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REVIEW PAPER

BIOETHANOL PRODUCTION FROM RESIDUAL LIGNOCELLULOSIC MATERIALS: A REVIEW – PART 1

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Lignocellulosic materials (LCM) are produced in large quantities and without clear application and their use as raw material for bioethanol production shows economic and ecologic benefits. LCM are composed mainly of three polymers: cellulose made up of glucose units, hemicellulose made up of several sugars (as xylose or arabinose), and lignin made up of phenylpropane units, interconnected in a strong structure. Pretreatment is an important step for bioethanol production from LCM, causing the solubilisation of hemicellulosic fraction (leading to the recovery of hemicellulose-derived saccharides) in order to obtain a solid phase enriched in cellulose and more susceptible to enzymatic attack. This study provides a comparative data regarding the chemical composition of various LCM used for bioethanol production, as well as different pretreatment technologies for improving the enzymatic hydrolysis of LCM.

Keywords: bioethanol, lignocellulosic material, pretreatment

Introduction

The control of European energy consumption and the increased use of energy from renewable sources are important components of measures needed to reduce greenhouse gas emission and to meet the Kyoto Protocol of the United Nation Framework Convention on Climate Change. Improving technological process, the use of energy efficiency technologies and the use of energy from renewable sources in transportation are the most effective measures to reduce dependency from fossil fuels. The European Council of March 2007 established a mandatory target of a 20% share of energy from renewable sources in energy consumption by 2020 and a mandatory 10% minimum target for the share of biofuels in transport petrol and diesel consumption by 2020. Member States may encourage the use of biofuels made from wastes, residues, non-food cellulosic material and lignocellulosic material (LCM) (EU- Directive 2009/28/EC).

LCM can be classified into six main groups: crop residues (corn stover, corn cobs, rice husks, barley husks, rye straw, oat straw, rice straw, wheat straw, corn stalks, cotton stalks, soya stalks, sugarcane bagasse), hardwoods (eucalyptus, acacia, poplar, black locust), softwoods (salix, spruce, pine), cellulose wastes (newspaper, waste office paper, recycled paper sludge), herbaceous biomass (alfalfa stems, switch grass), and municipal solid wastes (MSW) (Sánchez, & Cardona, 2008).

The joint U.S. Department of Energy and U.S. Department of Agriculture billion ton study (Biomass as Feedstock, 2005) found that in the United States about 368 million dry tons of sustainably removable biomass could be produced on forestlands and about 998 million dry tons could come from agricultural lands. Forestlands include 52 million dry tons of fuel wood harvested from forests, 145 million dry tons of residues from wood processing, 47 million dry tons of urban wood residues, 64 million dry tons of residues from logging, and 60 million dry tons of biomass from fuel treatment operations. Agricultural lands in the United States could produce nearly 1 billion dry tons of biomass annually, including 428 million dry tons of primary crop residues, 377 million dry tons of perennial energy crops and the rest of biomass from agricultural lands (Foust *et al.* 2006).

Based on the Pan-European natural resources database and Geographic Information System for European Union, land productivity potentials (Fischer *et al.*, 2010a) and land use scenarios (Fischer *et al.*, 2010b) were elaborated resulting in 42.7 million hectares of energy crops, such as herbaceous and woody lignocellulosic feedstock, and 166 million dry tons of agricultural residues available for biofuel production in EU27, Switzerland and Norway by 2030 (Gnansounou, 2010). In Romania and Bulgaria, the analysis suggests that almost 8 million hectares of arable land for bioenergy feedstock production could be released by 2030 without influencing the food production (Fischer *et al.*, 2010b).

Physico-chemical and chemical processes have been used for pretreatment of lignocellulosic materials. Physico-chemical methods include: steam explosion (autohydrolysis), liquid hot water (LHW), CO_2 explosion and AFEX (Ammonia fiber/ freeze explosion). Chemical methods are based on: acid pretreatment, alkaline pretreatment, wet oxidation, ozonolysis and organosolv.

This review is focused on pretreatment methods for bioethanol production from lignocellulosic materials because they represent the largest amount of residues remaining after harvesting, with great potential, both economic and industrial, in order to produce bioethanol as an alternative fuel source.

