ΠΡΟΓΡΑΜΜΑ ΔΙΑ ΒΙΟΥ ΜΑΘΗΣΗΣ ΑΕΙ ΓΙΑ ΤΗΝ ΕΠΙΚΑΙΡΟΠΟΙΗΣΗ ΓΝΩΣΕΩΝ ΑΠΟΦΟΙΤΩΝ ΑΕΙ (ΠΕΓΑ)

«Οι σύγχρονες τεχνικές βιο-ανάλυσης στην υγεία, τη γεωργία, το περιβάλλον και τη διατροφή»

Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Biotechnology

Advances in ethanol production

Claudia C Geddes, Ismael U Nieves and Lonnie O Ingram

Barriers to the commercialization of lignocellulosic ethanol include the development of more robust biocatalysts, reduction of cellulase costs, and high capital cost associated with a complex process. Improvements have been made in all areas during the past two years. Oxidoreductases, transporters, and regulators have been identified that can increase the tolerance of biocatalysts to inhibitors formed during pretreatment. Biocatalysts are being developed that grow under conditions that are optimal for cellulase activity and others have been engineered to produce glycoside hydrolases. Ethanol yields resulting from most current process configurations are similar, approximately 0.21 g ethanol/g dry cellulosic feedstock. Potentially, this can be increased to at least 0.27 g ethanol/g biomass (83 gal/ton) using simpler processes.

Address
Department of Microbiology & Cell Science, University of Florida, Box 110700, Gainesville, FL 32611, United States

Corresponding author: Ingram, Lonnie O (ingram@ufl.edu)

Current Opinion in Biotechnology 2011, **22**:312–319

This review comes from a themed issue on
Energy biotechnology
Edited by Peter Dürre and Tom Richard

Available online 19th May 2011

0958-1669/$ – see front matter

DOI 10.1016/j.copbio.2011.04.012

Introduction

Lignocellulosic biomass (LCB) is an abundant, renewable source of carbohydrates for microbial conversion to chemicals and fuels. However, lignocellulose (cellulose and hemicellulose) is designed by nature to resist depolymerization. Processes that produce fermentable sugars from LCB tend to be complex and capital intensive. Chemical pretreatment is essential to increase cellulase access [1–3]. Pretreatments that hydrolyze hemicellulose into sugar syrups also form side products that retard fermentation. A solid-liquid separation and partial sugar purification are typically included to mitigate toxins, followed by the separate fermentation of C-5 (hemicellulose) and C-6 (cellulose) sugars.

Approximately 200 million dry tons of LCB are produced in the U.S. each year that could be used to produce 16 billion gallons of ethanol (Oak Ridge National Laboratory; URL: http://www.ornl.gov/~webworks/cppr/y2001/rpt/123021.pdf). Starch-based ethanol (U.S.) and sugarcane-based ethanol (Brazil) are now mature industries but both compete with food uses. Companies such as Abengoa, BP-Verenium, Coskata, Dupont-Danisco and Poet are attempting to commercialize cellulosic ethanol in the next decade (Gigaom; URL: http://gigaom.com/cleantech/12-companies-racing-to-build-cellulosic-ethanol-plants-in-the-us/). Pilot and demonstration plants will serve as platforms to identify bottlenecks and potential barriers to full commercialization.

This review highlights advances in the fermentative production of ethanol from lignocellulose during the past two years. Improvements are noted in the areas of pretreatment, biocatalysts, saccharification and liquefaction, and process simplification.

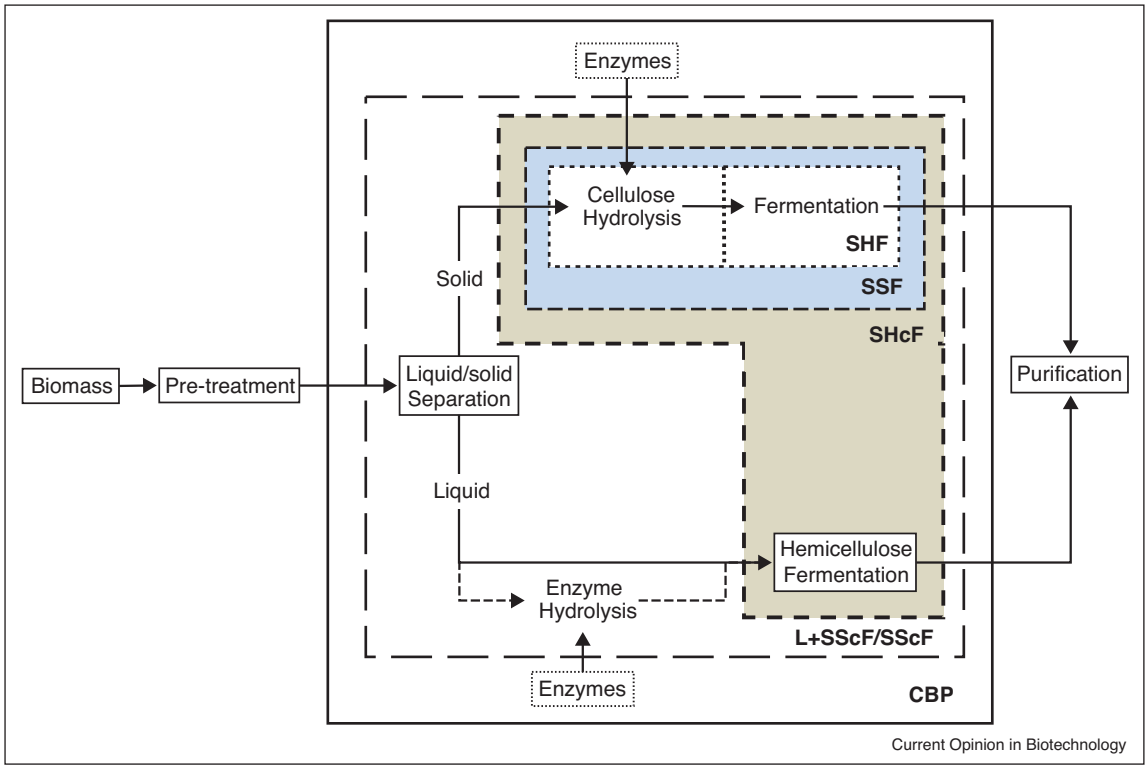
Advances in pretreatment

Pretreatments using dilute sulfuric acid require reactors made of exotic metals. Although all mineral acids have been explored to some extent, recent studies have proposed the use of phosphoric acid [4••,5]. As a weaker acid, phosphoric acid pretreatment produces lower levels of toxic side products than sulfuric acid pretreatment and can be used with a stainless steel reactor [4••]. Autohydrolysis produces the lowest levels of side products [2]. With autohydrolysis, hemicellulose components are solubilized as oligosaccharides that require further hydrolysis with enzymes or acid. Ethanologenic *Escherichia coli* strains have been adapted to phosphoric acid hydrolysates and can now ferment hemicellulose and cellulose derived sugars together in a single vessel, termed simultaneous saccharification and co-fermentation (SScF). The use of phosphoric acid could eliminate the need for separation of hydrolysates from pretreated fiber, detoxification, and reactors of exotic metals resulting in a simpler process quite analogous to that for corn ethanol (Figure 1). Using this process, ethanol yields of up to 0.27 g/g bagasse (dry weight) have been obtained (83 gal/ton) [6••].

