Available online at www.sciencedirect.com

SciVerse ScienceDirect

Current Opinion in Biotechnology

# Recent progress in consolidated bioprocessing

Daniel G Olson[1,4], John E McBride[2], A Joe Shaw[2] and Lee R Lynd[1,2,3,4]

Consolidated bioprocessing, or CBP, the conversion of lignocellulose into desired products in one step without added enzymes, has been a subject of increased research effort in recent years. In this review, the economic motivation for CBP is addressed, advances and remaining obstacles for CBP organism development are reviewed, and we comment briefly on fundamental aspects. For CBP organism development beginning with microbes that have native ability to utilize insoluble components of cellulosic biomass, key recent advances include the development of genetic systems for several cellulolytic bacteria, engineering a thermophilic bacterium to produce ethanol at commercially attractive yields and titers, and engineering a cellulolytic microbe to produce butanol. For CBP organism development, beginning with microbes that do not have this ability and thus requiring heterologous expression of a saccharolytic enzyme system, high-yield conversion of model cellulosic substrates and heterologous expression of CBH1 and CBH2 in yeast at levels believed to be sufficient for an industrial process have recently been demonstrated. For both strategies, increased emphasis on realizing high performance under industrial conditions is needed. Continued exploration of the underlying fundamentals of microbial cellulose utilization is likely to be useful in order to guide the choice and development of CBP systems.

**Addresses**
[1] Thayer School of Engineering at Dartmouth College, Hanover, NH 03755, United States
[2] Mascoma Corporation, Lebanon, NH 03766, United States
[3] Department of Biology, Dartmouth College, Hanover, NH 03755, United States
[4] BioEnergy Science Center, Oak Ridge, TN 37830, United States

Corresponding author: Lynd, Lee R (Lee.R.Lynd@Dartmouth.EDU)

**Current Opinion in Biotechnology** 2011, **23**:1–10

This review comes from a themed issue on
Energy biotechnology
Edited by Jim Liao and Joachim Messing

0958-1669/$ – see front matter
Published by Elsevier Ltd.

**DOI** 10.1016/j.copbio.2011.11.026

## Introduction

Cellulosic plant biomass has many desirable features as an energy source [1,2], but is difficult to efficiently convert into liquid fuels. Biological processing is a promising technology option for achieving this conversion, but still poses great challenges. These challenges arise primarily because plants have evolved to be recalcitrant to attack by the elements, and in particular by microbes and their enzymes.

The recalcitrance barrier can in principle be overcome by thermochemical technologies involving reactive intermediates other than sugars (e.g. synthesis gas, pyrolysis oil), fermentative processes (e.g. acid hydrolysis, phosphoric acid swelling, ionic liquid pretreatment) that overcome recalcitrance primarily via innovations in the realm of process engineering, and fermentative processes that overcome recalcitrance primarily via innovations in the realm of biotechnology. In the latter category, processes in which cellulosic biomass is fermented to desired products in one step without adding externally produced enzymes are of obvious appeal. Indeed, such 'consolidated bioprocessing' (CBP) is widely recognized as the ultimate configuration for low cost hydrolysis and fermentation of cellulosic biomass [3••].

A CBP-enabling microbe must be able to both solubilize a practical biomass substrate and produce desired products at high yield and titer under industrial conditions. Since microbes with these properties have not been found in nature, genetic engineering is required via one of the two strategies. The native strategy: beginning with microbes that have native ability to utilize insoluble components of cellulosic biomass, and the recombinant strategy: beginning with microbes that do not have this ability and thus require heterologous expression of a saccharolytic enzyme system. The CBP strategy is in principle applicable to production of a broad range of products from plant biomass, but has received the most attention with respect to ethanol production and is being implemented commercially for this product first.

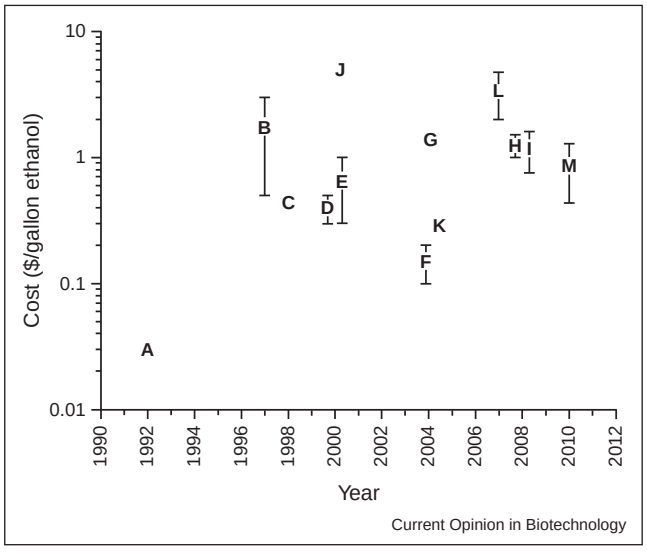
We endeavor here to provide an overview of technological and scientific advances relevant to CBP. Since our last such review [3••], the wholesale price of gasoline has more than doubled underscoring that both the need and opportunity for low cost biomass processing have grown considerably.

## Economic motivation

CBP has potential to lower the cost of biomass processing compared to process configurations featuring a dedicated step for cellulase production due to elimination of operating and capital costs associated with dedicated enzyme production and/or more effective biomass solubilization.

Estimates of the cost of added enzymes for lignocellulose conversion vary widely and do not show a decreasing

**Figure 1**




Historical estimates of the contribution of cellulase enzyme cost to final ethanol cost. Letters refer to individual references. Error bars represent the extent of the high and low estimates when given. A [52], B [53], C [54], D [55], E [56], F [57], G [58], H [59], I [60], J [61], K [61], L [62], M [62].

trend over time (Figure 1). Any cost savings estimate for CBP depends, however, on the choice of both the advanced technology configuration assumed as well as the base-case to which it is compared. If the base case has high loading of added enzymes, projected savings from eliminated cellulase production by CBP are potentially large. If a base case has low loading of added enzyme, projected savings from a CBP process achieving high rates and yields are primarily in the form of decreased capital and operating costs related to fermentation and feedstock. This dynamic partially explains why enzyme cost estimates differ so widely. Another implication is that the cost savings of CBP can be much larger than the cost of added enzyme if the basis of comparison is a process designed to minimize enzyme addition. In any case, all indications are that the cost of added enzyme continues to be a major constraint to cost-effective processing of cellulosic biomass (Figure 1). At the low end of recent estimates, 50 cents per gallon ethanol, the cost of cellulase is comparable to the purchase cost of feedstock. One is hard pressed to come up with an example of a commodity process where the catalyst cost is comparable to that of the raw material.

More effective biomass solubilization by CBP may arise because cellulase loadings are higher in CBP than is practical in other configurations, use of complexed cellulase systems, high temperature, enzyme-microbe synergy [4], or a combination of these. Economic benefits of more effective cellulose solubilization may be realized as a result of higher rate, higher yield, less expensive pretreatment, greater savings due to eliminated enzyme production, or a combination of these — again depending on the base case to which CBP is compared.

## The native strategy

The main challenges for the native strategy include the availability of tools to do genetic modification, and the application of these tools so that a desired fuel is produced with high yield, titer and robustness under industrial conditions. Candidates for the native strategy can be organized into three groups: fungi, free-enzyme bacteria and cellulosome-forming bacteria.

