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REVIEW PAPER

BIOETHANOL PRODUCTION FROM RESIDUAL LIGNOCELLULOSIC MATERIALS: A REVIEW – PART 2

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Lignocellulosic material (LCM) can be employed as feedstock for biorefineries, a concept related to industries designed to process biomass for producing chemicals, fuels and/or electrical power. According to this philosophy, LCM can be fractionated and the resulting fractions employed for specific applications. Bioethanol production from cellulosic fraction of LCM involves: hydrolysis of polysaccharides and fermentation of the monomers into bioethanol. Enzymatic hydrolysis is catalyzed by cellulolytic enzymes and fermentation is carried out by bacteria, yeasts or fungi. The main objective of this article is to review different process integration technologies for bioethanol production from LCM. This paper include: separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF), and simultaneous saccharification and co-fermentation (SSCF) methods. Furthermore, the fermentation process and a comparative data of cellulases, hemicellulases and ethanol producing-microorganisms were presented.

Keywords: bioethanol, enzymatic hydrolysis, fermentation, separate hydrolysis and fermentation, simultaneous saccharification and fermentation, simultaneous saccharification and co-fermentation

Introduction

Each fuel has its intrinsic properties that play an important role in determining the internal combustion engine performance, performance that depends on fuel quality. An example of engine performance related to fuel quality is the relationship between compression ratio and fuel octane. Other physical and chemical characteristics are related to energy density, vaporization heat, molecular ratio

between reactants and combustion products, specific energy, flammability limits, flame transmission speed, flame temperature and the hydrogen and carbon content. The use of bioethanol mixed with gasoline has a number of advantages: reduction by 8-30% of carbon monoxide emissions when using 10% ethanol in gasoline; reduction by 5-15% of toxic emissions (sulfur and aromatic compounds such as benzene, toluene, xylene) without influencing fuel performance; reduction of the ozone layer; reduction of pollution. Fuel bioethanol is currently used worldwide in E 10 mixture (10% anhydrous ethanol and 90% gasoline). This mixture (E 10) contains 3.5% oxygen compared to 2.7% for gasoline. Bioethanol can be mixed with gasoline in higher proportions: 85% (E 85), 95% (E 95) and used entirely (E 100) as an alternative fuel source (Banu *et al.*, 2006).

After pretreatment, the next steps in the biochemical process of bioethanol production from lignocellulosic materials (LCM) are: enzymatic hydrolysis of polysaccharides and fermentation of monosaccharides into bioethanol. They can be performed separately or simultaneously (Tomás-Pejó *et al.*, 2008). Enzymatic hydrolysis can be applied at different levels of process integration: separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF), simultaneous saccharification and co-fermentation (SSCF) (Hamelinck *et al.*, 2005). Enzymatic hydrolysis is catalyzed by cellulolytic enzymes and the fermentation is carried out by yeast or bacteria. Cellulases are composed of a mixture of enzyme proteins responsible for the degradation of cellulose to glucose (Howard *et al.*, 2003). The efficiency of enzymatic hydrolysis depends on the appropriate proportional ratio of the cellulose components. SHF process is performed in two different vessels and each step can be done under optimal conditions of pH and temperature. In the SSF, the enzymatic saccharification and fermentation process are run in the same vessel and glucose released by the action of cellulases is converted directly into ethanol by the fermenting microorganisms. These advantages result in an increased rate of saccharification and ethanol productivity compared to SHF (Wyman, 1996). SSCF process combines the hydrolysis of cellulose to glucose and the co-fermentation of pentose and hexose sugars to ethanol in one reaction vessel (Lynd, 1996; Spatari *et al.*, 2010). The main advantages of these processes are: in SHF each stage can be processed at its optimal operating conditions and separate steps minimize interaction between the hydrolysis and fermentation, compared to SSF where can be obtained higher ethanol yields due to removal of end product inhibition of saccharification process and can be reduced the number of reactors required (Sarkar *et al.*, 2012).

This review is focused on bioethanol production from cellulosic fraction of lignocellulosic materials (LCM) based on hydrolysis of polysaccharides (usually using enzymes) and fermentation of the monomers into bioethanol.