The SPORL process (sulfite pretreatment to overcome recalcitrance of lignocellulose) and SO_2 impregnation use sulfur compounds to disrupt the LCB structure [7••,8]. SPORL is better suited for biomass with high lignin content and SO_2 impregnation for agricultural residues [8]. SPORL pretreatment was shown to increase sugar yields (from 57% to 88%) and reduce inhibitors by up to 65% compared to dilute sulfuric acid pretreatment of softwood [7••,9].

Ammonia-based AFEX pretreatment (ammonia fiber expansion) is very effective at increasing fiber digestion while producing lower levels of inhibitors than sulfuric

Figure 1



Lignocellulose to ethanol process configurations. The cellulose could be hydrolyzed alone before fermentation (separate hydrolysis and fermentation, SHF) or with the hemicellulose (separate hydrolysis and co-fermentation, SHcF) followed by fermentation of the resulting slurry. Cellulose hydrolysis could also occur simultaneously with fermentation in the presence (SScF) or absence (SSF) of hemicellulose. In the liquefaction followed by simultaneous saccharification and co-fermentation (L + SScF) process, there is a cellulose prehydrolysis step in the presence of hemicellulose hydrolysate followed by fermentation but the cellulases continue to hydrolyze the cellulose during fermentation. The consolidated bioprocessing process involves a biocatalyst that is capable of producing all the hydrolytic enzymes required for cellulose hydrolysis and is also capable of fermenting all the resulting sugars in the presence of hydrolysate inhibitors.
Adapted from [2].

acid pretreatments [10,11]. Hemicellulose oligomers produced by the AFEX process require further hydrolysis into monomers [2]. After AFEX pretreatment and enzymatic digestion, over 80% of the carbohydrate in the fiber was recovered as soluble sugar [11]. Subsequent studies have identified compounds (4-hydroxybenzaldehyde, lactate, and acetate) formed during AFEX pretreatment that inhibited fermentations with *E. coli* KO11 [12••]. AFEX remains a highly effective pretreatment for grasses.

Advances in biocatalyst

The need for more robust biocatalysts is one of the weakest links in the LCB to ethanol process. These biocatalysts need to be resistant to inhibitors formed during lignocellulose pretreatments, co-utilize a variety of sugars at high yields, secrete cellulase enzymes, and remain active under conditions that are near optimal for cellulase function (pH 5, 50 °C). Much of the complexity in lignocellulosic ethanol processes stems from the need for toxin mitigation (solid liquid separation after pretreatment; sugar cleanup) before fermentation. Additional complexity comes from the requirement for external sources of cellulase enzymes. Developing biocatalysts that ferment under conditions that are near optimal for fungal cellulase activity can reduce the requirement for external enzymes. Engineering the fermenting biocatalyst to produce some or all of the cellulase enzymes provides a complementary route to further reduce enzyme cost. The development of a fermentation-based lignocellulose to ethanol industry depends on research advances to minimize these biological impediments.

Developing tolerance to hydrolysate inhibitors

Furans from sugar dehydration, acetate, and soluble products from lignin are the primary inhibitors in hemicellulose hydrolysates from dilute acid pretreatment [13]. Of these, furans appear to be particularly important and have been the focus of many recent papers. Biocatalysts have been developed with increased resistance to furfural [14••,15,16••,17,18,19••], 5-hydroxymethylfurfural [15,19••,20], acetate [21•], and to unfractionated dilute acid hydrolysate [6••,15,19••,22••,23••].

Furans can be reduced to less toxic furan alcohols by most organisms using native enzymes. Expression arrays have noted many changes among oxidoreductases in response to furans with yeast [14,15,24,25••], *Zymomonas mobilis* [19••,21•], and ethanologenic *E. coli* [16••,17,18,20]. In some cases, regulators have been identified [18,19••]. Several of the furfural reductase enzymes in *E. coli* have sufficiently low K_m values for NADPH that growth is inhibited until furan metabolism has been completed. Silencing of these low K_m enzymes (less than 20% of total furfural reductase activity) was beneficial for furfural tolerance in *E. coli* [17]. Other higher K_m enzymes (NADPH), NADH-dependent enzymes [15], and transhydrogenase enzymes have been shown to confer partial resistance to furfural [16••,18,20].

Reduced sulfur compounds have long been used to improve the fermentation of dilute sulfuric acid hydrolysates of wood although the mode of action remains unknown [23••,26••,27]. *Saccharomyces cerevisiae* fermentation of acid hydrolysates (sugarcane bagasse and spruce wood) was improved by the direct addition of reduced sulfur compounds to slurries containing complex media, termed 'in situ detoxification' [26••]. The use of sodium metabisulfite and sodium hydrosulfite was also shown to improve the fermentation of slurries containing phosphoric acid pretreated sugarcane bagasse (L + SScF) using *E. coli* in mineral salt medium [23••]. Surprisingly, addition of metabisulfite did not decrease the toxicity of furfural or acetate when each was tested alone. It is possible that metabisulfite neutralizes the toxicity of soluble products from lignin.

Improving hexose and pentose sugar co-utilization

The co-utilization of hexose and pentose (xylose and arabinose) sugars remains a challenge for biocatalysts, especially in the presence of hydrolysate inhibitors. Derivatives of *S. cerevisiae* have been previously engineered to ferment xylose and these continue to be improved by additional genetic changes in xylose metabolism [28••,29,30]. Although ethanologenic *E. coli* have the native ability to metabolize all sugars from LCB, xylose utilization lags behind glucose and was also improved by further genetic changes [31]. Inhibitors present in acid hydrolysates retarded xylose metabolism during *E. coli* fermentation even with this genetic change. The lag in xylose metabolism was substantially relieved by injection of small amounts of air during fermentation, termed microaeration [6••,32].