Progress in the development of genetic tools for fungal systems has recently been reviewed and will not be discussed in detail [5]. Although to date, most engineering efforts have focused on increasing cellulase production, there is also interest in engineering biofuel production in fungal systems such as *Fusarium oxysporum* [6] and *Trichoderma reesei* [7•].

There has also been substantial progress in the development of genetic tools for free-enzyme bacterial systems, including *Clostridium phytofermentans* [8], *Clostridium japonicus* [9], and *Thermoanaerobacter* and *Thermanaerobacterium* sp. [10–12]. *Thermoanaerobacterium saccharolyticum*, a thermophilic anaerobe that utilizes a broad range of substrates including xylan and all naturally occurring sugars present in biomass (although not crystalline cellulose), provides a prominent example of engineering an organism with recently developed genetic tools to produce a biofuel at high yield. Shaw *et al.* [13•] eliminated acetic and lactic acid production resulting in a strain that produces ethanol at a yield of 0.46 g ethanol/g xylose or other sugars. Such yield was observed under a variety of conditions (e.g. different substrate concentrations, batch and continuous culture), although it was not demonstrated in industrial growth media. In another notable genetic engineering feat reported by Cripps *et al.* [14•], *Geobacillus thermoglucosidasius*, a thermophile capable of oligosaccharide fermentation, was engineered by deletion of *ldh* and *pfl* and upregulation of *pdh* to produce industrially relevant yields of 0.42–0.47 g ethanol/g hexose sugar, although the reported yield was slightly less for pure pentose sugars.

Study of *Caldicellulosiruptor* sp. as potential CBP organisms has recently been initiated, motivated in part by their having the highest temperature optima among described cellulolytic microbes, and the finding that cultures are able to achieve substantial solubilization of lignocellulose without pretreatment [15•]. The architecture of the cellulase system of members of this genus features multiple catalytic enzyme modules in single enzymes [16], and appears to be different from both the noncomplexed paradigm exemplified by the cellulases of *T. reesei* and other aerobic fungi, and cellulosome



Please cite this article in press as: Olson DG, et al. Recent progress in consolidated bioprocessing, Curr Opin Biotechnol (2011), doi:10.1016/j.copbio.2011.11.026

**Table 1**

**Recent results from native cellulose-fermenting CBP organisms**

| Organism | Relevant genotype | Substrate | (g/L) | Conversion (%)[a] | Product | Titer (g/L) | Metabolic yield, (%)[b] | Rate (g $L^{-1}$ $h^{-1}$) | Reference |
|---|---|---|---|---|---|---|---|---|---|
| *C. thermocellum* M1570 and *T. saccharolyticum* ALK2 co-culture | M1570 *Δldh Δpta* ALK2 *Δldh Δpta Δack* | Avicel | 92.2 | 90 | Ethanol | 38.1 | 82 | 0.26 | Agyros *et al.*, *AEM*, in press |
| *C. cellulolyticum* H10 | Wildtype | Cellulose MN301 | 50 | 26 | Ethanol | 0.51 | 7 | 1.1E−03 | Guedon *et al.* [73] |
| *C. phytofermentans* ATCC 700394 | Wildtype | AFEX-Corn Stover | 8* | 81 | Ethanol | 2.8 | 85 | 1.2E−02 | Jin *et al.* [74] |
| *C. thermocellum* DSM 1313 | Wildtype | Avicel | 19.5 | 90 | Ethanol | 1.3 | 15 | 1.8E−02 | Agyros *et al.*, *AEM*, in press |
| *C. cellulolyticum* CC-pMG8 | *pdc adh* | Cellulose MN301 | 50 | 64 | Ethanol | 0.83 | 5 | 1.8E−03 | Guedon *et al.* [73] |
| *Geobacillus sp. R7* | Wildtype | Prairie cord grass | 10.8* | ND | Ethanol | 0.035 | ND | 2.1E−04 | Zambare *et al.* [75] |
| *T. hirsuta* | Wildtype | Rice straw | 10.3* | ND | Ethanol | 3 | ND | 3.1E−02 | Okamoto *et al.* [76] |
| *C. japonicus* Ueda107 | Wildtype | Avicel | 10 | ND | Ethanol | 0.0002 | ND | 4.2E−06 | Gardner and Keating [9] |
| *C. phytofermentans* ATCC 700394 | Wildtype | Filter paper | 10 | 63 | Ethanol | 2.9 | 81 | 4.3E−03 | Tolonen *et al.* [77] |
| *C. cellulolyticum* pWH320 | *kivd yqhD alsS ilvCD* | Sigma 50 | 10 | ND | Isobutanol | 0.66 | ND | 4.6E−03 | Higashide *et al.* [19] |
| *C. japonicus* MSB280 | *pdc adhB* | Avicel | 10 | ND | Ethanol | 0.0035 | ND | 7.3E−05 | Gardner and Keating [9] |
| *C. thermocellum* M1570 | *Δldh Δpta* | Avicel | 19.5 | 94 | Ethanol | 5.6 | 60 | 7.8E−02 | Agyros *et al.*, *AEM*, in press |
| *C. acetobutylicum 7* | Wildtype | Grass | 30 | ND | 1-Butanol | 0.6 | ND | ND | Berezina *et al.* [81] |

ND: Not determined, insufficient data.

[a] (starting substrate − ending substrate)/starting substrate × 100%.

[b] Product titer/(consumed substrate × theoretical yield).

* Concentration of total carbohydrates.

paradigm exemplified by *C. thermocellum* and other anaerobes. Active investigation is underway to develop genetic tools for manipulation of this organism with promising results presented at scientific meetings and submitted.

Among cellulosome-forming bacteria, *C. thermocellum* and *C. cellulolyticum* are the most well-developed as potential CBP hosts. There has been substantial recent progress with respect to development of tools for genetic manipulation [17] and subsequent metabolic engineering [18••,19], including production of butanol in the case of *C. cellulolyticum* [19]. Reported values of key performance parameters are shown in Tables 1 and 2, which includes both native cellulose-utilizing microbes and native hemicellulose-utilizing microbes. An early comprehensive analysis of the economic benefits of CBP described an advanced scenario with a very low projected cellulosic ethanol selling price based on a titer of 50 g/L, 92% hydrolysis yield after pretreatment, 90% metabolic yield after fermentation, and a rate of 1.39 g/L/h [20]. The Argyros *et al*. result with a co-culture of *C. thermocellum* and *T. saccharolyticum* [18••] is remarkably close to meeting these milestones, although it has yet to be shown with a real-world substrate under industrial conditions.

The concept of a 'titer gap' — a difference between the maximum concentration of a compound that is tolerated when it is *added* to a culture and the maximum concentration of that compound that is *produced* — is relevant when considering organism development for CBP via the native strategy. The titer gap may be defined with respect to either growth or metabolism, and is often not the same for these two points of reference. A substantial titer gap is a salient feature of most microbes of interest for the native strategy In the case of *C. thermocellum*, for example, several studies have established that strains able to grow in the presence of added ethanol at concentrations exceeding 50 g/L can readily be obtained by serial transfer [21,22], and uncoupled metabolism at yet higher concentrations seems reasonable to expect. However, the maximum concentration of ethanol produced by this organism in pure culture remains at about 25 g/L [21,23]. Production of ethanol at concentrations at or exceeding the tolerance exhibited in exogenous addition experiments has been observed in the case of engineered strains of *T. saccharolyticum*, for which titers ≥65 g/L have been obtained (Hogsett, DA, abstract 2.3.2.3, Biochemical Conversion Platform Peer Review, Denver, CO, February 2011).