Enzymatic hydrolysis

Cellulases and cellulase-producing microorganisms

Cellulases represent an enzymatic complex that is capable of hydrolyzing cellulose. This enzyme complex includes:

- Endo-1,4-β-glucanases (EG), acting within polyglucidic chains forming cellulose, making the final breaking of β-1,4-glucosidic bonds and formation of shorter chains with free reducing ends;
- Exo-1,4-β-D-glucanases or cellobiohydrolases (CBH), which include two enzymes: 1,4-β-D-glucanglucohydrolase which releases glucose units from the reducing end of cellulose chains, but slowly hydrolyses cellobiose formed by another enzyme; and 1,4-β-D-glucancellobiohydrolase which releases cellobiose units from the reducing end of cellulase chains;
- β-D-glucosidases (BGL) and β-D-glucosideglucohydrolase (cellobiase) that hydrolyze cellobiose and short chains of oligosaccharides to glucose (Zhang & Lynd, 2004; Howard *et al.*, 2003; Heikinheimo, 2002; Gusakov *et al.*, 2007; Gusakov *et al.*, 2005; Valjamae *et al.*, 2001).

When the enzymatic system (cellulase) acts *in vitro* on insoluble cellulose substrate, three significant processes occur simultaneously: chemical and physical changes in the cellulosic fraction; primary hydrolysis, which involves the release of soluble sugars from the surface of cellulosic molecules; secondary hydrolysis, which involves hydrolysis of soluble sugars to lower molecular weight sugars, and finally to glucose (Mosier *et al.*, 2002).

Several species of bacteria such as *Clostridium*, *Cellulomonas*, *Thermonospora*, *Bacillus*, *Bacteriodes*, *Ruminococcus*, *Erwinia*, *Acetovibrio*, *Microbispora*, *Streptomyces* species, and fungi such as *Trichoderma*, *Penicillium*, *Fusarium, Humicola*, *Phanerochaete*, *Schizophillum* species are able to produce cellulases (Rabinovich *et al.*, 2002; Sun & Cheng, 2002). Cellulases can also be produced by aerobic bacteria such as *Pseudomonas* and *Actinomycetes* species as well as fungi of *Aspergillus* species (Sun, 2002).

Hemicellulases and hemicellulase-producing microorganisms

Hemicellulases can be classified into three categories:

- Endo-hemicellulases which act within the hemicellulose chain and have limited activity on short chain oligomers;
- Exo-hemicellulases which act progressively outside the hemicellulose chain;
- Hemicellulases that hydrolyze hemicellulose from native plants (acetyl esterases and esterases).

Several species of bacteria (Table 1) such as *Agrobacterium*, *Bacillus*, *Bifidobacterium*, *Clostridium*, *Streptomyces*, *Cellulomonas*, *Geobacillus* species, and fungi such as *Aspergillus* and *Trichoderma* species are able to produce hemicellulases (Shallom & Shoham, 2003).

The main hemicellulase-producing microorganisms from several species of fungi are as follows: *Aspergillus niger*, *Trichoderma reesei* and *Trichoderma viride* species (Banu *et al.*, 2006).

Table 1. Comparative data of different microorganisms with the highest specific activity for cellulases and hemicellulases (*source*: Howard *et al.*, 2003; Menon, & Rao, 2012)