Advances in saccharification

Reducing the cost of enzyme production

The cost of cellulase enzymes remains a major concern for the commercialization of LCB ethanol processes. Cost estimation software (*e.g.* Aspen Plus and Aspen Icarus Process Evaluator) has been used to compare the minimum ethanol selling prices of processes involving the purchase of commercial cellulase and on-site cellulase production [33]. International enzyme companies such as Novozymes and Genencor have formed partnerships with Poet LLC and Dupont Danisco Cellulosic Ethanol LLC, respectively, to commercialize lignocellulosic ethanol (The New York Times; URL: http://www.nytimes.com/cwire/2010/02/16/16climatewire-economics-improve-for-first-commercial-cellu-93478.html?scp=1&sq=cellulosic%20ethanol%20plants&st=cse). Both have reported that enzymes will cost approximately $0.50/gallon of ethanol. This represents an 80% price reduction during the last two years.

Efforts continue to reduce the cellulase requirement. Novel cellulases have been isolated from a variety of organisms using improved screening methods [34]. Current research has focused on developing improved cellulase enzyme cocktails, development of biocatalysts with fermentations that match the optimal conditions for cellulase activity, and novel cellulases [34,35••]. *Trichoderma reesei* is currently the primary industrial organism used for the production of cellulase enzymes. Sequencing of the *T. reesei* genome will facilitate further improvements in enzyme production [36].

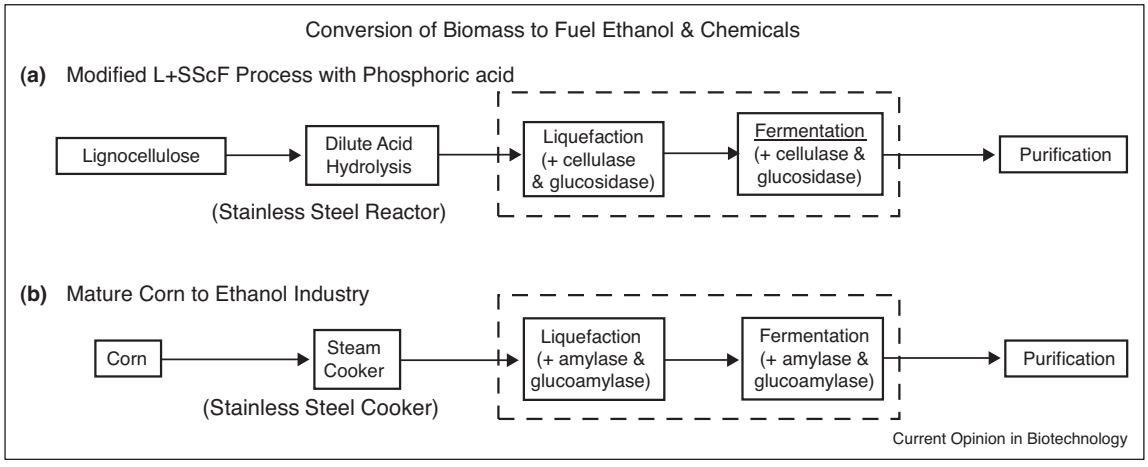
Improving cellulase performance

Compounds have been identified that increase cellulase effectiveness and enzyme usage [37,38••,39]. These include surfactants (Tween 80, cetylpyridinium chloride, and cetyl trimethylammonium bromide) and divalent metals (calcium and magnesium). Up to 35% improvement in saccharification was reported. All are proposed to act by reducing the nonproductive binding of cellulases to lignin. *Bacillus coagulans* is a thermotolerant biocatalyst capable of growth at temperatures and pH (55 °C, pH 5.0) that are optimal for fungal cellulases. Using this organism, the cellulase was reduced to 5 FPU/g cellulose during lactate production [35••,40]. Similar benefits would be expected for ethanol production after further metabolic engineering, and for other biocatalysts that can function under these conditions.

Toward consolidated bioprocessing

Consolidated bioprocessing without the need for externally supplied enzymes remains a goal for many scientists [41•,42•,43••,44,45]. Expression of endoglucanase I and II genes from *T. reesei* QM6a allowed the resulting strain to ferment phosphoric acid swollen cellulose (amorphous) when beta-glucosidase was supplied [41•]. Tsai *et al.* and Wen *et al.* reported the development of recombinant *S. cerevisiae* strains capable of displaying functional mini-cellulosomes on their surface exhibiting enzyme synergy and producing 3.5 g/L and 1.8 g/L ethanol, respectively, using phosphoric acid swollen cellulose [42•,43••]. Previous studies have demonstrated up to 11 g/L ethanol production from phosphoric acid swollen cellulose using *Klebsiella oxytoca* strain SZ21 expressing endoglucanase

Figure 2



Comparison of ethanol production from lignocellulose and corn. **(a)** Simplified process using phosphoric acid hydrolysis of hemicellulose and enzymatic hydrolysis of cellulose. Enzymatic liquefaction was added before co-fermentation of hexose and pentose sugars in a single vessel (L + SScF) and **(b)** enzymatic liquefaction of hydrated corn before simultaneous saccharification and fermentation (L + SSF). Adapted from [21•].

genes from *Erwinia chrysanthemi* (*celY*, *celZ*) [44]. Synergies between purified cellulases and xylanases from the thermophilic bacterium *Thermobifida fusca* displayed on 'designer cellulosomes' were found to possess higher activity on wheat straw than the corresponding free enzymes [45].

Process simplification

Reducing process complexity remains a major challenge for the commercialization of LCB to ethanol. Current research is focused on eliminating the need for detoxification of hydrolysates, developing robust biocatalysts capable of fermenting pentose and hexose sugars simultaneously, reducing water usage, increasing ethanol yield and titer, and decreasing cellulase usage. Considerable progress has been made during the past two years by developing robust biocatalysts capable of fermenting pentose and hexose sugars simultaneously. Further progress is needed to increase ethanol titers and to decrease water and cellulase usage. Collaborative research projects have focused on comparing pretreatment options for specific biomass types (*e.g.* corn stover or poplar wood [1,46••]). Various process configurations are shown in Figure 2. These decrease in complexity from separate hydrolysis and fermentation (SHF) to consolidated bioprocessing (CBP). The SHF process involves separation of the cellulose-rich solid from the hemicellulose hydrolysate and separate fermentation trains. L + SScF and SScF processes combine C-6 and C-5 sugar fermentations in a single vessel [6••,22••,23••]. The consolidation of bioprocessing steps is hindered by the fibrous nature of suspensions at loadings of 10–20% solids [47,48]. Models have been described relating viscosity, solubilized sugars, time, and enzyme loadings for slurries of sugarcane bagasse [4••,48,49]. On the basis of these studies, a partial saccharification step using a CSTR (one to six hours residence) was proposed [4••]. This liquefaction step can produce slurries containing 10–15% solids (solids plus solubles) that can be readily pumped and mixed.