Experience with industrial microorganism development provides increasing support for the proposition that with sufficient effort, stoichiometric yields of engineered products can be achieved, and the titer gap closed.



Please cite this article in press as: Olson DG, et al. Recent progress in consolidated bioprocessing, Curr Opin Biotechnol (2011), doi:10.1016/j.copbio.2011.11.026

**Table 2**

**Recent results from native hemicellulose-fermenting CBP organisms**

| Organism | Relevant genotype | Substrate | (g/L) | Conversion (%)[a] | Product | Titer (g/L) | Metabolic yield (%)[b] | Rate, (g $L^{-1}$ $h^{-1}$) | Reference |
|---|---|---|---|---|---|---|---|---|---|
| *T. saccharolyticum* M1442 | *Δldh Δpta Δack* | Hardwood hydrolysate | 13.8 | 93 | Ethanol | 7.4 | 113 | 0.11 | Lee *et al.* [80] |
| *T. aotearoense Δldh* mutant | *Δldh* | Xylose | 9.7 | >99 | Ethanol | 3.5 | 71 | 0.15 | Cai *et al.* [78] |
| *T. saccharolyticum* | Wildtype | Xylose | 11.1 | >99 | Ethanol | 3.4 | 60 | 0.31 | Shaw *et al.* [13•] |
| *T. saccharolyticum* ALK2 | *Δldh Δpta Δack* | Xylose | 12 | >99 | Ethanol | 5.5 | 90 | 0.55 | Shaw *et al.* [13•] |
| *G. thermoglucosidasius* TM242 | *Δldh Δpfl pdh-upregulated* | Xylose | 29 | 95 | Ethanol | 9.6 | 69 | 0.76 | Cripps *et al.* [14•] |
| *C. phytofermentans* ATCC 700394 | Wildtype | Birch Wood Xylan | 3 | ND | Ethanol | 0.46 | 27 | 1.9E−02 | Tolonen *et al.* [77] |
| *T. mathranii* BG1L1 | *Δldh* | Xylose | 5.2 | >99 | Ethanol | 2.2 | 84 | 4.6E−02 | Yao and Mikkelsen [79] |
| *T. mathranii* BG1 | Wildtype | Xylose | 5.5 | >99 | Ethanol | 2.3 | 83 | 4.8E−02 | Yao and Mikkelsen [79] |
| *T. aotearoense* SCUT27 | Wildtype | Xylose | 8.6 | 88 | Ethanol | 1.5 | 39 | 6.3E−02 | Cai *et al.* [78] |

ND: Not determined, insufficient data.

[a] (starting substrate − ending substrate)/starting substrate × 100%.

[b] Product titer/(consumed substrate × theoretical yield).

* Concentration of total carbohydrates.

Prominent examples include ethanol production in yeast and *E. coli*, and, more recently, engineering of *E. coli* to produce propane diol at 81% of theoretical yield and a titer of 135 g/L [24]. It can be expected that this is also true for less well-established organisms of interest for the native CBP development strategy, with *T. saccharolyticum* providing the most fully developed example to date (Hogsett, DA, abstract 2.3.2.3, Biochemical Conversion Platform Peer Review, Denver, CO, February 2011). Progress with hosts for the native CBP strategy will be slower because tools are less developed, although this will probably become less true over time. The case for eventual success via the native strategy is somewhat less clear with respect to industrial robustness, including compatibility with practical pretreatments, fermentation at high substrate (and hence solids) concentration in industrial growth media, and strain management and stability. We suggest that these and other dimensions of industrial robustness are a key area for investigation aimed at advancing the native strategy.

## The recombinant strategy

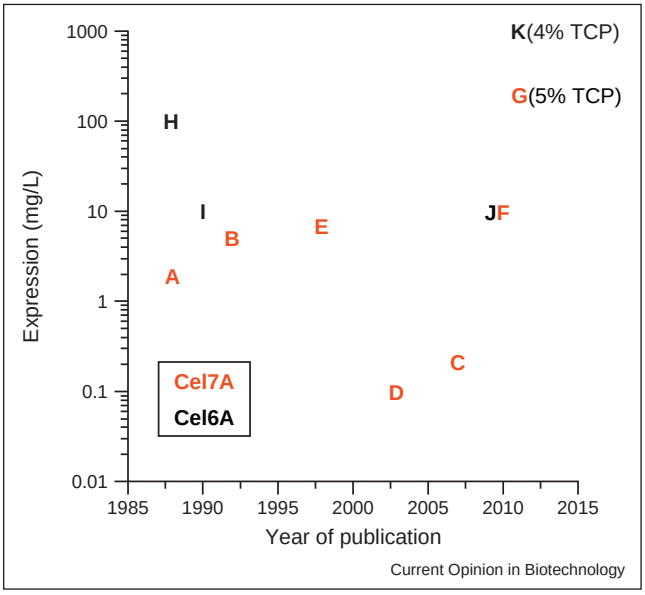
The primary challenge for the 'recombinant strategy' is heterologous expression of sufficient quantities of several types of cellulase and/or hemicellulase enzymes to permit rapid growth and conversion of pretreated lignocelluloses. Total enzyme activity produced by the host can be increased by improving both total expression and specific activity of the enzyme system. Moreover, specific activity of the system is a function of both the composition of the system and the specific activity of the components. Given the expense of aerating large culture volumes, as well as loss of product yield and feedstock energy as a result of aerobic respiration, non-oxidative metabolism is highly desirable and is likely required for many applications. The recombinant strategy has been pursued in a number of host organisms including *S. cerevisiae*, *E. coli*, and *Bacillus subtilis*, with work in *S. cerevisiae* the most advanced to date.

Significant advances have recently been made with regard to expression level of cellulases in *S. cerevisiae*. As shown in Figure 2, the report of Ilmen *et al.* represents a large increase in the maximum titer achieved for two critical cellulases Cel6A (CBH1) and Cel7a (CBH2). The cellulase expression levels achieved in this study correspond to 4–5% of total cell protein, which meets the calculated levels for growth on cellulose at rates required for an industrial process [3••]. However, data for heterologous cellulase production in yeasts in Figure 2 and in the literature in general have been reported for aerobic cultures with cell densities in the range of approximately 5 g/L (shake flask) to 50 g/L (fed batch, bioreactor), whereas CBP will involve anaerobic cultures with cell densities at the low end of this range. Assessing the impact of the advances reported by Ilmen *et al.*, and future advances, will require data obtained under anaerobic conditions



Please cite this article in press as: Olson DG, et al. Recent progress in consolidated bioprocessing, Curr Opin Biotechnol (2011), doi:10.1016/j.copbio.2011.11.026

**Figure 2**




Comparison of expression levels for Cel7A and Cel6A in *S. cerevisiae* in terms of both the titer achieved and the percentage of total cell protein (TCP). A [63], B [64], C [65], D [66], E [67], F [68], G [69], H [63], I [70], J [71], K [69]. Note: percentage of TCP for Cel7A in Ilmen *et al*. was calculated by assuming a cell yield of 0.45 g cells/g glucose, and a protein content of dry cell weight of 42% [72].

under industrial conditions. While such data have not been reported to date in the literature, the authors of this study have observed anaerobic production of CBHs at roughly equivalent levels on a percentage to total cell protein (TCP) basis to the maximum shown in Figure 2 (unpublished data).