Organism	Enzyme	Substrate	Specific activity ($\mu mol.min^{-1}.mg^{-1}$)
Cellulase-producing microorganisms			
Aspergillus niger	Cellulase	Carboxymethylcellulose / cellohexaose / cellopentaose / Cellotetraose / cellotriose / cellulose	194
Achlya bisexualis	1,3-β-glucan glucohydrolase	Glucan / laminarin / neutral glucan / phosphoglucan	7840
Rhizopus chinensis	1,3-β-D-glucan glucanohydrolase	β-glucan	4800
Penicillium brefeldianum	1,6-β-D-glucan glucanohydrolase	β-glucan/gentiobiose / pachyman	405
Bacillus subtilis	Mannan endo-1,4-β-mannosidase	Galactoglucomannan / glucomannans / mannans	514
Clostridium thermocellum	Cellulase	Avicel / carboxymethylcellulose / cellulose / cellopentaose / Cellotetraose / cellotriose	428
Streptomyces murinus	1,3-β-glucan glucohydrolase	Laminarin	6.7
Bacillus macerans	1,3-1,4-β-D-glucan glucanohydrolase	β-D-glucan/lichenan	5030
Hemicellulases-producing microorganisms			
Trichoderma longibrachiatum	Endo-1,4-β-xylanase	1,4-β-D-xylan	6630
Aspergillus nidulans	β-1,4-xylosidase	p-nitrophenyl-β-D-xylopyranoside	107.1
Aspergillus niger	Exo-β-1,4-mannosidase	β-D-Man-(1-4)-β-D-GlcNAc-(1-4)-β-DGlcNAc-Asn-Lys	188
Aspergillus niger	Endo-α-1,5-arabinanase	1,5-α-L-arabinan	90.2
Sclerotium rolfsii	Endo-β-1,4-mannanase	Galactoglucomannan / mannans / galactomannans / glucomannans	380
Phanerochaete chrysosporium	α-Glucuronidase	4-O-methyl-glucuronosyl-xylobiose	4.5
Mortierella vinacea	α-Galactosidase	Melibiose	2000
Humicola insolvens	β-glucosidase	(2-hydroxymethylphenyl)-β-D-glucopyranoside	266.9
Schizophyllum commune	Acetyl xylan esterase	4-methylumbelliferyl acetate / 4-nitrophenyl acetate	227
Clostridium stercorarium	Feruloyl esterase	Ethyl ferulate	88
Thermoanaerobacter ethanolicus	β-1,4-xylosidase	o-nitrophenyl-β-D-xylopyranoside	1073
Fibrobacter succinogenes	Acetyl xylan esterase	Acetylxylan / α-naphthyl acetate	2933
Pyrococcus furiosus	Exo-β-1,4-mannosidase	p-nitrophenyl-β-D-galactoside	31.1
Bacillus subtilis	Endo-β-1,4-mannanase	Galactoglucomannan / glucomannans / mannan	514
Bacillus subtilis	Endo-α-1,5-arabinanase	1,5-α-L-arabinan	429
Bacillus subtilis	Endo-galactanase	Arabinogalactan	1790
Bacillus polymyxa	β-Glucosidase	4-nitrophenyl-β-D-glucopyranoside	2417
Bacillus pumilus	Endo-1,4-β-xylanase	β-1,4-D-xylan	1780
Escherichia coli	α-Galactosidase	Raffinose	27350

Fermentation

Hydrolysate obtained by acid pretreatment is used for fermentation by microorganisms. Because the hydrolysate contains not only glucose, but also different monosaccharides such as xylose, galactose, mannose, arabinose and oligosaccharides, microorganisms are necessary to ferment these sugars (Katahira *et al.*, 2006). These microorganisms can use carbohydrates with 6-carbon atoms, one of the most common being glucose. Cellulosic materials containing high levels of glucose or glucose precursors are most easily converted into bioethanol (Balat *et al.*, 2008). There is a number of microorganisms that produce significant amounts of bioethanol (Steward & Russell, 1987). Xylose fermenting microorganisms are bacteria, yeasts and filamentous fungi (Hahn-Hagerdal *et al.*, 2006). One of the most efficient ethanol producing yeast is *Saccharomyces cerevisiae* which has a high tolerance to ethanol and other inhibitory compounds resulting from acid hydrolysis of LCM. Since wild strains of this yeast cannot ferment pentose such as xylose, arabinose and oligosaccharides, production of bioethanol from lignocellulosic hydrolysate is inadequate (Katahira *et al.*, 2006).