SScF of lignocellulosic biomass

Pretreatment processes typically require solid–liquid separations and neutralization of hydrolysate toxins before fermentation. With the development of hydrolysate resistant biocatalysts such as *E. coli* MM160 [22••,23••] and *S. cerevisiae* 424A [43••,44,45,46••,47], comparable yields could be obtained with less process complexity. The development of robust biocatalysts allowed the fiber and liquid from pretreatment to be fermented without separation [6••,22••,23••]. The resulting process is analogous to the mature corn dry milling ethanol process (Figure 1) that combines all components in a single vessel after an initial liquefaction step (L + SScF process).Ethanol yields for LCB processes have continued to improve during the past two years (Table 1). Despite differences in process complexity, similar ethanol yields were obtained by most researchers, approximately 0.21 g/g (63 gal/ton). Higher yields are obtained when purified cellulose was used (*e.g.* paper sludge [55••]) or starch combined with lignocellulose (*e.g.* corn silage and whole corn plant [53]). The use of SPORL pretreatment is making similar progress toward process simplifications (*e.g.* L + SScF) although part of the hydrolysate was removed before fermentation [56]. AFEX treated corn stover supplemented with corn steep liquor was fermented after an initial 96 h prehydrolysis (cellulases and hemicellulases added) to produce 40 g/L ethanol

Table 1

Comparison of ethanol yields from SScF processes.

Feedstock	Pretreatment	Biocatalyst	Ethanol		Reference
			Titer (g/L)	Yield (g/g untreated feedstock)	
Rice straw	Dilute acid	*M. indicus*	11	0.11	[60]
Spruce	SO_2 impregnation	*S. cerevisiae* TMB3400	45	Not calculated[c]	[61]
Forage sorghum	Aqueous ammonia	*S. cerevisiae*	–	0.13	[62••]
Hybrid poplar	Aqueous ammonia[a]	*E. coli* KO11	16	0.24 calculated[d]	[63]
Rice straw	Aqueous ammonia[a]	*S. cerevisiae* D5A	12	0.12	[64]
Corn stover	AFEX	*S. cerevisiae* 424A(LNH-ST)	40	0.20	[51••]
Wheat straw	Steam explosion[a]	*K. marxianus* CECT 10875	36	0.18 calculated[d]	[65••]
Rice straw	AFEX	*S. cerevisiae* 424A(LNH-ST)	37	0.21 calculated[d]	[54]
Rice straw	AFEX	*P. stipitis* FPL-061	30	0.17 calculated[d]	[54]
Rice straw	AFEX	*P. stipitis* DX-26	28	0.16 calculated[d]	[54]
Switchgrass	Hydrothermolysis	*S. cerevisiae* D5A	22	0.17 calculated[d]	[66]
Switchgrass	Hydrothermolysis	*K. marxianus* IMB	19	0.15 calculated[d]	[66]
Barley straw	Steam explosion	*K. marxianus* CECT 10875	22	0.17 calculated[d]	[67]
Sugarcane bagasse	Dilute phosphoric	*E. coli* MM160 (KO11 derivative)	29	0.21	[22••]
Sugarcane bagasse	Dilute phosphoric	*E. coli* MM160 (KO11 derivative)	20	0.20	[23••]
Sugarcane bagasse	Dilute phosphoric	*E. coli* MM170 (KO11 derivative)	27	0.27	[6••]
Spruce	SO_2 impregnation	*S. cerevisiae* (bakers's yeast)	18	0.18 calculated[d]	[68••]
Switchgrass	AFEX	*S. cerevisiae* 424A(LNH-ST)	36	0.19	[50]
Distillers grains	Liquid hot water	*S. cerevisiae* D5A	14	0.09 calculated[d]	[69]
Distillers grains	AFEX	*S. cerevisiae* D5A	14	0.09 calculated[d]	[69]
Corn stover	AFEX	*S. cerevisiae* 424A(LNH-ST)	40	0.22 calculated[d]	[70]
Corn stover	AFEX	*E. coli* KO11	31	0.17 calculated[d]	[70]
Corn stover	AFEX	*Z. mobilis* AX101	32	0.18 calculated[d]	[70]
Forage sorghum	AFEX	*S. cerevisiae* 424A(LNH-ST)	31	0.17 calculated[d]	[52]
Sweet sorghum bagasse	AFEX	*S. cerevisiae* 424A(LNH-ST)	42	0.15 calculated[d]	[52]
Forage sorghum	AFEX	*S. cerevisiae* 424A(LNH-ST)	31	0.18 calculated[d]	[52]
Sweet sorghum bagasse	AFEX	*S. cerevisiae* 424A(LNH-ST)	29	0.18 calculated[d]	[52]
Rice straw	Dilute acid	*C. tropicalis* ATCC 13803	20	0.20	[71]
Corn silage	AFEX	*S. cerevisiae* 424A(LNH-ST)	28	0.31 calculated[d]	[53]
Whole corn plant	AFEX	*S. cerevisiae* 424A(LNH-ST)	30	0.32 calculated[d]	[53]
Lodgepole pine	SPORL[b]	*S. cerevisiae* Y5	21	0.21	[7]
Paper sludge	No additional treatments	*S. cerevisiae* RWB222	45	0.26 calculated[d]	[55••]
Paper sludge	No additional treatments	*Z. mobilis* 8b	46	0.27 calculated[d]	[55••]
Lodgepole pine	SPORL[b,e]	*S. cerevisiae* D5A	–	0.22	[56]
Corn stover	AFEX	*C. phytofermentans*	2.8	0.17	[72]
Corn stover	AFEX	*S. cerevisiae* 424A(LNH-ST)	3.9	0.24	[72]

[a] Solids were washed after pretreatment.
[b] Solid-liquid separation after pretreatment.
[c] Unable to calculate with reported data.
[d] Results presented were used to calculate yields on an original biomass basis.
[e] Detoxification of hydrolysate before fermentation.

