cellulases, hemicellulases and ligninases and have been well reviewed elsewhere (Figure 2) [33,43–48]. The cost of lignocellulosic enzymes remains the bottleneck for bioethanol production and remains one of the main challenges for large scale production of 2nd generation biofuels from LCW [49]. Enzymatic hydrolysis therefore requires excessive use of high-cost enzymes to complete the breakdown of polysaccharide biopolymers into fermentable sugars [15,18,50,51]. At a minimum, the cooperative action of a battery of a cellulolytic enzyme (at least three cellulases) is required to complete the bioconversion of cellulose into glucose. The cellulases enzymes are endo-1,4- β -glucanase or carboxymethylcellulases (EC 3.2.1.4), exoglucanase or cellobiohydrolase (EC 3.2.1.91) and β -glucosidase (EC 3.2.1.21) [44–46]. The endoglucanase acts on amorphous cellulose regions, attacking the glucose-polymer chain randomly,

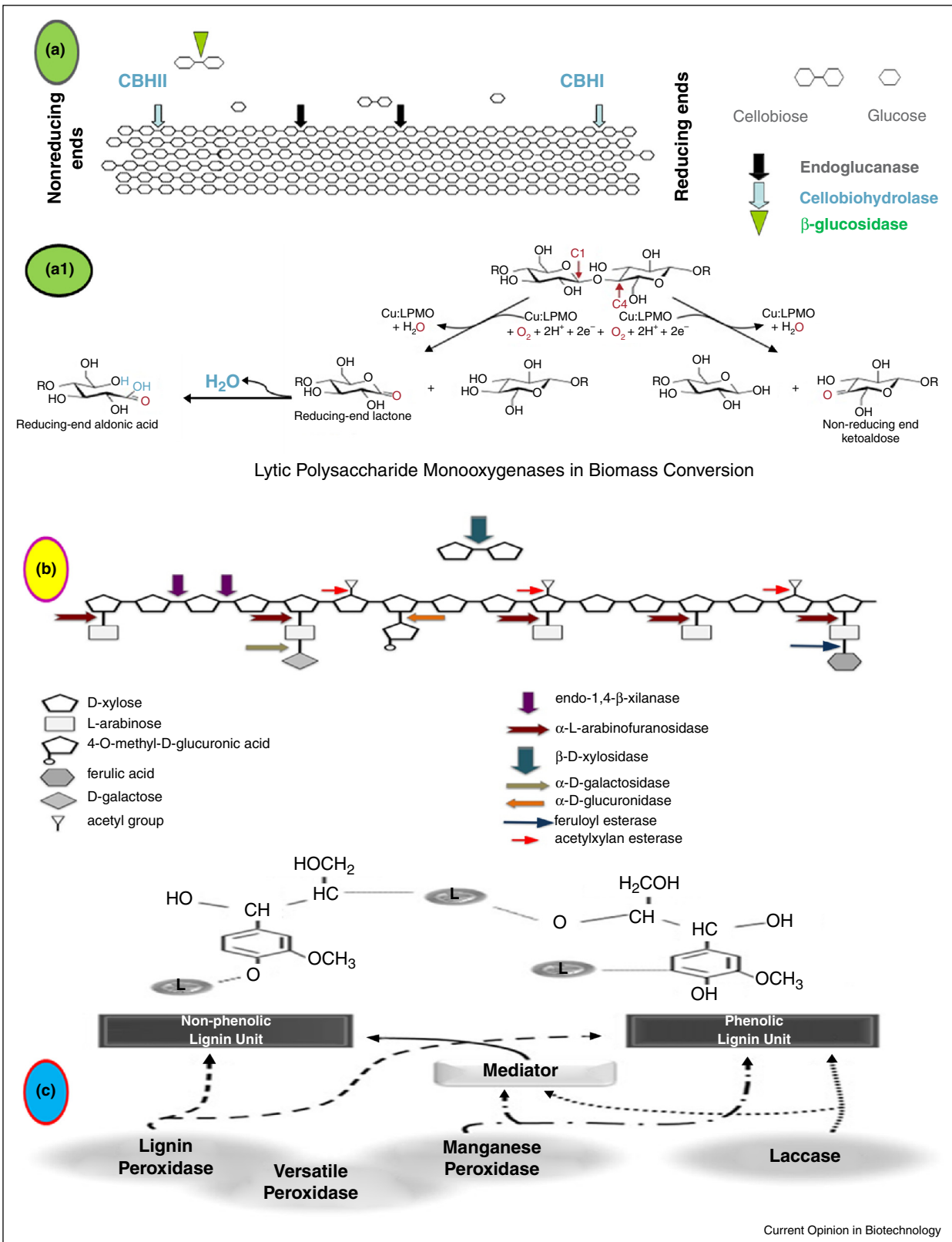
which releases small chains consisting of more reducing and non-reducing ends. These reducing and non-reducing ends are exposed to the activity of cellobiohydrolase enzymes (CBHI attack the reducing ends and CBHII attack non-reducing ends), producing cellobiose. The third component of the cellulase cocktail is β -glucosidase, which hydrolyzes cellobiose, producing glucose as the final product of cellulose bioconversion. LPMOs work in synergy with the hydrolytic cellulases by producing more free ends for subsequent hydrolysis (Figure 2A and A1) [41,44].