One approach to increasing the specific activity of recombinantly expressed enzyme systems is to mimic systems already available in nature, and in some cases already industrially applied. Table 3 depicts the ongoing work to create a complete enzymatic system for *S. cerevisiae*, relying on data generated by studies that have examined enzymatic systems for the breakdown of lignocellulose via proteomic analysis. Enzyme diversity from *Trichoderma reesei*, an organism whose enzymes will be used to hydrolyze lignocellulose at an industrial scale, and from the rapid cellulose utilizer *C. thermocellum* was compared to the enzyme diversity functionally expressed in *S. cereivisae*. Compared to the Herpoel-Gimbert study [25] 100% of the functional enzyme classes identified in *T. reesei* have been expressed in *S. cerevisiae*, whereas 80% of those identified in Nagendran *et al*. have been expressed in *S. cerevisiae* [26]. Of particular note is recent work that has shown expression of many enzyme categories in *S. cerevisiae*, utilizing >300 genes in the process (Brevnova *et al*. patent applied for). In contrast, but not surprisingly, expression of complexed enzyme system components is not as advanced as for non-complexed components. Using the enzymatic components of the *C. thermocellum* system as an example, only about 20% of the diversity has been functionally expressed.

Another approach to increasing specific activity is to mimic the cellulosome system of complexed enzymes bound to the cell surface. This approach has shown promising results with activity enhancement of 4.5-fold for complexed systems compared with the same enzymes in a non-complexed system [27]. Surface display and endoglucanase activity have been demonstrated in yeast in several instances [28–30]. Recombinant cellulosomes capable of solubilizing 30% of 10 g/L PASC in 73 hours were developed by expressing a scaffoldin and 3 enzymatic subunits in a single yeast strain [31]. By expressing cellulosome components separately, a different group was able to achieve 60% solubilization of 10 g/L PASC in 50 hours [32]. However in both of these cases, the cellulosome-displaying yeast were unable to grow on PASC, and hydrolysis required a high cell density ($OD_{600} \sim 50$) using cells pre-cultured in rich media.

Once an enzyme system is created, the components of that system must be combined in an organism to allow cellulose and/or hemicellulose hydrolysis. The first report of anaerobic conversion of phosphoric acid swollen cellulose (PASC) into ethanol using *S. cerevisiae* at low cell densities, to our knowledge, was published in 2007, where the authors demonstrated ~27% conversion of PASC in 192 hours [33]. As per recent reports, more rapid conversion of PASC into ethanol was achieved by optimizing the level and ratios of cellulase enzymes in *S. cerevisiae* using delta-integration of cellulase genes followed by screening for PASC solubilization [34]. The yield of 75% of theoretical ethanol suggests that further increases in enzyme production are necessary and/or additional enzymes to achieve complete conversion. Expression of a single engineered endoglucanase, Bscel5 at very high levels (~5.9% of total cell protein) in *Bacillus subtilis* has been shown to allow conversion of 7 g/L reactive amorphous cellulose (RAC) into 4 g/L lactate in 144 hours [35]. A recent patent application describes the construction of *S. cerevisiae* strains expressing cellulases that can produce small amounts of ethanol directly from Avicel (a crystalline cellulose) by virtue of these expressed enzymes, which to our knowledge is the first report of its kind [36]. Using strains described in this patent application the authors have observed 80% conversion of PASC into ethanol by recombinant *S. cerevisiae* in 96 hours, and 90% conversion of bacterial microcrystalline cellulose into ethanol in 144 hours, using low inoculums (5% v/v) (Unpublished results). While these studies demonstrate the principle that cellulose chains can be hydrolyzed by recombinant enzyme systems, they have all been carried out on extremely reactive substrates, and all but one on amorphous cellulose. Substrates available in



Please cite this article in press as: Olson DG, et al. Recent progress in consolidated bioprocessing, Curr Opin Biotechnol (2011), doi:10.1016/j.copbio.2011.11.026

**Table 3**

**Expression of cellulase components in *S. cerevisiae***

| Type of Activity | Cazy family/ Gene Name* | Actively Expressed in *S. cerevisiae*** | *T. reesei* Herpoel-Gimbert (2008) | *T. reesei* Nagendran 2009 | *C. thermocellum* Raman et al. 2009 (fig 5&6)*** |
|---|---|---|---|---|---|
| Cellobiohydrolase/ Exoglucanase | GH7 | 1 | | | |
| | GH6 | 1 | | | |
| | GH5 | | | | |
| | GH9 | | | | |
| | GH48 | | | | |
| Endoglucanase | GH7 | 2 | | | |
| | GH5 | 2 | | | |
| | GH12 | 2 | | | |
| | GH61 | 2 | | | |
| | GH45 | 2 | | | |
| | GH6 | 2 | | | |
| | GH? | 2 | | | |
| | GH9 | 2 | | | |
| | GH8 | 2 | | | |
| xyloglucanase | GH74 | 2 | | | |
| β-glucosidase | GH3 | 3 | | | |
| xylanase | GH11 | 4 | | | |
| | GH10 | 2 | | | |
| β-xylosidase | GH3 | 5 | | | |
| | GH43 | 2 | | | |
| β-mannanase | GH26 | | | | |
| | GH5 | 6 | | | |
| β-mannosidase | GH2 | | | | |
| | GH26 | 2 | | | |
| acetylxylanesterase | CE5 | | | | |
| | CE1 | 2 | | | |
| arabinofuranosidase | GH54 | 2 | | | |
| | GH43 | | | | |
| ferulicacid/cinnamoyl esterase | CE1 | 2 | | | |
| Swollenin | SWO | 8 | | | |
| glucuronoyl esterase | CIP2 | 2 | | | |
| Arabinosidase | GH62 | | | | |
| Polygalacturonase | GH28 | 11 | | | |
| α-mannosidase | GH47 | ***** | | | |
| β 1,3-glucanase | GH55 | | | | |
| α-galactosidase | | 7 | | | |
| β-galactosidase | GH35 | 12 | | | |
| α-glucuronidase | GH67 | 9 | | | |
| Acetyl esterase | CE16 | | | | |
| Scaffoldin | CipA | 10**** | | | |
| Anchor protein | SdbA | | | | |
| Anchor protein | Orf2p | | | | |
| Anchor protein | OlpB | | | | |
| Lichenase | GH16 | | | | |
| Putative Esterase | CE12 | | | | |
| Putative Esterase | CE3 | | | | |
| Putative Glycosidase | GH43 | | | | |
| Putative Pectinase | PL11 | | | | |
| Putative Pectinase | PL1 | 2 | | | |
| Putative Pectinase | PL1, PL9 | | | | |
| Serpin Proteinase Inhibitor | | | | | |
| Multifunctional (Endoglucanse, ?) | GH9, GH44 | | | | |
| Multifunctional (Endoglucanse, Esterase) | GH5, CE2 | | | | |
| Multifunctional (Endoglucanse, ?) | GH5, GH26 | | | | |
| Multifunctional (Endoglucanse, arabinofuranosidase) | GH5, GH43 | | | | |
| Multifunctional (Xylanase, Esterase) | GH10, CE1 | | | | |
| Multifunctional (Xylanase, Esterase) | GH11, CE4 | | | | |