Ethanologenic bacteria which are currently used for bioethanol production from LCM are: *Escherichia coli*, *Klebsiella oxytoca* and *Zymomonas mobilis* (Dien *et al.*, 2003). *Zymomonas mobilis* is well known for its ability to quickly and efficiently produce ethanol from raw materials based on glucose and comparative tests have shown that *Zymomonas mobilis* can achieve yields higher than 5% and a productivity up to five times higher compared to traditional yeast fermentation. *Zymomonas mobilis* showed ethanol yields up to 97% and ethanol concentrations up to 12% for glucose fermentation (Mohagheghi *et al.*, 2002). *Zymomonas mobilis* can efficiently produce ethanol from hexose: glucose and fructose, but not from pentose (Hahn-Hagerdal *et al.*, 2006). *Zymomonas mobilis* ferments only glucose, fructose and sucrose. In the recent years, researchers at NREL have successfully designed *Zymomonas mobilis* strains capable of fermenting xylose and arabinose (Dien *et al.*, 2003). *Escherichia coli* and *Klebsiella oxytoca* metabolize arabinose so that ethanologenic strains ferment all lignocellulose sugars (Hahn-Hagerdal *et al.*, 2006). *Pichia stipitis*, *Candida parapsilosis* and *Candida shehatae* can metabolize xylose by the action of xylose reductase to convert xylose to xylitol and by the action of xylitol dehydrogenase to convert xylitol to xylulose. Xylose fermentation can be carried out successfully by recombinant *Saccharomyces cerevisiae* which takes xylose reductase and xylitol-dehydrogenase from *Pichia stipitis*, and xylulokinase from *Sacharomyces cerevisiae* (Katahira *et al.*, 2006).

Table 2. *Ethanol-producing microorganisms for the bioconversion of LCM (source: Waites et al., 2001)*

Microorganism	Fermented substrate
Bacteria	
Clostridium thermohydrosulfuricum (thermophilic)	Glucose, xylose sucrose and cellobiose
Clostridium thermocellum (thermophilic)	Glucose and cellobiose

Microorganism	Fermented substrate
Thermoanaerobacter ethanolicus	Xylose
Thermoanaerobium brockii (thermophilic)	Glucose, sucrose and cellobiose
Thermobacteroides aceroethylicus (thermophilic)	Glucose, sucrose and cellobiose
Zymomonas mobilis	Glucose, fructose and sucrose
Yeasts	
Candida pseudotropicalis	Glucose and galactose
Candida tropicalis	Glucose, xylose and xylulose
Kluyveromyces fragilis	Glucose and galactose
Pachysolen tannophilus	Xylose*
Pichia stipitis	Xylose*
Saccharomyces cerevisiae	Glucose, sucrose, galactose, fructose, xylulose, maltose and maltotriose
Saccharomyces caerlbergensis	Glucose, galactose, fructose, xylulose, maltose and maltotriose
Saccharomyces rouxii (osmophilic)	Glucose, sucrose, fructose and maltose
Filamentous fungi	
Fusarium sp.	Xylose*
Mucor sp.	Xylose and arabinose*

* In addition to hexose monosaccharides and disaccharides

Process integration

Separate hydrolysis and fermentation (SHF)

Enzymatic hydrolysis of cellulose consists in a heterogeneous reaction where endoglucanases randomly attack the inner links of the chain and exoglucanases catalyze the oligosaccharides from the end of the polymer chains releasing cellobiose and shorter chains of cellulose which are soluble or do not depend on its degree of polymerization. Cellobiose is passing in the aqueous solution and is hydrolyzed in the homogeneous phase by β-1,4-glucosidase which catalyzes the hydrolysis of cellobiose to glucose. Most investigated cellulase systems were extracted from fungi such as *Trichoderma viride*, *Trichoderma reesei* and *Fusarium solani* (Gan *et al.*, 2003). Enzymatic hydrolysis is carried out under conditions of moderate temperature and pH in non-corrosive medium (temperature = 45-50°C and pH = 4.8) (Duff & Murray, 1996). This represents significant savings in energy and equipment. Furthermore, the enzymatic hydrolysis will only attack polysaccharides without altering the phenolic fraction, resulting cleaner and easily fermentable solutions (Vazquez *et al.*, 1991). In the first step glucose solution enters in fermentation reactor and the mixture is then distilled to remove bioethanol. In the second step, xylose is fermented with the production of bioethanol and then the bioethanol is distilled (Hamelinck *et al.*, 2003; Hamelinck *et al.*, 2005). The main advantage of SHF is that hydrolysis and fermentation take place under optimum conditions. The disadvantage is that cellulolytic enzymes are inhibited, so that the rate of hydrolysis is reduced when glucose and cellobiose are accumulating (Hahn-Hagerdal *et al.*, 2006; Balat *et al.*, 2008) (Figure 1).