In comparison to cellulose, hemicellulose is more heterogeneous, requiring more specific enzymes (with a specific ratio of enzymes) to achieve the complete biodegradation of hemicellulose into pentoses, hexoses or uronic acids. For instance, endo-1,4- β -xylanase or endoxylanase (E.C.3.2.1.8), xylan 1,4- β -xylan esterases, ferulic and p-coumaric esterases, α -1-arabinofuranosidases, α -glucuronidase (E.C.3.2.1.139), α -arabinofuranosidase (E.C.3.2.1.55), acetylxylan esterase (E.C.3.1.1.72) and α -4-O-methyl glucuronosidases xylosidase (E.C.3.2.1.37) are all required in specific ratios for the complete hydrolysis of hemicellulose (Figure 2B) [33,47,48]. The structural complexity of lignin also requires a cocktail of oxidising enzymes working cooperatively with the hydrolytic enzymes to break down the physical barrier around the polysaccharides allowing the hydrolytic enzymes access to the carbohydrate biopolymer substrates. The main enzymes involved in lignin biodegradation are lignin peroxidases, manganese peroxidases and laccases (Figure 2C) [43].

Limitations to the enzymatic saccharification of lignocellulosic biomass

Unlike using edible resources as in the first generation biofuels (corn and sugarcane) where one enzyme is effective for the bioconversion step, lignocellulosic material is composed mainly of cellulose, hemicellulose and lignin and each component is theoretically hydrolysed by specific hydrolytic and oxidative enzymes working in a synergy to bioconvert the carbohydrates biopolymers to their monomer sugars [52]. Therefore biodegradation of LCW remains the bottleneck for the commercial production of cellulosic biofuels. Bioethanol production from LCW is dependent on the potential economic efficiency of enzymatic hydrolysis of cellulose and hemicellulose by using defined microbial consortia, novel microbial isolates or a unique mixture of commercially exploited hydrolytic enzymes in specific ratios. The cost of these hydrolysis enzymes was initially too high (about 50% of the total hydrolysis cost; \$5 per each gallon or \$0.75 per litre of bioethanol) to be used commercially for the bioconversion process, making the production of cellulosic bioethanol economically unfeasible [3,15,53–55]. Despite the successful collaboration between Novozymes and National Renewable Energy Laboratory (NREL) to reduce the cost of cellulase enzyme by 30-fold, (to \$0.10–0.18 per gallon or \$0.027 per litre of bioethanol), the prediction of producing 2nd generation

Figure 2



Enzymes involved in the biodegradation of cellulose (A), Lytic Polysaccharide Monooxygenases (LPMOs) in Biomass Conversion (A1), hemicellulose (B) and lignin (C) (Adapted from [28,33,41,43,48]).

bioethanol on a large scale by 2010 was not realised, and unfortunately we are still a long way from those goals [54]. With government subsidies from the US, Novozymes and Genencor have pledged to develop a commercial enzyme mixture containing up to 80 different enzymes (in specific ratios) for cellulosic biofuel production from a specific substrate — corn stover — to enhance commercial production at low-enzyme load, high efficiency and cost-effectiveness [52]. Van Dyk and Pletschke highlighted the fact that a large number of enzymes are required to bioconvert lignocellulose's carbohydrates into monomeric sugars effectively. Furthermore, the hydrolysis activity of multiple enzyme combinations working cooperatively together can be higher than adding individual enzymes, with the hydrolysis yield depending on the specific characteristics of the enzymes involved, their ratios and the characteristics of the substrate [52].

Conclusions

Cellulosic bioethanol has not been produced on a large scale due to technical constraints on LCW deconstruction which makes the commercial production economically infeasible in present market conditions. Despite extensive research on optimising the hydrolytic enzymes that have the capability to bioconvert LCW effectively, novel hydrolytic-oxidative enzyme mixtures for the complete bioconversion of carbohydrate biopolymer substrates (cellulose and hemicellulose) into its reducing sugars efficiently are lacking. Thus, the enzymatic approach to LCW bioconversion remains commercially limited and an area requiring further research.

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