*Enzymes of unreported function are not included
**Numbers given are example references
*** C. thermocellum contains multiple versions of several
****Chimeric scafolding expressed, much smaller than
*****Native S. cerevisiae?-mannosidase is localized to ER

**Reference**


1 Ilmen et al. (2011)
2 Accessories application
3 van Rooyen R, Hahn-Hägerdal B, La Grange DC, van Zyl WH. (2005) J Biotechnol
4 La Grange DC, PretoriusI S, Van Zyl WH (1996) Appl Environ Microbiol 62:1036
5 Margolles-Clark E, Tenkanen M, Nakari-Setala T, Penttilä M (1996) Appl Environ
6 Romanos MA, Makoff AJ, Fairweather NF, Beesley KM, Slater DE, Rayment FB, Payne
7 Margolles-Clark E, Tenkanen M, Luonteri E, Penttilä M (1996) Eur J Biochem 240:104
8 Saloheimo M, Paloheimo M, Hakola S, Pere J, Swanson B, Nyyssönen E, Bhatia A, Ward
9 De Wet BJM, Van Zyl WH, Prior BA (2006) Enzyme Microb Technol 38:649
10 Lilly M, Fierobe HP, van Zyl WH, Volschenk H. (2009) FEMS Yeast Res 9:1236
11 Nagai M, Ozawa A, Katsuragi T, Kawasaki H, Sakai T. (2000) Biosci Biotechnol Biochem
12 Becerra M, Diaz Prado S, Cerdan E, Gonzalez Siso M (2001) Biotech Lett 23:33




Please cite this article in press as: Olson DG, et al. Recent progress in consolidated bioprocessing, Curr Opin Biotechnol (2011), doi:10.1016/j.copbio.2011.11.026

industrial processes are unlikely to be as amorphous in character or as reactive as these model substrates, however, and development of CBP organisms using PASC may mask the need for enzymes targeting crystalline cellulose.

In addition to the advances in cellulose utilization, there has been some work directed toward obtaining polymeric hemicellulose utilization. An obvious prerequisite to hemicellulose utilization is the ability to use the monomer sugars, including xylose, galactose, arabinose, mannose, and others resulting from hydrolysis. It is also often necessary to be able to consume these sugars in the presence of glucose, or cello-oligomers. While several candidate organisms such as *E. coli* and *B. subtilis* already possess this ability, it must be engineered into *S. cerevisiae*. Pentose fermentation by *S. cerevisiae* is relatively advanced, and has been reviewed elsewhere [37]. However, one new direction for the field is the creation of strains that co-ferment cellobiose and xylose [38]. In these strains a cellodextrin transporter is coupled with xylose fermentation capability, relieving inhibition of xylose utilization by glucose. Because cellobiose is a potent inhibitor of cellulases, industrial processes where separate hydrolysis and fermentation are used are unlikely to have high concentrations of cellobiose and xylose, but this technology may be applicable to CBP processes if cellobiose or other cello-oligomers can be created and rapidly co-fermented with xylose.

As discussed above (Table 3), many if not all of the necessary enzyme classes for hemicellulose hydrolysis have been expressed in *S. cerevisiae*. A recent publication demonstrates that a combination of xylanase, β-xylosidase, and β-glucosidase in a xylose utilizing background yielded 76% conversion of oligomers from pretreated rice straw [39], although these results were obtained using very high cell densities (100 g/L wet cell weight). In addition to this study, several xylan fermenting *E. coli* strains have been developed. A binary culture of *E. coli* strains expressing xylanase enzymes was shown to allow 63% of the birchwood xylan initially present to be converted into ethanol without the addition of exogenous enzymes [40]. Another example of hemicellulase engineering in *E. coli* demonstrated the production of fatty acid ethyl esters from birchwood xylan by expressing the endoxylanase Xyn10B and the xylanases Xsa. In this case, the expression of just two enzymes was sufficient to support growth on xylan [41•].

## Microbial cellulose utilization fundamentals

There are substantial and perhaps underestimated additional phenomena that arise when considering solubilization of cellulose and other insoluble biomass components by cultures of saccharolytic microbes as compared to solubilization by cell-free enzymatic systems. Examples of these phenomena include bioenergetics [42], implications of attachment of cells to the surface of insoluble substrates and expression of saccharolytic enzymes on the cell surface with respect to kinetics and substrate capture [43], and metabolic control related to the choice between investing cellular resources in cellulase or growth. There are also some foundational questions of great relevance to CBP for which there are not yet definitive answers, notably including:

- Under what conditions (e.g. temperature, pH, substrate particle size, presence of multiple microorganisms with complementary capabilities) does microbial cellulose utilization proceed fastest?
- How do saccharolytic microbes compare to cell-free cellulase systems as agents of biomass solubilization with respect to key variables such as rate, yield, and the extent of pretreatment required?

While outstanding issues remain, understanding microbial cellulose utilization has received an increased level of effort in the last five years with many exciting findings in the publication pipeline. Highlights of published work during this period include systematic study of the implications of enzymes displayed on the cell surface [31,32,43], indications of cellulose binding-triggered gene expression [44,45,46,47•,48,49,50], quantitative demonstration of enzyme-microbe synergy [4], the first studies involving targeted knockout of cellulase components in an obligate anaerobe [8,51•], and demonstration of high conversion of unpretreated lignocellulose by microbial cultures [15•]. A particularly important focus research during the coming years will be to extend fundamentals-inclusive studies to include lignocellulosic substrates and operation under conditions approaching those that would occur in an industrial process.

## Conclusions

During the last five years, key advances have been made in organism development for CBP, while at the same time remaining barriers have been brought into focus. For the native strategy, key advances include development of genetic systems for several cellulolytic anaerobic bacteria, engineering a cellulolytic host to produce butanol, and engineering of a thermophilic bacterium to produce ethanol at commercially attractive yields and titers. For the recombinant strategy, key advances include high-yield conversion of model cellulosic substrates and heterologous expression of CBH1 and CBH2 in yeast at levels believed to be sufficient for an industrial process. For both strategies, increased emphasis on realizing high performance under industrial conditions is needed. Demonstrating high fermentation yields and titer from practical, pretreated lignocellulosic feedstocks is a particular priority for the native strategy. Co-expression of multiple proteins allowing utilization of such pretreated feedstocks with high hydrolysis yields and reasonable rates is a particular priority for the recombinant



Please cite this article in press as: Olson DG, et al. Recent progress in consolidated bioprocessing, Curr Opin Biotechnol (2011), doi:10.1016/j.copbio.2011.11.026

strategy. Continued exploration of the underlying fundamentals of microbial cellulose utilization is likely to be useful in order to guide the choice and development of CBP systems.

## Acknowledgements

This work was supported by the United States Department of Energy BioEnergy Science Center and the Mascoma Corporation. The BioEnergy Science Center is a U.S. Department of Energy Bioenergy Research Center supported by the Office of Biological and Environmental Research in the DOE Office of Science.