Lignocellulosic material

LCM structure consists of three basic polymers: cellulose, hemicellulose and lignin found in stalks, stems and leaves (Demirbas, 2005; Arin & Demirbas, 2004). Cellulose is a homopolysaccharide composed of β-D-glucopyranose units linked together by (1 → 4)-glycosidic bonds. Glucose anhydride, formed by removing water from glucose, is polymerized into long chains of cellulose containing 5000-10000 glucose units. The basic unit of the cellulose polymer consists of two anhydride glucose units called cellobiose units. Hemicellulose is a mixture of various polymerized monosaccharide such as glucose, mannose, galactose, xylose, arabinose, 4-O-methyl glucuronic acid and galacturonic acid (Mohan *et al.*, 2006). Lignin is a mononuclear aromatic polymer located in the cell walls of biomass and is connected to cellulose fibers (Yaman, 2004; Balat *et al.*, 2008).

LCM can vary in composition and moisture content according to: region, fertilization practices, harvesting, storage and storage time (Table 1).

Table 1. Composition data of several lignocellulosic materials for bioethanol production

Feedstock	**Content (dry wt %)**			**References**
	Cellulose	**Hemicellulose**	**Lignin**	
Hardwoods				
Eucalyptus globulus	46.30	25.83	22.90	Garrote *et al.*, 2007
Acacia dealbata	50.50	19.30	21.90	Muñoz *et al.*, 2007
Poplar	44.05	15.71	20.95	Pan *et al.*, 2006
Black locust	41.61	17.66	26.70	Hamelinck *et. al.*, 2005
Softwoods				
Salix	42.50	25.00	26.00	Sassner *et al.*, 2008
Spruce	44.00	24.60	27.50	Sassner *et al.*, 2008
Pine	44.55	21.90	27.67	Hamelinck *et. al.*, 2005
Agro-industrial residues				
Corn stover	40.00	29.60	23.00	Sassner *et al.*, 2008
Corn cobs	34.40	40.75	18.80	Parajó *et al.*, 2004
Rice husks	36.70	20.05	21.30	Parajó *et al.*, 2004
Barley husks	21.40	36.62	19.20	Parajó *et al.*, 2004
Rye straw	41.10	30.20	22.90	Gullón *et al.*, 2010
Oat straw	39.40	27.10	17.50	Nigam *et al.*, 2009
Rice straw	36.20	19.00	9.90	Nigam *et al.*, 2009
Wheat straw	32.90	24.00	8.90	Nigam *et al.*, 2009
Corn stalks	35.00	16.80	7.00	Nigam *et al.*, 2009
Cotton stalks	58.50	14.40	21.50	Nigam *et al.*, 2009
Soya stalks	34.50	24.80	19.80	Nigam *et al.*, 2009
Sunflower stalks	42.10	29.70	13.40	Nigam *et al.*, 2009
Sugarcane bagasse	40.00	27.00	10.00	Nigam *et al.*, 2009
Ethiopian mustard	32.70	21.90	18.70	González-García *et al.*, 2010
Flax shives	47.70	17.00	26.60	González-García *et al.*, 2010
Hemp hurds	37.40	27.60	18.00	González-García *et al.*, 2010
Dedicated energy crops				
Alfalfa stems	27.50	23.00	15.80	González-García *et al.*, 2010
Switch grass	31.98	25.19	18.13	Hamelinck *et. al.*, 2005
Waste papers				
Newspaper	61.30	9.80	12.00	Kim & Moon, 2003

A recent study of the National Renewable Energy Laboratory (NREL) together with the U.S. Department of Energy (DOE) presents the composition of corn stover from a variety of commercial hybrid varieties using a rapid compositional analysis method known as NIR/PLS (Near-Infrared Spectroscopy/Projection-to-Latent-Structures modeling) (Templeton *et al.*, 2009). This study evaluated the compositions of 508 corn stover samples (Table 2) collected from 47 regions of 8 corn growing countries, after 2001, 2002 and 2003 harvesting. Another recent study of NREL (National Renewable Energy Laboratory) shows a comparison between the average composition of 9 corn stover samples obtained in 2012 from two lots, compared to 2011, obtained from one sample from overall distribution (Humbird *et al.*, 2011).

