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BIOTECHNOLOGY and BIOENGINEERING

The Challenge of Enzyme Cost in the Production of Lignocellulosic Biofuels

Daniel Klein-Marcuschamer,[1,2] Piotr Oleskowicz-Popiel,[1,2] Blake A. Simmons,[1,2,3] Harvey W. Blanch[1,2,4]

[1]Joint Bioenergy Institute, 5885 Hollis St. Emeryville, California 94608

[2]Physical Biosciences Division, Lawrence Berkeley National Laboratory, 1 Cyclotron Road, Berkeley, California 94720

[3]Sandia National Laboratories, Biomass Science and Conversion Technology Department, Livermore, California

[4]Department of Chemical Engineering, University of California, Berkeley, California 94720. T: (510) 486-7069; F: (510) 486-4252; e-mail: blanch@berkeley.edu

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ABSTRACT: With the aim of understanding the contribution of enzymes to the cost of lignocellulosic biofuels, we constructed a techno-economic model for the production of fungal cellulases. We found that the cost of producing enzymes was much higher than that commonly assumed in the literature. For example, the cost contribution of enzymes to ethanol produced by the conversion of corn stover was found to be $0.68/gal if the sugars in the biomass could be converted at maximum theoretical yields, and $1.47/gal if the yields were based on saccharification and fermentation yields that have been previously reported in the scientific literature. We performed a sensitivity analysis to study the effect of feedstock prices and fermentation times on the cost contribution of enzymes to ethanol price. We conclude that a significant effort is still required to lower the contribution of enzymes to biofuel production costs.

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KEYWORDS: cellulase; process model; techno-economic analysis; biofuels; enzyme costs; enzyme loading

The contribution of enzyme costs to the economics of lignocellulosic biofuel production continues to be a much-debated topic. Whereas some authors argue that the cost of enzymes is a major barrier for biofuel production (Brijwani et al., 2010; Cherry and Fidantsef, 2003; Fang et al., 2009), others implicitly assume that it is not, either because they estimate the cost to be relatively low or because they assume that it will decrease with technological innovation or other advances (Aden and Foust, 2009; Schubert, 2006). Other studies simply acknowledge that publicly available detailed information on the cost of enzymes is limited (Gusakov, 2011; Klein-Marcuschamer et al., 2010; Lynd et al., 2008). Adding to the confusion is the fact that costs of enzymes are almost exclusively accounted for and reported in terms of dollars per gallon of biofuel (ethanol in particular), a "top-down" measure that depends on many factors besides the cost of the enzymes themselves, including the choice of feedstock, the enzyme loading, and the overall biofuel yield. Literature estimates for the cost contribution of enzymes to the production of lignocellulosic ethanol vary significantly, including $0.10/gal (Aden and Foust, 2009), $0.30/gal (Lynd et al., 2008), $0.32/gal (Dutta et al., 2010), $0.35/gal (Klein-Marcuschamer et al., 2010), and $0.40/gal (Kazi et al., 2010).

This inconsistency in the cost of enzymes for biofuel applications seriously hampers robust techno-economic analysis of biofuel production processes. This, in turn, adds uncertainty in decision-making at many levels: From researchers choosing where to focus their efforts and policymakers negotiating biofuel subsidies to investors funding a project and peripheral industry players projecting the proliferation of fossil fuel alternatives. The development of a techno-economic model for the production of cellulases enables the determination of "bottom-up" enzyme costs based on process characteristics, and provides a framework that can be used to refine costs as better information becomes available. Other authors have analyzed the economics of cellulase production, though studies commonly cover the topic tangentially to the issue of biofuel production itself (Kazi et al., 2010; Wooley et al., 1999). The process model here presented includes all unit operations required for the production of cellulases from steam-exploded poplar by *Trichoderma reesei*, including feedstock transport, steam explosion, fungal growth, cellulase production, and downstream processing. The latter includes biomass filtration and incineration (as a biosafety measure)

Correspondence to: H.W. Blanch

and concentration of the proteins to commercially relevant titers (~150 g/L). The model is available, for non-commercial use, at JBEI's techno-economic analysis wiki site (http://econ.jbei.org).