## 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. Lynd LR, Weimer PJ, van Zyl WH, Pretorius IS: **Microbial cellulose utilization: fundamentals and biotechnology**. *Microbiol Mol Biol Rev* 2002, **66**:506.

2. Demain AL: **Biosolutions to the energy problem**. *J Ind Microbiol Biotechnol* 2009, **36**:319-332.

3. •• Lynd LR, van Zyl WH, McBride JE, Laser M: **Consolidated bioprocessing of cellulosic biomass: an update**. *Curr Opin Biotechnol* 2005, **16**:577-583.
This review continues to be an authoritative reference for fundamentals of microbial cellulose degradation and its implications for biotechnological applications.

4. Lu YP, Zhang YHP, Lynd LR: **Enzyme-microbe synergy during cellulose hydrolysis by *Clostridium thermocellum***. *Proc Natl Acad Sci USA* 2006, **103**:19605.

5. Kuck U, Hoff B: **New tools for the genetic manipulation of filamentous fungi**. *Appl Microbiol Biotechnol* 2010, **86**:51-62.

6. Xiros C, Christakopoulos P: **Enhanced ethanol production from brewer's spent grain by a *Fusarium oxysporum* consolidated system**. *Biotechnol Biofuels* 2009, **2**:4.

7. • Xu Q, Singh A, Himmel ME: **Perspectives and new directions for the production of bioethanol using consolidated bioprocessing of lignocellulose**. *Curr Opin Biotechnol* 2009, **20**:364-371.
An in-depth review of the state of the art in fungal CBP progress and aspirations.

8. Tolonen AC, Chilaka AC, Church GM: **Targeted gene inactivation in *Clostridium phytofermentans* shows that cellulose degradation requires the family 9 hydrolase Cphy3367**. *Mol Microbiol* 2009, **74**:1300-1313.

9. Gardner JG, Keating DH: **Requirement of the Type II secretion system for utilization of cellulosic substrates by *Cellvibrio japonicus***. *Appl Environ Microbiol* 2010, **76**:5079-5087.

10. Shaw AJ, Covalla SF, Hogsett DA, Herring CD: **Marker removal system for *Thermoanaerobacterium saccharolyticum* and development of a markerless ethanologen**. *Appl Environ Microbiol* 2011, **77**:2534-2536.

11. Shaw AJ, Hogsett DA, Lynd LR: **Natural competence in *Thermoanaerobacter* and *Thermoanaerobacterium* species**. *Appl Environ Microbiol* 2010, **76**:4713-4719.

12. Peng H, Fu BL, Mao ZG, Shao WL: **Electrotransformation of *Thermoanaerobacter ethanolicus* JW200**. *Biotechnol Lett* 2006, **28**:1913-1917.

13. • Shaw AJ, Podkaminer KK, Desai SG, Bardsley JS, Rogers SR, Thorne PG, Hogsett DA, Lynd LR: **Metabolic engineering of a thermophilic bacterium to produce ethanol at high yield**. *Proc Natl Acad Sci* 2008, **105**:13769-13774.
An example of how applying genetic tools in a non-model organism can enable CBP biocatalyst creation.

14. • Cripps RE, Eley K, Leak DJ, Rudd B, Taylor M, Todd M, Boakes S, Martin S, Atkinson T: **Metabolic engineering of *Geobacillus thermoglucosidasius* for high yield ethanol production**. *Metabolic Eng* 2009, **11**:398-408.
An excellent example of engineering high product yields in a thermophilic microrganism.

15. • Yang S-J, Kataeva I, Hamilton-Brehm SD, Engle NL, Tschaplinski TJ, Doeppke C, Davis M, Westpheling J, Adams MWW: **Efficient degradation of lignocellulosic plant biomass, without pretreatment, by the thermophilic anaerobe *Anaerocellum thermophilum* DSM 6725**. *Appl Environ Microbiol* 2009, **75**:4762-4769.
Eliminating the requirement for pretreatment is a potentially disruptive advance for lignocellulosic bioprocessing, and the ulitmate embodiment of CBP.

16. Kataeva IA, Yang SJ, Dam P, Poole FL II, Yin Y, Zhou F, Chou WC, Xu Y, Goodwin L, Sims DR *et al.*: **Genome sequence of the anaerobic, thermophilic, and cellulolytic bacterium *Anaerocellum thermophilum* DSM 6725**. *J Bacteriol* 2009, **191**:3760-3761.

17. Tripathi SA, Olson DG, Argyros DA, Miller BB, Barrett TF, Murphy DM, McCool JD, Warner AK, Rajgarhia VB, Lynd LR *et al.*: **Development of pyrF-based genetic system for targeted gene deletion in *Clostridium thermocellum* and creation of a pta mutant**. *Appl Environ Microbiol* 2010, **76**:6591-6599.

18. •• Argyros DA, Tripathi SA, Barrett TF, Rogers SR, Feinberg LF, Olson DG, Foden JM, Miller BB, Lynd LR, Hogsett DA, et al.: **High ethanol titers from cellulose using metabolically engineered thermophilic, anaerobic microbes**. *Appl Environ Microbiol* 2011, http://aem.asm.org/content/early/2011/09/30/AEM.00646-11.short?rss=1, in press.
The best example of CBP to date in terms of yield, titer, and productivity from crystalline cellulose.

19. Higashide W, Li YC, Yang YF, Liao JC: **Metabolic engineering of *Clostridium cellulolyticum* for production of isobutanol from cellulose**. *Appl Environ Microbiol* 2011, **77**:2727-2733.

20. Lynd LR, Elander RT, Wyman CE: **Likely features and costs of mature biomass ethanol technology**. *Appl Biochem Biotechnol* 1996, **57**:741-761.

21. Brown SD, Guss AM, Karpinets TV, Parks JM, Smolin N, Yang SH, Land ML, Klingeman DM, Bhandiwad A, Rodriguez M *et al.*: **Mutant alcohol dehydrogenase leads to improved ethanol tolerance in *Clostridium thermocellum***. *Proc Natl Acad Sci USA* 2011, **108**:13752-13757.

22. Williams T, Combs J, Lynn B, Strobel H: **Proteomic profile changes in membranes of ethanol-tolerant *Clostridium thermocellum***. *Appl Microbiol Biotechnol* 2007, **74**:422-432.

23. Demain AL, Newcomb M, Wu JHD: **Cellulase, clostridia, and ethanol**. *Microbiol Mol Biol Rev* 2005, **69**:124-154.

24. Nakamura CE, Whited GM: **Metabolic engineering for the microbial production of 1,3-propanediol**. *Curr Opin Biotechnol* 2003, **14**:454-459.

25. Herpoel-Gimbert I, Margeot A, Dolla A, Jan G, Molle D, Lignon S, Mathis H, Sigoillot J-C, Monot F, Asther M: **Comparative secretome analyses of two *Trichoderma reesei* RUT-C30 and CL847 hypersecretory strains**. *Biotechnol Biofuels* 2008, **1**:18.

26. Nagendran S, Hallen-Adams HE, Paper JM, Aslam N, Walton JD: **Reduced genomic potential for secreted plant cell-wall-degrading enzymes in the ectomycorrhizal fungus *Amanita bisporigera*, based on the secretome of *Trichoderma reesei***. *Fungal Genet Biol* 2009, **46**:427-435.