Table 2. Chemical composition of corn stover
(*source*: Aden *et al.*, 2002; Humbird *et al.*, 2011)

Component	**Content (dry wt %)**		
	whole stover	**2002**	**2011**
Glucan	31.90	37.40	35.05
Xylan	18.90	21.07	19.53
Arabinan	2.80	2.92	2.38
Galactan	1.50	1.94	1.43
Mannan	0.30	1.56	0.60
Sucrose	3.60	-	0.77
Acetyl groups	2.20	2.93	1.81
Lignin	13.30	17.99	15.76
Ash	3.90	5.23	4.93
Protein	3.70	3.10	3.10
Extractives	8.60	4.68	14.65

Biochemical process

The main biochemical processes consisting in transformation of LCM into bioethanol (Figure 1) are (Wooley *et al.*, 1999; Wooley *et al.*, 2000; Aden *et al.*, 2002; Aden, 2008; Humbird & Aden, 2009; Humbird *et al.*, 2011):

- *Pretreatment*. LCM is treated at higher temperatures for a short time to release hemicellulosic sugars to enzymatic hydrolysis (Figure 2).
- *Simultaneous saccharification and fermentation (SSF).* Enzymatic hydrolysis is initiated in a continuous bioreactor using enzymes and then the biomass is inoculated with fermenting microorganisms. At this stage, most of the cellulose and xylose are converted into bioethanol.

1. Pretreatment

The first step in the conversion of LCM into bioethanol is reducing the size by pretreatment (Graf & Koehler, 2000). The goal of any pretreatment technology is to alter or remove structural and compositional obstacles for hydrolysis in order to improve the rate of enzymatic hydrolysis and the increasing yields of fermenting sugars from cellulose and hemicellulose (Mosier *et al.*, 2005a). This is an important tool for cellulose conversion processes, changing the structure of cellulosic biomass, making it more accessible for enzymes that convert polysaccharide into fermentable sugars (Patel *et al.*, 2007; Balat *et al.*, 2008).

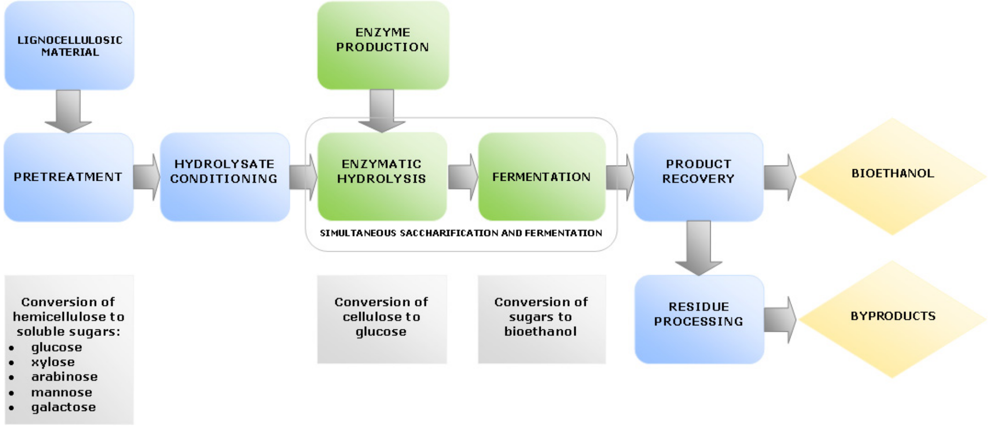

Figure 1. Biochemical conversion of lignocellulosic material into bioethanol (*source*: Foust *et al.*, 2009)

Pretreatment (Figure 3) must meet the following requirements (Silverstein, 2004):

- Improving the ability to form carbohydrates by hydrolysis;
- Avoiding degradation or loss of carbohydrates;
- Avoiding the formation of byproducts that inhibit hydrolysis and fermentation process.

Pretreatment can be done in various ways, such as: steam explosion (Brownell & Saddler, 1987; Zhang *et al.*, 2007); liquid hot water; CO_2 explosion; AFEX (Alizadeh *et al.*, 2005; Teymouri *et al.*, 2004; Teymouri *et al.*, 2005; Indacoechea *et al.*, 2006); acid pretreatment (Martín *et al.*, 2007a); alkaline pretreatment (Silverstein *et al.*, 2007; Champagne, 2007); wet oxidation; ozonolysis; organosolv.

1.1. Physico-chemical pretreatment

Steam explosion pretreatment

In this process which takes place at high pressure, is introduced steam at high temperature into a closed room which contains LCM. After 1-5 min, the steam is released from the matrix chains causing breakage and separation of fibers with minimal loss of material (Mabee *et al.*, 2006; Balat *et al.*, 2008).