After modeling the process of enzyme production, we used data from our previously constructed model for corn stover ethanol production with dilute acid pretreatment (Klein-Marcuschamer et al., 2010) to contextualize the costs of enzymes based on typical ethanol yields. At 20% solids loading during saccharification, a typical enzyme loading is 10 FPU/g cellulose, equivalent to ~20 mg enzyme/g cellulose (Gusakov, 2011; Roche et al., 2009). The typical yield for the saccharification of cellulose at this enzyme loading is 70% after 5 days (Roche et al., 2009). It must be noted that the optimal value of enzyme loading varies depending on feedstock, solids loading, and pretreatment technology, among other variables (Kazi et al., 2010; Kristensen et al., 2009; Wyman et al., 1992). As the costs of enzymes on a $/gal basis depend on the yield of ethanol, we considered four scenarios in the following discussion: (1) The theoretical maximum yield based on conversion of all C5 and C6 sugars present in corn stover; (2) the yield based on conversion of C6 sugars at a 95% efficiency, but not C5 sugars; (3) the yield based on conversion of all C5 and C6 sugars after a saccharification cellulose conversion of 70%; and (4) the yield based on conversion of C5 and C6 sugars expected from a typical saccharification and a typical fermentation using engineered yeast [based on our previous model, (Klein-Marcuschamer et al., 2010)]. Scenario (2) exemplifies the case in which saccharification efficiency is very high, but fermentation efficiency remains close to what is expected today for a corn ethanol process; scenario (3) explores the case in which saccharification efficiency does not improve, but fermentation efficiency is greatly enhanced. Henceforth, we refer to these scenarios by their number only. Based on dry corn stover, the ethanol yields are: (1) 111.4 gal/DT (dry tonne); (2) 67.13 gal/DT; (3) 88.9 gal/DT; and (4) 51.6 gal/DT. Comparing the yields of scenarios (1) and (3) with those of (2) and (4) highlight that the main technological bottlenecks for improving overall yield are associated with fermentation, not saccharification, at least at these relatively high enzyme loadings. For all practical purposes, scenario (1) is unattainable and is only included as a metric for comparison. When discussing avenues to decrease the cost contribution of enzymes, it is more relevant to look at the costs given by scenarios (2) and (4), since focusing on scenario (3) would merely shift the burden for improvement to fermentation technologies. Efficient fermentation of available sugars is indeed important, but does not impact the cost of enzyme production.

We began our analysis by benchmarking the cost of any enzyme to the cost of one of the cheapest proteins available today: Soy protein. Market prices for soy in 2011 have averaged ~$500/MT [www.indexmundi.com, (IndexMundi)], which, at a protein content of ~40% [USDA National Nutrient Database, (USDA)], amounts to ~$1.25/kg of protein. We do not expect any protein produced by a biotechnological (fermentation) process to approach this level of production, but if cellulases could be produced at this cost, they would amount to (1) $0.08/gal, (2) $0.14/gal, (3) $0.11/gal, or (4) $0.18/gal. As can be seen from this analysis, some of the cost contribution values in the literature are close to those that would be expected if cellulases could be produced as inexpensively as soy protein and the ethanol yield was close to the theoretical maximum. This is clearly unrealistic.

Barring a revolutionary shift in the paradigm of industrial protein production, it is unreasonable to assume that enzyme costs compared to that of soy-derived protein would be achieved in practice, regardless of technological advances or campaigns of process optimization. With that in mind, one can analyze the cost of production of biotechnologically derived enzymes using our process model (Fig. 1). The baseline production cost of enzyme was found to be $10.14/kg, which translates to (1) $0.68/gal, (2) $1.13/gal, (3) $0.85/gal, and (4) $1.47/gal. Figure 2 shows the breakdown of the annual operating cost of the baseline case of a cellulase production facility (see Materials and Methods section). Nearly half of the cost of production is associated with the capital investment (depreciation, insurance, maintenance, etc.), while the cost of raw materials accounts for almost a third of the cost. This partly explains why it is not possible to produce enzymes cheaper than plant-derived proteins: It is quite expensive simply to purchase the equipment for the