(0.22 g ethanol/g corn stover [51••]) and dilute acid pretreated sugarcane bagasse was fermented to high ethanol yields (0.27 g ethanol/g bagasse) when air was added to the headspace during a L + SScF process [6••].

Dual uses of process residues, chemicals, and water

Beneficial products must be derived from all materials entering LCB to ethanol processes. Vinasse, stillage from sugarcane ethanol processes, has been used for many years as a fertilizer for biomass crops [57]. Pretreatment processes with phosphoric acid offer a similar opportunity by producing an ammonium phosphate fertilizer that includes magnesium sulfate and trace metals [4••,22••,49]. A phosphoric acid LCB-ethanol process can be viewed as a temporary stop for water and fertilizer *en route* to farms for new crop growth, sharing the cost of these materials. Lignin-rich residues can be used as boiler fuel or converted to higher value products [58,59]. Lignin could also be formed into inert blocks as an effective means for carbon sequestration.

Conclusions

The challenge of producing 36 billion gallons of ethanol by the year 2022 is being met with an expansion of research in the biofuel arena. Major improvements have been made as researchers learn more about the genetic basis of resistance to inhibitors in acid hydrolysates and pentose utilization. Pretreatment processes have been optimized to minimize inhibitor formation and to improve enzymatic hydrolysis of cellulose. The cost of cellulase enzymes remains a concern. Approaches have been proposed to minimize external enzyme usage by

producing enzymes in the biocatalyst and by providing conditions that increase the effectiveness of cellulases.

Acknowledgements

The authors gratefully acknowledge support by grants from the U.S. Department of Energy (DE-FG36-08GO88142), Verenium Corporation, Myriant Technologies, and the Florida Universities Board of Governors.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Dale BE: **Editorial: consortium for applied fundamentals and innovation (CAFI)**. *Biotechnol Prog* 2009, **25**:301.

2. Girio FM, Fonseca C, Carvalheiro F, Duarte LC, Marques S, Bogel-Lukasik R: **Hemicelluloses for fuel ethanol: a review**. *Bioresour Technol* 2010, **101**:4775-4800.

3. Zhang YH, Berson E, Sarkanen S, Dale BE: **Sessions 3 and 8: pretreatment and biomass recalcitrance: fundamentals and progress**. *Appl Biochem Biotechnol* 2009, **153**:80-83.

4. •• Geddes CC, Peterson JJ, Roslander C, Zacchi G, Mullinnix MT, Shanmugam KT, Ingram LO: **Optimizing the saccharification of sugar cane bagasse using dilute phosphoric acid followed by fungal cellulases**. *Bioresour Technol* 2010, **101**:1851-1857.
The authors show a decrease in side reactions during dilute acid pretreatment when phosphoric acid is used instead of sulfuric acid. Sugar yields higher than 60% were obtained for total sugars when using 0.5 FPU/g WIS.

5. Romero I, Moya M, Sanchez S, Ruiz E, Castro E, Bravo V: **Ethanolic fermentation of phosphoric acid hydrolysates from olive tree pruning**. *Ind Crop Prod* 2007, **25**:160-168.

6. •• Nieves IU, Geddes CC, Mullinnix MT, Hoffman RW, Tong X, Shanmugam KT, Ingram LO: **Injection of air into the headspace improves fermentation of phosphoric acid pretreated sugarcane bagasse by Escherichia coli MM170**. *Bioresour Technol* 2011 doi: 10.1016/j.biortech.2011.04.036.
The authors were able to improve sugar utilization by injecting small amounts of air into the headspace, resulting in increased ethanol yields. The process was successfully scaled up to 80 L with yields of up to 0.27 g ethanol/g bagasse.

7. •• Tian S, Luo XL, Yang XS, Zhu JY: **Robust cellulosic ethanol production from SPORL-pretreated lodgepole pine using an adapted strain *Saccharomyces cerevisiae* without detoxification**. *Bioresour Technol* 2010, **101**:8678-8685.
Successful SHcF process of softwood using an inhibitor tolerant strain *S. cerevisiae* Y5. A yield of 270 L/tonne wood was obtained using 15 FPU cellulase/g wood.

8. Zhu JY, Pan XJ: **Woody biomass pretreatment for cellulosic ethanol production: technology and energy consumption evaluation**. *Bioresour Technol* 2010, **101**:4992-5002.

9. Shuai L, Yang Q, Zhu JY, Lu FC, Weimer PJ, Ralph J, Pan XJ: **Comparative study of SPORL and dilute-acid pretreatments of spruce for cellulosic ethanol production**. *Bioresour Technol* 2010, **101**:3106-3114.

10. Balan V, Bals B, Chundawat SP, Marshall D, Dale BE: **Lignocellulosic biomass pretreatment using AFEX**. *Methods Mol Biol* 2009, **581**:61-77.

11. Lau MW, Gunawan C, Dale BE: **The impacts of pretreatment on the fermentability of pretreated lignocellulosic biomass: a comparative evaluation between ammonia fiber expansion and dilute acid pretreatment**. *Biotechnol Biofuels* 2009, **2**:30.

12. •• Lau MW, Dale BE: **Effect of primary degradation-reaction products from ammonia fiber expansion (AFEX)-treated corn stover on the growth and fermentation of *Escherichia coli* KO11**. *Bioresour Technol* 2010, **101**:7849-7855.
Using an engineered *S. cerevisiae* strain, the authors were able to ferment corn stover without detoxification or added nutrients to obtain a yield of 238 L/tonne corn stover.

13. Mills TY, Sandoval NR, Gill RT: **Cellulosic hydrolysate toxicity and tolerance mechanisms in *Escherichia coli***. *Biotechnol Biofuels* 2009, **2**:26.

14. •• Heer D, Heine D, Sauer U: **Resistance of *Saccharomyces cerevisiae* to high concentrations of furfural is based on NADPH-dependent reduction by at least two oxireductases**. *Appl Environ Microbiol* 2009, **75**:7631-7638.
The authors identified *ADH7* and an uncharacterized open reading frame, *YKL071W*, as genes that conferred furfural resistance to *S. cerevisiae*.

15. Liu ZL, Ma M, Song M: **Evolutionarily engineered ethanologenic yeast detoxifies lignocellulosic biomass conversion inhibitors by reprogrammed pathways**. *Mol Genet Genomics* 2009, **282**:233-244.