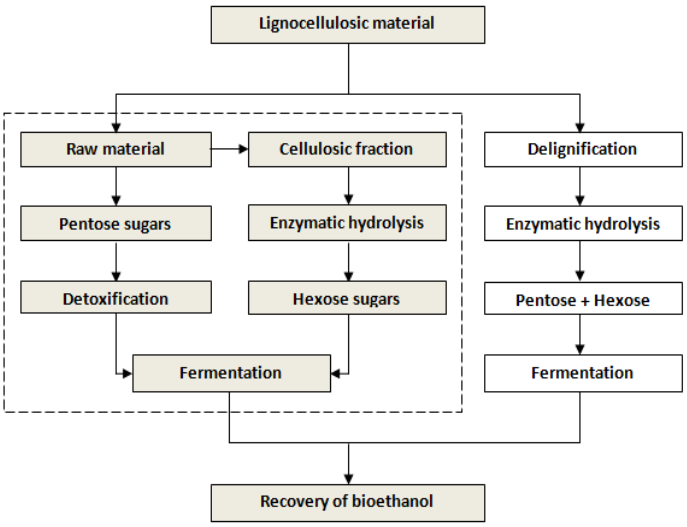

Figure 1. Separate hydrolysis and fermentation (SHF) with separate pentose and hexose sugars and combined sugar fermentation (*source*: Chandel *et al.*, 2007; Balat *et al.*, 2008)

Simultaneous saccharification and fermentation (SSF)

Enzymatic hydrolysis of LCM is a very slow process because cellulose hydrolysis is prevented by the structural parameters of the substrate, such as lignin and hemicellulose content as well as by crystallinity of cellulose (Pan *et al.*, 2006). Enzymatic hydrolysis usually occurs at pH = 4.8 and temperature of 45-50˚C (Sun, 2002). Carbohydrates of pretreatment and enzymatic hydrolysis steps are fermented by enzymes produced by certain microorganisms (bacteria, yeasts or filamentous fungi) (Hahn-Hagerdal *et al.*, 2006). SSF provides higher bioethanol yields and requires small amounts of enzyme (Dien *et al.*, 2003; Chandel *et al.*, 2007). SSF process can be carried out in batch mode with multiple additions of solid substrate or in fed-batch mode with higher concentrations of final product. The optimum enzyme loading depends on the conditions during SSF such as water-insoluble solids (WIS) and inhibitory compounds concentration in the SSF medium (Hoyer *et al.*, 2010).

SSF is a process that uses natural materials containing heterogeneous polymer complex, such as lignin, pectin and lignocellulose (Sabu *et al.*, 2006) (Figure 2).

Major advantages of SSF are (Sun & Cheng, 2002): increase of hydrolysis rate by conversion of sugars; small amounts of enzymes used; high yields of the products obtained; lower requirements under sterile conditions because glucose is removed immediately by producing bioethanol; short processing time; lower volume bioreactor. The main disadvantage of SSF process is to choose the optimal temperature (Krishna *et al.*, 2001). In many cases, pH < 5 and temperatures higher than 40˚C may be favorable for enzymatic hydrolysis, since low pH can inhibit the production of lactic acid and high temperatures can adversely affect the growth of microorganisms (Huang *et al.*, 2005). Cellulase from *Trichoderma reesei*, which is the most active enzyme preparation, has optimal activity at pH = 4.5 and

temperature of 55˚C. For *Saccharomyces cerevisiae* cultures, the process is controlled at pH = 4.5 and temperature of 37˚C (Dien *et al.*, 2003).

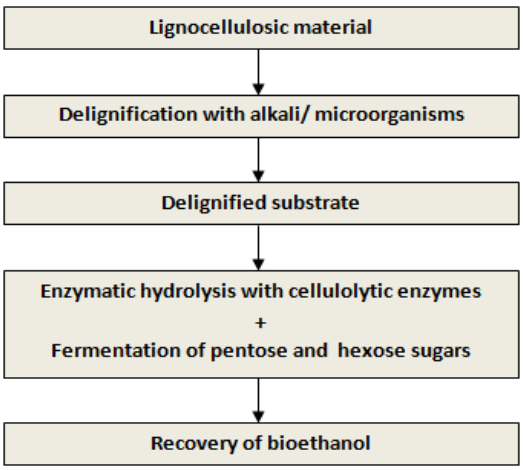

Figure 2. Simultaneous saccharification and fermentation (SSF) with combined sugars fermentation (*source*: Chandel *et al.*, 2007; Balat *et al.*, 2008)

Several species of bacteria such as *Clostridium*, *Thermoanaerobacter*, *Thermoanaerobium*, *Thermobacteroides* species, yeasts such as *Candida*, *Kluyveromyces*, *Pachysolen*, *Pichia*, *Saccharomyces* species and fungi such as *Fusarium* and *Mucor* species are able to produce ethanol (Waites *et al.*, 2001).