27. Fierobe HP, Mingardon F, Mechaly A, Belaich A, Rincon MT, Pages S, Lamed R, Tardif C, Belaich JP, Bayer EA: **Action of designer cellulosomes on homogeneous versus complex substrates — controlled incorporation of three distinct enzymes into a defined trifunctional scaffoldin**. *J Biol Chem* 2005, **280**:16325-16334.

28. Hyeon JE, Jeon WJ, Whang SY, Han SO: **Production of minicellulosomes for the enhanced hydrolysis of cellulosic substrates by recombinant *Corynebacterium glutamicum***. *Enzyme Microb Technol* 2011, **48**:371-377.



Please cite this article in press as: Olson DG, et al. Recent progress in consolidated bioprocessing, Curr Opin Biotechnol (2011), doi:10.1016/j.copbio.2011.11.026

29. Lilly M, Fierobe HP, van Zyl WH, Volschenk H: **Heterologous expression of a *Clostridium minicellulosome* in *Saccharomyces cerevisiae***. *Fems Yeast Res* 2009, **9**: 1236-1249.

30. Tsai SL, Oh J, Singh S, Chen RZ, 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.

31. Wen F, Sun J, Zhao H: **Yeast surface display of trifunctional minicellulosomes for simultaneous saccharification and fermentation of cellulose to ethanol**. *Appl Environ Microbiol* 2009, **76**:1251-1260.

32. Tsai SL, Goyal G, Chen W: **Surface display of a functional minicellulosome by intracellular complementation using a synthetic yeast consortium and its application to cellulose hydrolysis and ethanol production**. *Appl Environ Microbiol* 2010, **76**:7514-7520.

33. Den Haan R, Rose SH, Lynd LR, van Zyl WH: **Hydrolysis and fermentation of amorphous cellulose by recombinant *Saccharomyces cerevisiae***. *Metab Eng* 2007, **9**:87-94.

34. Yamada R, Yamakawa S-I, Tanaka T, Ogino C, Fukuda H, Kondo A: **Direct and efficient ethanol production from high-yielding rice using a *Saccharomyces cerevisiae* strain that express amylases**. *Enzyme Microb Technol* 2011, **48**:393-396.

35. Zhang XZ, Sathitsuksanoh N, Zhu ZG, Zhang YHP: **One-step production of lactate from cellulose as the sole carbon source without any other organic nutrient by recombinant cellulolytic *Bacillus subtilis***. *Metab Eng* 2011, **13**:364-372.

36. McBride JE, Brevnova E, Ghandi C, Mellon M, Froehlich A, Deleault K, Rajgharia V, Flatt J, Van Zyl E, Den Haan R, *et al.*: **Yeast expressing cellulases for simultaneous saccharification and fermentation using cellulose**. *US Patent* 2010.

37. Matsushika A, Inoue H, Kodaki T, Sawayama S: **Ethanol production from xylose in engineered *Saccharomyces cerevisiae* strains: current state and perspectives**. *Appl Microbiol Biotechnol* 2009, **84**:37-53.

38. Ha S-J, Galazka JM, Rin Kim S, Choi J-H, Yang X, Seo J-H, Louise Glass N, Cate JHD, Jin Y-S: **Engineered *Saccharomyces cerevisiae* capable of simultaneous cellobiose and xylose fermentation**. *Proc Natl Acad Sci* 2011, **108**:504-509.

39. Sakamoto T, Hasunuma T, Hori Y, Yamada R, Kondo A. **Direct ethanol production from hemicellulosic materials of rice straw by use of an engineered yeast strain codisplaying three types of hemicellulolytic enzymes on the surface of xylose-utilizing *Saccharomyces cerevisiae* cells.** *J Biotechnol*, http://www.sciencedirect.com/science/article/pii/S0168165611003245, in press.

40. Shin H-D, McClendon S, Vo T, Chen RR: ***Escherichia coli* binary culture engineered for direct fermentation of hemicellulose to a biofuel**. *Appl Environ Microbiol* 2010, **76**:8150-8159.

41. • Steen EJ, Kang YS, Bokinsky G, Hu ZH, Schirmer A, McClure A, del Cardayre SB, Keasling JD: **Microbial production of fatty-acid-derived fuels and chemicals from plant biomass**. *Nature* 2010, **463**: 559-U182.
A great example of how biotechnology enables the flexibility to engineer both biomass degradation and advanced fuel production characteristics in a single microbe

42. Zhang YHP, Lynd LR: **Cellulose utilization by *Clostridium thermocellum*: bioenergetics and hydrolysis product assimilation**. *Proc Natl Acad Sci USA* 2005, **102**:9430.

43. Fan ZL, McBride JE, van Zyl WH, Lynd LR: **Theoretical analysis of selection-based strain improvement for microorganisms with growth dependent upon extracytoplasmic enzymes**. *Biotechnol Bioeng* 2005, **92**:35-44.

44. Raman B, McKeown CK, Rodriguez M, Brown SD Jr, Mielenz JR: **Transcriptomic analysis of *Clostridium thermocellum* ATCC 27405 cellulose fermentation**. *BMC Microbiol* 2011, **11**:.

45. Raman B, Pan C, Hurst GB, Rodriguez M, McKeown CK Jr, Lankford PK, Samatova NF, Mielenz JR: **Impact of pretreated switchgrass and biomass carbohydrates on *Clostridium thermocellum* ATCC 27405 cellulosome composition: a quantitative proteomic analysis**. *PLoS ONE* 2009, **4**:e5271.

46. Brown SD, Raman B, McKeown CK, Kale SP, He ZL, Mielenz JR: **Construction and evaluation of a Clostridium thermocellum ATCC 27405 whole-genome oligonucleotide microarray**. *Appl Biochem Biotechnol* 2007:663-674.

47. • Nataf Y, Bahari L, Kahel-Raifer H, Borovok I, Lamed R, Bayer EA, Sonenshein AL, Shoham Y: ***Clostridium thermocellum* cellulosomal genes are regulated by extracytoplasmic polysaccharides via alternative sigma factors**. *Proc Natl Acad Sci USA* 2010, **107**:18646-18651.
This study provides a deeper understanding of how the highly complex multi-protein cellulosome, perhaps the most specalized lignocellulose degrading system, is regulated in *C. thermocellum.*

48. Abdou L, Boileau C, de Philip P, Pages S, Fierobe HP, Tardif C: **Transcriptional regulation of the *Clostridium cellulolyticum* cip-cel operon: a complex mechanism involving a catabolite-responsive element**. *J Bacteriol* 2008, **190**:1499-1506.

49. Gold ND, Martin VJJ: **Global view of the *Clostridium thermocellum* cellulosome revealed by quantitative proteomic analysis**. *J Bacteriol* 2007, **189**:6787-6795.

50. Newcomb M, Chen CY, Wu JHD: **Induction of the celC operon of *Clostridium thermocellum* by laminaribiose**. *Proc Natl Acad Sci USA* 2007, **104**:3747-3752.