16. •• Miller EN, Jarboe LR, Turner PC, Pharkya P, Yomano LP, York SW, Nunn D, Shanmugam KT, Ingram LO: **Furfural inhibits growth by limiting sulfur assimilation in ethanologenic *Escherichia coli* strain LY180**. *Appl Environ Microbiol* 2009, **75**:6132-6141.
The authors proposed a model of furfural inhibition in which growth is limited by the assimilation of sulfur into amino acids due to the depletion of NADPH during furfural reduction.

17. Miller EN, Jarboe LR, Yomano LP, York SW, Shanmugam KT, Ingram LO: **Silencing of NADPH-dependent oxidoreductase genes (yqhD and dkgA) in furfural-resistant ethanologenic *Escherichia coli***. *Appl Environ Microbiol* 2009, **75**:4315-4323.

18. Turner PC, Miller EN, Jarboe LR, Baggett CL, Shanmugam KT, Ingram LO: **YqhC regulates transcription of the adjacent *Escherichia coli* genes *yqhD* and *dkgA* that are involved in furfural tolerance**. *J Ind Microbiol Biotechnol* 2011, **38**:431-439.

19. •• Yang S, Pelletier DA, Lu TY, Brown SD: **The *Zymomonas mobilis* regulator *hfq* contributes to tolerance against multiple lignocellulosic pretreatment inhibitors**. *BMC Microbiol* 2010, **10**:135.
This study reports the involvement of the global regulator Hfq in the resistance of inhibitors. For the purpose of this study, the authors created a plasmid for gene expression and mutation complementation in *Z. mobilis*.

20. Miller EN, Turner PC, Jarboe LR, Ingram LO: **Genetic changes that increase 5-hydroxymethyl furfural resistance in ethanol-producing *Escherichia coli* LY180**. *Biotechnol Lett* 2010, **32**:661-667.

21. • Yang S, Land ML, Klingeman DM, Pelletier DA, Lu TY, Martin SL, Guo HB, Smith JC, Brown SD: **Paradigm for industrial strain improvement identifies sodium acetate tolerance loci in *Zymomonas mobilis* and *Saccharomyces cerevisiae***. *Proc Natl Acad Sci U S A* 2010, **107**:10395-10400.
The authors used a combination of microarray-based CGS and next-generation 454 pyroresequencing to identify genes related to acetate tolerance in *Z. mobilis*.

22. •• Geddes CC, Mullinnix MT, Nieves IU, Peterson JJ, Hoffman RW, York SW, Yomano LP, Miller EN, Shanmugam KT, Ingram LO: **Simplified process for ethanol production from sugarcane bagasse using hydrolysate-resistant *Escherichia coli* strain MM160**. *Bioresour Technol* 2011, **102**:2702-2711.
Successful L + SScF of sugarcane bagasse without detoxification. A yield of 260 L ethanol per tonne bagasse was obtained using 10 FPU/g bagasse and a solids loading of 14%.

23. •• Nieves IU, Geddes CC, Miller EN, Mullinnix MT, Hoffman RW, Fu Z, Tong Z, Ingram LO: **Effect of reduced sulfur compounds on the fermentation of phosphoric acid pretreated sugarcane bagasse by ethanologenic Escherichia coli**. *Bioresour Technol* 2011, **102**:5145-5152.
The authors tested the effect of various reduced sulfur compounds on the performance of an ethanologenic *E. coli* strain during the fermentation of phosphoric acid pretreated sugarcane bagasse. Addition of sodium metabisulfite at the start of fermentation resulted in increased ethanol yields.

24. Liu ZL, Moon J: **A novel NADPH-dependent aldehyde reductase gene from *Saccharomyces cerevisiae* NRRL Y-12632 involved in the detoxification of aldehyde inhibitors derived from lignocellulosic biomass conversion**. *Gene* 2009, **446**:1-10.

25. •• Ma M, Liu ZL: **Comparative transcriptome profiling analyses during the lag phase uncover *YAP1*, *PDR1*, *PDR3*, *RPN4*, and *HSF1* as key regulatory genes in genomic adaptation to the**

lignocellulose derived inhibitor HMF for *Saccharomyces cerevisiae*. *BMC Genomics* 2010, **11**:660.
The authors identified several transcription factor genes that play a role in inhibitor tolerance.

26. •• Alriksson B, Cavka A, Jonsson LJ: **Improving the fermentability of enzymatic hydrolysates of lignocellulose through chemical in-situ detoxification with reducing agents**. *Bioresour Technol* 2011, **102**:1254-1263.
The authors reported the use of sulfur reducing agents for detoxification of hydrolysate without the need for additional steps.

27. Barbosa MF, Beck MJ, Fein JE, Potts D, Ingram LO: **Efficient fermentation of *Pinus* sp. acid hydrolysates by an ethanologenic strain of *Escherichia coli***. *Appl Environ Microbiol* 1992, **58**:1382-1384.

28. •• Bettiga M, Bengtsson O, Hahn-Hagerdal B, Gorwa-Grauslund MF: **Arabinose and xylose fermentation by recombinant *Saccharomyces cerevisiae* expressing a fungal pentose utilization pathway**. *Microb Cell Fact* 2009, **8**:40.
The authors express a fungal pathway for pentose utilization, resulting in a decrease in L-arabitol yield when compared to previously engineered strains with D-xylose reductase activity co-fermenting L-arabinose and D-xylose.

29. Brat D, Boles E, Wiedemann B: **Functional expression of a bacterial xylose isomerase in *Saccharomyces cerevisiae***. *Appl Environ Microbiol* 2009, **75**:2304-2311.

30. Runquist D, Fonseca C, Radstrom P, Spencer-Martins I, Hahn-Hagerdal B: **Expression of the Gxf1 transporter from *Candida intermedia* improves fermentation performance in recombinant xylose-utilizing *Saccharomyces cerevisiae***. *Appl Microbiol Biotechnol* 2009, **82**:123-130.

31. Yomano LP, York SW, Shanmugam KT, Ingram LO: **Deletion of methylglyoxal synthase gene (*mgsA*) increased sugar co-metabolism in ethanol-producing *Escherichia coli***. *Biotechnol Lett* 2009, **31**:1389-1398.