Simultaneous saccharification and co-fermentation (SSCF)

Recently, SSF technology has proven to be advantageous for simultaneous fermentation of hexose and pentose sugars, this process is called simultaneous saccharification and co-fermentation (SSCF). In SSCF, enzymatic hydrolysis is releasing continuously hexose sugars, which increase the rate of glycolysis, so that pentose sugars are fermented faster and with better yields (Hahn-Hagerdal *et al.*, 2006). In this process, cellulose is converted into glucose using enzymes (cellulase). An enzyme preparation (cellulase) is a mixture of enzymes (catalytic proteins) that work together to break the cellulose fibers in cellobiose and glucose. Glucose and carbohydrates resulted in hydrolysis of hemicellulose during pretreatment are fermented to bioethanol. Bacteria used for co-fermentation of cellulose are *Zymomonas mobilis* because this organism and its genome is largely accessible (Urbanchuk, 2006). "Co-fermentation" means that the microorganism can ferment simultaneously glucose and xylose to bioethanol. For fermentation of cellulose are considered other cultures such as genetically modified strains of *Saccharomyces cerevisiae* (McAloon *et al.*, 2000; Balat *et al.*, 2008).

Enzymatic hydrolysis is initiated in a continuous reactor. Diluted and neutralized hydrolysate is cooled with water and introduced into the reactor at a concentration of 20%. Originally, the enzyme (cellulase) is mixed with the hydrolysate at a temperature of 48°C (Humbird *et al.*, 2011). The quantity of enzyme used is

determined by the quantity of cellulose present in the hydrolysate and the specific activity of the enzyme. During enzymatic hydrolysis, temperature is maintained by cooling water. Studies by NREL showed optimum temperature at 48 °C for a range of commercial enzymes. A deviation from this temperature would reduce the efficiency of converting cellulose into bioethanol. The hydrolysate is containing 11.7% total soluble carbohydrates (including oligomers) with 6.7% glucose and 3.7% xylose. The hydrolysate is cooled to a temperature of 32°C for fermentation (Humbird *et al.*, 2011). Recombinant bacteria *Zymomonas mobilis* can simultaneously ferment glucose and xylose to bioethanol. In order to ensure an appropriate volume of 10% inoculum for fermentation, 10% of hydrolysate is sent to prepare the culture medium. In addition to being fermented to ethanol, sugars can be converted into products of contamination by microorganisms. Co-fermentation process lasts 36 hours. Inoculum is fed with corn syrup (CSL) and ammonium phosphate (DAP). It is assumed that the viscosity reduction of hydrolysate occurs in continuous hydrolysis reactor and stirring is not required in anaerobic fermentation with *Zymomonas mobilis*. Fermented syrup, having a 5.4% ethanol concentration, is sent to the storage tank (Humbird *et al.*, 2011).

Olofsson *et al.* (2010a) demonstrates a new approach for controlling the glucose release rate from the enzymatic hydrolysis by controlling the addition of enzymes in SSCF using spruce as feedstock and a recombinant xylose fermenting strain *Saccharomyces cerevisiae* TMB3400. The results showed that the total xylose uptake could be increased from 40% to 80% by controlling the enzyme feed.

Jin *et al.* (2012) presented information on the performance of industrial xylose fermenting strain in lignocellulosic hydrolyzates. Xylose consumption by *Saccharomyces cerevisiae* 424A(LNH-ST), which is a widely used genetically modified xylose-fermenting yeast strain for ethanol production from AFEX^{TM} pretreated biomass, during SSCF of AFEX^{TM} pretreated switchgrass was inhibited by unhydrolyzed solids. Such inhibitory effects were not found in unhydrolyzed solids from AFEX^{TM} pretreated corn stover.