Another type of steam explosion pretreatment refers to a technique in which LCM is rapidly heated with high pressure steam without adding any chemicals. Mixture of biomass/steam is kept for a period of time to promote hydrolysis of hemicellulose and the process is completed by decompression. Steam explosion involves chemical effects because acetic acid is generated by hydrolysis of the acetyl groups associated with hemicellulose and can catalyze the hydrolysis and degradation of glucose or xylose (Mosier *et al.*, 2005a).

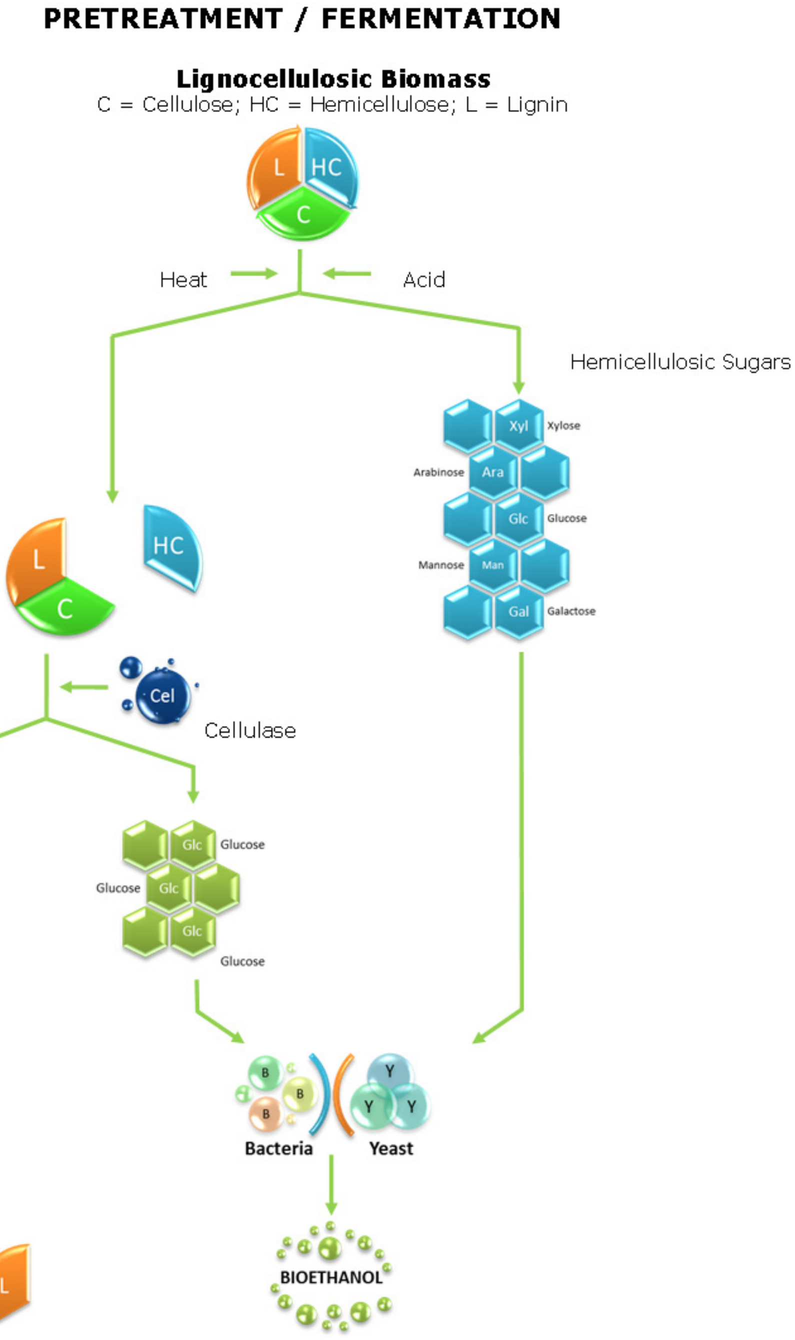

Figure 2. Pretreatment - Fermentation Cycle
(*source*: NREL - National Renewable Energy Laboratory)