32. Okuda N, Ninomiya K, Takao M, Katakura Y, Shioya S: **Microaeration enhances productivity of bioethanol from hydrolysate of waste house wood using ethanologenic *Escherichia coli* KO11**. *J Biosci Bioeng* 2007, **103**:350-357.

33. Barta Z, Kovacs K, Reczey K, Zacchi G: **Process design and economics of on-site cellulase production on various carbon sources in a softwood-based ethanol plant**. *Enzyme Res* 2010:734182.

34. Maki M, Leung KT, Qin W: **The prospects of cellulase-producing bacteria for the bioconversion of lignocellulosic biomass**. *Int J Biol Sci* 2009, **5**:500-516.

35. •• Ou MS, Mohammed N, Ingram LO, Shanmugam KT: **Thermophilic *Bacillus coagulans* requires less cellulases for simultaneous saccharification and fermentation of cellulose to products than mesophilic microbial biocatalysts**. *Appl Biochem Biotechnol* 2009, **155**:379-385.
The use of thermophilic bacteria during SSF decreased the optimum concentration of enzymes to less than 5 FPU/g cellulose.

36. Hemme CL, Mouttaki H, Lee YJ, Zhang G, Goodwin L, Lucas S, Copeland A, Lapidus A, Glavina del Rio T, Tice H *et al.*: **Sequencing of multiple clostridial genomes related to biomass conversion and biofuel production**. *J Bacteriol* 2010, **192**:6494-6496.

37. Knutsen JS, Liberatore MW: **Rheology modification and enzyme kinetics of high solids cellulosic slurries**. *Energy Fuels* 2010, **24**:3267-3274.

38. •• Tu M, Saddler JN: **Potential enzyme cost reduction with the addition of surfactant during the hydrolysis of pretreated softwood**. *Appl Biochem Biotechnol* 2010, **161**:274-287.
The author projected that using the surfactant Tween 80 during enzymatic hydrolysis could result in savings of 60% of the cost of enzyme.

39. Tu M, Zhang X, Paice M, McFarlane P, Saddler JN: **Effect of surfactants on separate hydrolysis fermentation and simultaneous saccharification fermentation of pretreated lodgepole pine**. *Biotechnol Prog* 2009, **25**:1122-1129.

40. Su Y, Rhee MS, Ingram LO, Shanmugam KT: **Physiological and fermentation properties of *Bacillus coagulans* and a mutant lacking fermentative lactate dehydrogenase activity**. *J Ind Microbiol Biotechnol* 2010.

41. • du Plessis L, Rose SH, van Zyl WH: **Exploring improved endoglucanase expression in *Saccharomyces cerevisiae* strains**. *Appl Microbiol Biotechnol* 2010, **86**:1503-1511.
The endoglucanase I and II genes of *T. reesei* QM6a were cloned and expressed in *Saccharomyces cerevisiae*. The authors used random mutagenesis to increase endoglucanase I activity and they were able to hydrolyze phosphoric-acid-swollen cellulose.

42. • Tsai SL, Oh J, Singh S, Chen R, Chen W: **Functional assembly of minicellulosomes on the *Saccharomyces cerevisiae* cell surface for cellulose hydrolysis and ethanol production**. *Appl Environ Microbiol* 2009, **75**:6087-6093.
The assembled minicellulosomes maintained the synergistic effect for cellulose hydrolysis and produced ethanol directly from phosphoric acid-swollen cellulose with a 95% conversion of the carbohydrate consumed.

43. •• Wen F, Sun J, Zhao H: **Yeast surface display of trifunctional minicellulosomes for simultaneous saccharification and fermentation of cellulose to ethanol**. *Appl Environ Microbiol* 2010, **76**:1251-1260.
The recombinant strain was able to create cell-associated multifunctional minicellulosomes and produce ethanol directly from phosphoric acid-swollen cellulose.

44. Zhou SD, Ingram LO: **Simultaneous saccharification and fermentation of amorphous cellulose to ethanol by recombinant *Klebsiella oxytoca* SZ21 without supplemental cellulase**. *Biotechnol Lett* 2001, **23**:1455-1462.

45. Morais S, Barak Y, Caspi J, Hadar Y, Lamed R, Shoham Y, Wilson DB, Bayer EA: **Cellulase-xylanase synergy in designer cellulosomes for enhanced degradation of a complex cellulosic substrate**. *mBio* 2010, **1**: e00285-00210.

46. •• Wyman CE, Dale BE, Elander RT, Holtzapple M, Ladisch MR, Lee YY, Mitchinson C, Saddler JN: **Comparative sugar recovery and fermentation data following pretreatment of poplar wood by leading technologies**. *Biotechnol Prog* 2009, **25**:333-339.
The authors obtained high ethanol yields (>80%) with all pretreatment options tested.

47. Kristensen JB, Felby C, Jorgensen H: **Yield-determining factors in high-solids enzymatic hydrolysis of lignocellulose**. *Biotechnol Biofuels* 2009, **2**:11.

48. Pereira LT, Sposina Sobral Teixeira R, Pinto da Silva Bon E, Pereira Freitas S: **Sugarcane bagasse enzymatic hydrolysis: rheological data as criteria for impeller selection**. *J Ind Microbiol Biotechnol* 2010:1-7.

49. Geddes CC, Peterson JJ, Mullinnix MT, Svoronos SA, Shanmugam KT, Ingram LO: **Optimizing cellulase usage for improved mixing and rheological properties of acid-pretreated sugarcane bagasse**. *Bioresour Technol* 2010, **101**:9128-9136.

50. Jin M, Lau MW, Balan V, Dale BE: **Two-step SSCF to convert AFEX-treated switchgrass to ethanol using commercial enzymes and *Saccharomyces cerevisiae* 424A(LNH-ST)**. *Bioresour Technol* 2010, **101**:8171-8178.

51. •• Lau MW, Dale BE: **Cellulosic ethanol production from AFEX-treated corn stover using *Saccharomyces cerevisiae* 424A(LNH-ST)**. *Proc Natl Acad Sci U S A* 2009, **106**:1368-1373.
This article describes a SHF process in which there is no need to add nutrients or detoxify the hydrolysate. AFEX treated corn stover was used to produce 0.19 g ethanol/g dry corn stover (40 g/L ethanol) using *S. cerevisiae* 424-A (LNH-ST).

52. Li BZ, Balan V, Yuan YJ, Dale BE: **Process optimization to convert forage and sweet sorghum bagasse to ethanol based on ammonia fiber expansion (AFEX) pretreatment**. *Bioresour Technol* 2010, **101**:1285-1292.