Table 3. Comparative data of ethanol production from LCM using different process configurations

Substrate	Microorganism	Process	Temp (°C)	Final ethanol conc (g L^{-1})	Ethanol yield (g g^{-1})[1]	Ethanol yield (%)[2]	References
Sugarcane bagasse	*Zymomonas mobilis* (immobilized in PVA)	SHF	EH:45; F:30	6.24	0.403	79.09	Wirawan *et al.*, 2012
Sugarcane bagasse	*Zymomonas mobilis* (immobilized in CA)	SHF	EH:45; F:30	5.52	0.356	69.96	Wirawan *et al.*, 2012
Sugarcane bagasse	*Zymomonas mobilis* (immobilized in PVA)	SSF	30	5.53	0.357	70.09	Wirawan *et al.*, 2012

Substrate	Microorganism	Process	Temp (°C)	Final ethanol conc (g L^{-1})	Ethanol yield (g g^{-1})[1]	Ethanol yield (%)[2]	References
Sugarcane bagasse	*Zymomonas mobilis* (immobilized in CA)	SSF	30	5.44	0.351	68.95	Wirawan *et al.*, 2012
Bermudagrass	*Saccharomyces cerevisiae*	SSF	38	16.10	0.480*	94.70	Li *et al.*, 2009
Reed	*Saccharomyces cerevisiae*	SSF	38	16.40	0.490*	96.40	Li *et al.*, 2009
Rapeseed	*Saccharomyces cerevisiae*	SSF	38	15.80	0.470*	92.90	Li *et al.*, 2009
Rice straw	*Saccharomyces cerevisiae*	SSF	38	12.70	0.420*	83.10	Ko *et al.*, 2009
Corn stover	*Saccharomyces cerevisiae*	SSF	35	33.80	0.410*	80.20	Öhgren *et al.*, 2007
Barley straw	*Saccharomyces cerevisiae*	SSF	35	22.40	0.410*	80.00	Linde *et al.*, 2007
Spruce	*Saccharomyces cerevisiae*	SSF	37	44.50	0.430	84.00	Rudolf *et al.*, 2005
Populus nigra	*Kluyveromyces marxianus* CECT 10875	SSF	42	19.00	0.360	71.20	Ballesteros *et al.*, 2004
Eucalyptus globulus	*Kluyveromyces marxianus* CECT 10875	SSF	42	17.00	0.320	62.50	Ballesteros *et al.*, 2004
Wheat straw	*Kluyveromyces marxianus* CECT 10875	SSF	42	18.10	0.320	62.50	Ballesteros *et al.*, 2004
Sweet sorghum bagasse	*Kluyveromyces marxianus* CECT 10875	SSF	42	16.20	0.310	60.90	Ballesteros *et al.*, 2004
Brassica carinata residue	*Kluyveromyces marxianus* CECT 10875	SSF	42	19.00	0.350	68.10	Ballesteros *et al.*, 2004
Spruce	*Saccharomyces cerevisiae* TMB3400	SSCF	34	32.90	0.390	77.00	Olofsson *et al.*, 2010a
Spruce	*Saccharomyces cerevisiae* TMB3400	SSCF	34	35.50	0.410	79.00	Olofsson *et al.*, 2010a
Wheat straw	*Saccharomyces cerevisiae* TMB3400	SSCF	34	38.00	0.350	69.00	Olofsson *et al.*, 2010b

* Not directly given in the reference article, calculated by the authors.
[1] g ethanol/g cellulose (in most cases expressed as potential glucose) in the pretreated raw material.
[2] based on maximum theoretical ethanol yield on available sugars (in most cases only glucose) in the pretreated raw material.
PVA – polyvinyl alcohol; CA – calcium alginate; EH – enzymatic hydrolysis; F – fermentation.

Conclusions

The challenges for different process integration technologies for bioethanol production from LCM are to obtain high degree of hydrolysis and high ethanol yields.
Compared with SHF technology, where each stage takes place under optimal operating conditions (minimizing the interaction between hydrolysis and fermentation), the main

SSF advantages are as follows: i) obtaining higher ethanol yields with small amount of enzymes; ii) increasing the hydrolysis rate by sugars conversion; and iii) lower requirements under sterile conditions, because glucose is removed immediately by producing bioethanol. In SSCF process, enzymatic hydrolysis is releasing continuously hexose sugars, so that pentose sugars are fermented faster and with better yields.

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