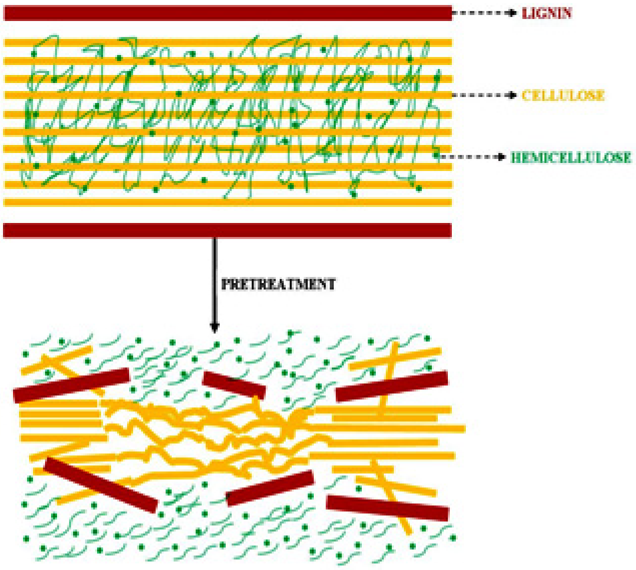

Figure 3. Effect of pretreatment on lignocellulosic materials

Liquid hot water pretreatment (LHW)

LHW is one of the hydrothermal pretreatment methods applied for pretreatment of LCM (Taherzadeh *et al.*, 2008) that benefit from the use of no chemical agents other than water, being more environmentally friendly technologies (Garrote *et al.*, 1999). The objective of LHW pretreatment is to solubilize mainly the hemicellulose, to make the cellulose more susceptible to enzymatic attack and to avoid the formation of inhibitors, such as hydroxymethylfurfural (HMF) and furfural (Alvira *et al.*, 2010). The LHW pretreatment involves saturation of lignocellulosic biomass with water, followed by heat treatment of the slurry at temperatures between 120 and 200°C for 5–15 min under pressure (Kim *et al.*, 2009). The pH is restricted to 4–7 to minimize formation of monomeric sugars and sugar decomposition products (Mosier *et al.*, 2005a; Mosier *et al.*, 2005b). This hydrothermal pretreatment is easy to perform: there is a low usage of energy; the process is without the difficult steps of handling and recovery of chemicals; equipment corrosion can be excluded (Cybulska *et al.*, 2010). The process is already applied to lignocellulosic biomass such as corn stover (Mosier *et al.*, 2005c), sugarcane bagasse (Laser *et al.*, 2002) and wheat straw (Pérez *et al.*, 2008).

CO_2 explosion pretreatment

This method is based on the use of CO_2 as a supercritical fluid. The supercritical CO_2 as well as its use in extraction as solvent is capable of enhancing the enzymatic hydrolysis of LCM. This process can remove the lignin and increase the digestibility of the substrate. Co-solvents such as ethanol, acetic acid and water improve the delignification of LCM. CO_2 forms carbonic acid in aqueous solutions, favoring hydrolysis of the polymers. CO_2 molecules are similar in size to those of water and ammonia, which allow them to penetrate in the same way the pores of

LCM. After the release of CO_2 under pressure, there is a change in the structure of cellulose and hemicellulose, this allows enzymes to have accessibility to the substrate surface. The main advantages of this treatment are: availability of technology at relatively low cost; it does not produce toxic degradation products; easy recovery after extraction; the process is considered environmentally friendly (Taherzadeh & Karimi, 2008).

AFEX pretreatment (Ammonia fiber/ freeze explosion)

AFEX pretreatment involves the injection of liquid ammonia (Hamelinck *et al.*, 2005). AFEX is a process in which LCM with moisture content of 15-30 % are placed in a pressure container with liquid ammonia. This system does not release any sugars but allows hemicellulose and cellulose to be attacked by enzymes and be reduced to soluble sugars (Dale & Moelhman, 2000; Balat *et al.*, 2008).

2. Chemical pretreatment

Acid pretreatment

The pretreatment process (Table 3) converts most of the hemicellulosic sugars from LCM into soluble sugars (glucose, xylose, arabinose, mannose) by hydrolysis reactions. Acetyl groups from hemicellulose are released as acetic acid. Sugar degradation products such as furfural and HMF can also be formed in the pretreatment (Humbird *et al.*, 2011). The aim of this process is to obtain high yields of sugars from LCM (Lee, 2005). There are several types of acid pretreatment: the use of sulfuric acid (Parajó *et al.*, 1993) hydrochloric acid (Kurakake *et al.*, 2005), peracetic acid (Teixeira *et al.*, 1999), nitric acid (Brink, 1993), or phosphoric acid (Hussein *et al.*, 2001). Dilute acid pretreatment is one of the most studied and widely used (Karimi *et al.*, 2006; Dale & Moelhman, 2000; Tucker *et al.*, 2003; Chung *et al.*, 2005; Kim *et al.*, 2005; Agbogbo & Wenger, 2006).