53. Shao Q, Chundawat SP, Krishnan C, Bals B, Sousa Lda C, Thelen KD, Dale BE, Balan V: **Enzymatic digestibility and ethanol fermentability of AFEX-treated starch-rich lignocellulosics such as corn silage and whole corn plant**. *Biotechnol Biofuels* 2010, **3**:12.

54. Zhong C, Lau MW, Balan V, Dale BE, Yuan YJ: **Optimization of enzymatic hydrolysis and ethanol fermentation from AFEX-treated rice straw**. *Appl Microbiol Biotechnol* 2009, **84**:667-676.

55. Zhang J, Lynd LR: **Ethanol production from paper sludge by simultaneous saccharification and co-fermentation using recombinant xylose-fermenting microorganisms**. *Biotechnol Bioeng* 2010, **107**:235-244.
The authors report the production of over 40 g/L ethanol using waste paper sludge as feedstock. Two recombinant strains, *Z. mobilis* 8b and *S. cerevisiae* RWB222, were compared.

56. Zhu JY, Zhu W, Obryan P, Dien BS, Tian S, Gleisner R, Pan XJ: **Ethanol production from SPORL-pretreated lodgepole pine: preliminary evaluation of mass balance and process energy efficiency**. *Appl Microbiol Biotechnol* 2010, **86**:1355-1365.

57. Silva CF, Arcuri SL, Campos CR, Vilela DM, Alves JG, Schwan RF: **Using the residue of spirit production and bio-ethanol for protein production by yeasts**. *Waste Manage* 2011, **31**:108-114.

58. Inyang M, Gao B, Pullammanappallil P, Ding W, Zimmerman AR: **Biochar from anaerobically digested sugarcane bagasse**. *Bioresour Technol* 2010, **101**:8868-8872.

59. Kim YS, Kadla JF: **Preparation of a thermoresponsive lignin-based biomaterial through atom transfer radical polymerization**. *Biomacromolecules* 2010, **11**:981-988.

60. Abedinifar S, Karimi K, Khanahmadi M, Taherzadeh MJ: **Ethanol production by *Mucor indicus* and *Rhizopus oryzae* from rice straw by separate hydrolysis and fermentation**. *Biomass Bioenergy* 2009, **33**:828-833.

61. Bertilsson M, Olofsson K, Liden G: **Prefermentation improves xylose utilization in simultaneous saccharification and co-fermentation of pretreated spruce**. *Biotechnol Biofuels* 2009, **2**:8.

62. •• Dien BS, Sarath G, Pedersen JF, Sattler SE, Chen H, Funnell-Harris DL, Nichols NN, Cotta MA: **Improved sugar conversion and ethanol yield for forage sorghum (*Sorghum bicolor* L. Moench) lines with reduced lignin contents**. *Bioenergy Res* 2009, **2**:153-164.
Forage sorghum mutants containing reduced lignin contents were dilute acid pretreated and digested with cellulases. Glucose yields increased by over 30% compared to wild-type. The authors obtained 0.13–0.16 g ethanol/g of dry sorghum in an SSF process using *S. cerevisiae* and pretreated mutant sorghum.

63. Gupta R, Lee YY: **Pretreatment of hybrid poplar by aqueous ammonia**. *Biotechnol Prog* 2009, **25**:357-364.

64. Ko JK, Bak JS, Jung MW, Lee HJ, Choi IG, Kim TH, Kim KH: **Ethanol production from rice straw using optimized aqueous-ammonia soaking pretreatment and simultaneous saccharification and fermentation processes**. *Bioresour Technol* 2009, **100**:4374-4380.

65. •• Tomas-Pejo E, Oliva JM, Gonzalez A, Ballesteros I, Ballesteros M: **Bioethanol production from wheat straw by the thermotolerant yeast *Kluyveromyces marxianus* CECT 10875 in a simultaneous saccharification and fermentation fed-batch process**. *Fuel* 2009, **88**:2142-2147.
The authors used a fed-batch SSF strategy to obtain 20% more ethanol using steam exploded wheat straw and *K. marxianus* CECT10875. They obtained 0.18 g ethanol/g dry wheat straw from a final solids loading of 14%.

66. Faga BA, Wilkins MR, Banat IM: **Ethanol production through simultaneous saccharification and fermentation of switchgrass using *Saccharomyces cerevisiae* D(5)A and thermotolerant *Kluyveromyces marxianus* IMB strains**. *Bioresour Technol* 2010, **101**:2273-2279.

67. García-Aparicio MP, Oliva JM, Manzanares P, Ballesteros M, Ballesteros I, González A, Negro MJ: **Second-generation ethanol production from steam exploded barley straw by *Kluyveromyces marxianus* CECT 10875**. *Fuel* 2011, **90**:1624-1630.

68. •• Hoyer K, Galbe M, Zacchi G: **Effects of enzyme feeding strategy on ethanol yield in fed-batch simultaneous saccharification and fermentation of spruce at high dry matter**. *Biotechnol Biofuels* 2010, **3**:14.
The authors compared a batch and fed-batch SSF process as well as enzyme feeding strategy using sulfite pretreated spruce and *S. cerevisiae*. They found no definite relationship between ethanol production and enzyme feeding strategy but found that the fed-batch mode provided the best mixing.

69. Kim Y, Hendrickson R, Mosier NS, Ladisch MR, Bals B, Balan V, Dale BE, Dien BS, Cotta MA: **Effect of compositional variability of distillers' grains on cellulosic ethanol production**. *Bioresour Technol* 2010, **101**:5385-5393.

70. Lau MW, Gunawan C, Balan V, Dale BE: **Comparing the fermentation performance of *Escherichia coli* KO11, *Saccharomyces cerevisiae* 424A(LNH-ST) and *Zymomonas mobilis* AX101 for cellulosic ethanol production**. *Biotechnol Biofuels* 2010, **3**:11.

71. Oberoi HS, Vadlani PV, Brijwani K, Bhargav VK, Patil RT: **Enhanced ethanol production via fermentation of rice straw with hydrolysate-adapted *Candida tropicalis* ATCC 13803**. *Process Biochem* 2010, **45**:1299-1306.

72. Jin MJ, Balan V, Gunawan C, Dale BE: **Consolidated Bioprocessing (CBP) Performance of *Clostridium phytofermentans* on AFEX-Treated Corn Stover for Ethanol Production**. *Biotechnol Bioeng* 2011, 108:1290-1297.