51. • Olson DG, Tripathi SA, Giannone RJ, Lo J, Caiazza NC, Hogsett DA, Hettich RL, Guss AM, Dubrovsky G, Lynd LR: **Deletion of the Cel48S cellulase from *Clostridium thermocellum***. *Proc Natl Acad Sci* 2010, **107**:17727-17732.
This study demonstrates the robustness and redundancy of the cellulase system of *C. thermocellum*.

52. Hinman N, Schell D, Riley J, Bergeron P, Walter P: **Preliminary estimate of the cost of ethanol production for ssf technology**. *Appl Biochem Biotechnol* 1992, **34**:639-649.

53. Hettenhaus JR, Glassner D: *Enzyme Hydrolysis of Cellulose: Short-Term Commercialization Prospects for Conversion of Lignocellulosics to Ethanol*. National Renewable Energy Laboratory; 1997.

54. Wiselogel A: *Bioethanol from the Corn Industry*. National Renewable Energy Laboratory; 1998.

55. Hettenhaus JR: *Biomass Commercialization Prospects in the Next 2–5 Years. Biomass Colloquies 2000*. National Renewable Energy Laboratory; 2000.

56. Tetarenko P: *Building a Bridge to the Corn Ethanol Industry*. Vogelbusch; 2000.

57. Nguyen C (Ed): *Genencor Celebrates Major Progressin the Conversion of Biomass to Ethanol. (Press release)*. Genecor; 2004.

58. Tuli R: *R&D for Bio-Energy*. National Botanical Research Institute; 2004

59. Petiot E: **On the road to cost-competitive cellulosic ethanol**. *Chimica Oggi-Chemistry Today* 2008, **26**:20-22.

60. Sheridan C: **Europe lags, US leads 2nd-generation biofuels**. *Nat Biotechnol* 2008, **26**:1319-1321.

61. McMillan JD: *Biotechnological Routes to Biomass Conversion. DOE/NASULGC Biomass and Solar Energy Workshops*. National Renewable Energy Laboratory; 2004.

62. Bryant C: **Putting the pieces together, cellulosic commercialization**. *National Ethanol Conference*. 2011.

63. Penttila ME, Andre L, Lehtovaara P, Bailey M, Teeri TT, Knowles JKC: **Efficient secretion of 2 fungal cellobiohydrolases by *Saccharomyces cerevisiae***. *Gene* 1988, **63**:103-112.

64. Reinikainen T, Ruohonen L, Nevanen T, Laaksonen L, Kraulis P, Jones TA, Knowles JKC, Teeri TT: **Investigation of the function of mutated cellulose-binding domains of *Trichoderma reesei* cellobiohydrolase I**. *Proteins Struct Funct Bioinform* 1992, **14**:475-482.

65. Den Haan R, McBride JE, La Grange DC, Lynd LR, Van Zyl WH: **Functional expression of cellobiohydrolases in *Saccharomyces***



Please cite this article in press as: Olson DG, et al. Recent progress in consolidated bioprocessing, Curr Opin Biotechnol (2011), doi:10.1016/j.copbio.2011.11.026

***cerevisiae* towards one-step conversion of cellulose to ethanol**. *Enzyme Microb Technol* 2007, **40**:1291-1299.

66. Hong J, Tamaki H, Yamamoto K, Kumagai H: **Cloning of a gene encoding a thermo-stable endo-beta-1,4-glucanase from *Thermoascus aurantiacus* and its expression in yeast**. *Biotechnol Lett* 2003, **25**:657-661.

67. Takada G, Kawaguchi T, Sumitani J, Arai M: **Expression of *Aspergillus aculeatus* no. F-50 cellobiohydrolase I (cbhI) and beta-glucosidase 1 (bgl1) genes by *Saccharomyces cerevisiae***. *Biosci Biotechnol Biochem* 1998, **62**:1615-1618.

68. Heinzelman P, Komor R, Kanaan A, Romero P, Yu XL, Mohler S, Snow C, Arnold F: **Efficient screening of fungal cellobiohydrolase class I enzymes for thermostabilizing sequence blocks by SCHEMA structure-guided recombination**. *Protein Eng Design Selection* 2010, **23**:871-880.

69. Ilmen M, den Haan R, Brevnova E, McBride J, Wiswall E, Froehlich A, Koivula A, Voutilainen S, Siika-aho M, la Grange D *et al.*: **High level secretion of cellobiohydrolases by *Saccharomyces cerevisiae***. *Biotechnol Biofuels* 2011, **4**:30.

70. Zurbriggen B, Bailey MJ, Penttila ME, Poutanen K, Linko M: **Pilot scale production of a heterologous *Trichoderma reesei* cellulase by *Saccharomyces cerevisiae***. *J Biotechnol* 1990, **13**:267-278.

71. Heinzelman P, Snow CD, Wu I, Nguyen C, Villalobos A, Govindarajan S, Minshull J, Arnold FH: **A family of thermostable fungal cellulases created by structure-guided recombination**. *Proc Natl Acad Sci USA* 2009, **106**:5610-5615.

72. Verduyn C, Stouthamer AH, Scheffers WA, Vandijken JP: **A theoretical evaluation of growth yields of yeasts**. *Antonie Van Leeuwenhoek Int J Gen Mol Microbiol* 1991, **59**:49-63.

73. Guedon E, Desvaux M, Petitdemange H: **Improvement of cellulolytic properties of Clostridium cellulolyticum by metabolic engineering**. *Appl Environ Microbiol* 2002, **68**:53-58.

74. 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.

75. Zambare VP, Bhalla A, Muthukumarappan K, Sani RK, Christopher LP: **Bioprocessing of agricultural residues to ethanol utilizing a cellulolytic extremophile**. *Extremophiles* 2011, **15**:611-618.

76. Okamoto K, Nitta Y, Maekawa N, Yanase H: **Direct ethanol production from starch, wheat bran and rice straw by the white rot fungus Trametes hirsuta**. *Enzyme Microb Technol* 2011, **48**:273-277.

77. Tolonen AC, Haas W, Chilaka AC, Aach J, Gygi SP, Church GM: **Proteome-wide systems analysis of a cellulosic biofuel-producing microbe**. *Mol Sys Biol* 2011, **6**.

78. Cai Y, Lai C, Li S, Liang Z, Zhu M, Liang S, Wang J: **Disruption of lactate dehydrogenase through homologous recombination to improve bioethanol production in Thermoanaerobacterium aotearoense**. *Enzyme Microb Technol* 2011, **48**:155-161.

79. Yao S, Mikkelsen MJ: **Metabolic engineering to improve ethanol production in Thermoanaerobacter mathranii**. *Appl Microbiol Biotechnol* 2010, **88**:199-208.

80. Lee JM, Venditti RA, Jameel H, Kenealy WR: **Detoxification of woody hydrolyzates with activated carbon for bioconversion to ethanol by the thermophilic anaerobic bacterium Thermoanaerobacterium saccharolyticum**. *Biomass Bioenergy* 2011, **35**:626-636.

81. Berezina O, Sineoky S, Velikodvorskaya G, Schwarz W, Zverlov V: **Extracellular glycosyl hydrolase activity of the Clostridium strains producing acetone, butanol, and ethanol**. *Appl Biochem Microbiol* 2008, **44**:42-47.



Please cite this article in press as: Olson DG, et al. Recent progress in consolidated bioprocessing, Curr Opin Biotechnol (2011), doi:10.1016/j.copbio.2011.11.026