There are two types of dilute acid pretreatment processes: at moderate temperatures (T < 160 ˚C) and higher temperatures (T > 160 ˚C) (Silverstein *et al.*, 2007). Dilute acid pretreatments at moderate temperatures are using sulfuric acid or phosphoric acid to convert LCM, including hemicellulosic fraction, into soluble sugars, followed by enzymatic hydrolysis of cellulosic fraction to glucose (Um *et al.*, 2002). Higher pretreatment temperatures have a higher yield of xylose recovery and increased enzymatic digestibility of cellulosic residues (Tucker *et al.*, 2003).

Alkaline pretreatment

Alkaline pretreatment uses temperatures and low pressures compared to other technologies. This process can remove lignin without having large effects on other components (McMillan, 1997). NaOH treatment increases internal surface of LCM, decreases the degree of crystallinity and breaks the lignin structure (Li *et al.*, 2004). Alkaline pretreatment reduces lignin and hemicellulose content of biomass, increasing the surface and allowing penetration of water molecules in the inner layer as well as breaking connections between hemicellulose and lignin (Lee, 2005). This treatment removes acetyl and uronic acid from hemicellulose, which slows the enzyme accessibility of hemicellulose and cellulose surface (Ramirez, 2005; Balat *et al.*, 2008).

Wet oxidation pretreatment

LCM is subjected to the action of water at elevated temperatures in the presence of oxygen (Schmidt & Thomsen, 1998). This treatment has the advantage of not generating the degradation products, such as furfural and HMF (Klinke *et al.*, 2002; Varga *et al.*, 2003). The presence of oxygen makes this process more expensive but allows working at lower temperatures than the autohydrolysis.

Ozonolysis pretreatment

Ozone can be used for the degradation of lignin and hemicellulose in many LCM. This treatment has a delignification effect on the feedstock and practically does not affect the cellulose. The main advantages of the ozonolysis process are: high efficiency in removing lignin; it does not produce toxic products which could affect the following processes; reactions are carried out under conditions of ambient temperature and atmospheric pressure (Vidal & Molinier, 1988).

Organosolv pretreatment

Organosolv pretreatment represents an alternative method for the delignification of LCM. This method is using an organic or aqueous organic solvent with addition of a catalyst (HCl or H_2SO_4) to break the internal lignin and hemicellulose bonds. Among the organic solvents used in the process are: methanol, ethanol, acetone and ethylene-glycol (Sun & Cheng, 2002).

Table 3. Comparison between different pretreatment methods for improving the enzymatic hydrolysis of LCM

Feedstock	Type of pretreatment	Temp (°C)	Time (min)	Solid loading	Enzyme loading	Yield (%)	References
Eucalyptus globulus	Steam explosion	210	4	10[1]	15[4]	62.5[13]	Ballesteros *et al.*, 2004
Populus nigra	Steam explosion	210	4	10[1]	15[4]	71.2[13]	Ballesteros *et al.*, 2004
Sorghum sp.	Steam explosion	210	2	10[1]	15[4]	62.5[13]	Ballesteros *et al.*, 2004
Brassica carinata	Steam explosion	210	8	10[1]	15[4]	60.9[13]	Ballesteros *et al.*, 2004
Wheat straw	Steam explosion	190	8	10[1]	15[4]	68.1[13]	Ballesteros *et al.*, 2004
Corn stover	Liquid hot water	195	15	13[1]	15[4]	201[15]	Xu *et al.*, 2010
Rapeseed straw	Liquid hot water	193	27	5[1]	15[4]	94.85[14]	Díaz *et al.*, 2010
Olive stones	Liquid hot water	200	2	-	82.8[8]	0.25[16]	Cuevas *et al.*, 2009
Wheat straw	Liquid hot water	220	-	4[1]	15[4]+15[8]	92[14]	Pérez *et al.*, 2008
Hybrid	Liquid hot	200	10	15[1]	40[4]	76.7[14]	Kim *et al.*,

Feedstock	Type of pretreatment	Temp (°C)	Time (min)	Solid loading	Enzyme loading	Yield (%)	References
poplar	water						2009
Olive tree residues	Liquid hot water	220	10	5[1]	15[4]+15[8]	75[14]	Cara *et al.*, 2007
Corn fiber	Liquid hot water	215	-	-	15[4]	86[17]	Allen *et al.*, 2001
Corn stover	AFEX	90	5	1[2]	31.3[5]+33.3[6]	88.5[14]	Balan *et al.*, 2009
Switch grass	AFEX	100	5	-	15[4]+40[4]	93[14]	Alizadeh *et al.*, 2005
Rice straw	CO_2 explosion	110	30	-	30[4]+15[7]	32.4[13]	Gao *et al.*, 2010
Sugarcane bagasse	CO_2 explosion	220	5	2[1]	65[4]+17[8]	97.2[13]	Ferreira-Leitão *et al.*, 2010
Wheat straw	Acid hydrolysis	121	60	-	2[4]+1[10]	76.5[14]	Saha *et al.*, 2005
Wheat straw	Wet oxidation	195	10	2[1]	67[4]+246[6]	90[14]	Pedersen & Meyer, 2009
Clover and ryegrass	Wet oxidation	195	10	2[1]	25[4]+0.46[9]	93.6[14]	Martín *et al.*, 2008
Sugarcane bagasse	Wet oxidation	195	15	2[1]	25[4]+0.46[9]	74.9[14]	Martín *et al.*, 2007b
Sugarcane bagasse	Organosolv	175	60	10[1]	15[4]+300[11]	92.8[13]	Mesa *et al.*, 2010
Pinus taeda	Organosolv	170	60	2[1]	8[4]+16[11]	70[14]	Sannigrahi *et al.*, 2010
Pinus rigida	Organosolv	180	-	1[1]	700[12]+250[7]	80[14]	Park *et al.*, 2010
Pinus radiata	Organosolv	195	5	2[1]	20[4]+40[11]	99.5[13]	Araque *et al.*, 2007
Pine beetle killed	Organosolv	187	60	2[3]	20[4]+40[11]	100[14]	Pan *et al.*, 2008
Lodgepole pine	Organosolv	187	60	2[3]	20[4]+40[11]	100[14]	Pan *et al.*, 2008
Miscanthus giganteus	Organosolv	170	60	2[3]	20[4]+40[11]	100[14]	Brosse *et al.*, 2009

[1] solid (g solid/100 g dissolution); [2] glucan (g glucan/100 g dissolution); [3] cellulose (g cellulose/100 g dissolution); [4] cellulase (FPU/g dry matter); [5] cellulase (mg protein/g of glucan); [6] β-glucosidase (mg protein/g of glucan); [7] β-glucosidase (CBU/g dry matter); [8] β-glucosidase (IU/g dry matter); [9] β-glucosidase (CBU/ml); [10] pectinase (g pectinase/100 g dry matter); [11] xylanase (IU/g dry matter); [12] endo-glucanase (EGU/g dry matter); [13] ethanol conversion (g ethanol/100 g theoretical ethanol); [14] glucose (g glucose/100 g potential glucose); [15] ethanol (g ethanol/kg of substrate); [16] ethanol (g ethanol/g of substrate); [17] ethanol (g ethanol/100 g potential ethanol); FPU – Filter Paper Units; IU – International Units; CBU – Cellobiase Units; EGU – Endo-Glucanase Units

Conclusions

Pretreatment process is an important step for bioethanol production from LCM, changing the structure of cellulosic biomass, making it more accessible for enzymes in order to improve the rate of enzymatic hydrolysis and the increasing yields of fermenting sugars from cellulose and hemicellulose. The main advantages of pretreatment methods for LCM are: steam explosion causes hemicellulose degradation and lignin transformation; liquid hot water, CO_2 explosion and wet oxidation do not cause formation of inhibitory compounds; AFEX increases accessible surface area; acid pretreatment hydrolyzes hemicellulose to xylose and other sugars; alkaline pretreatment removes hemicelluloses and lignin; ozonolysis reduces lignin content; organosolv hydrolyzes lignin and hemicellulose. LCM is economically attractive for obtaining bioethanol as an alternative fuel source.

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