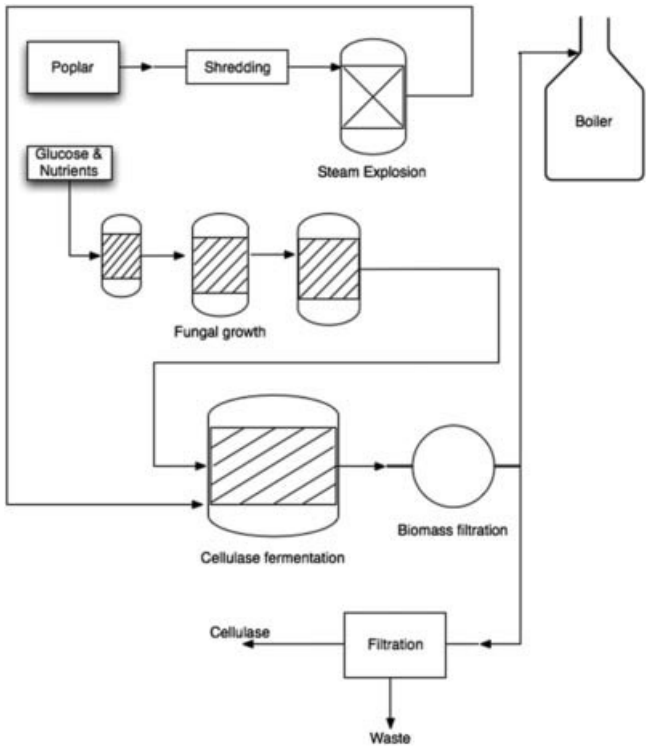

Figure 1. Process diagram for cellulase production.

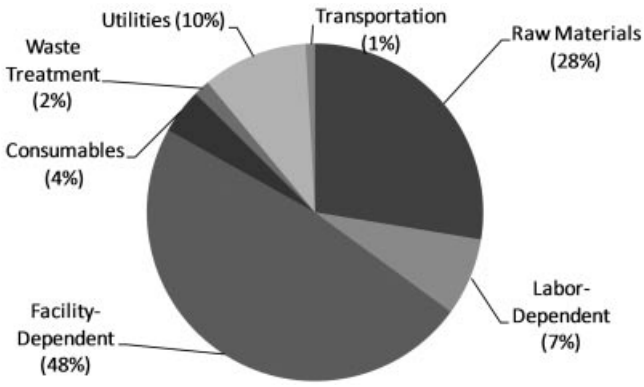

Figure 2. Breakdown of the annual operating cost (AOC) of the designed enzyme production facility.

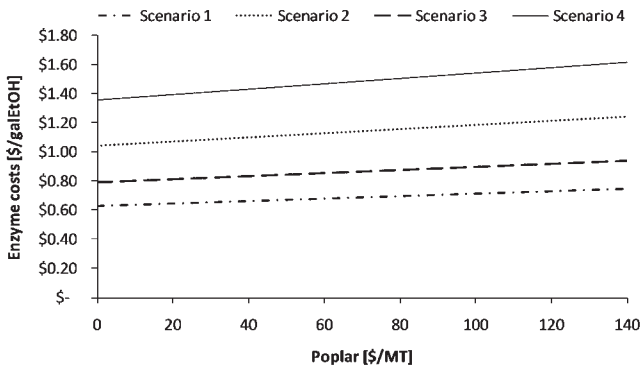

Figure 3. Sensitivity of the contribution of enzyme costs to poplar price using the ethanol yield scenarios: (1) Maximum theoretical yield; (2) conversion of C6 sugars at a 95% efficiency, no C5 conversion; (3) conversion of all C5 and C6 sugars after a saccharification cellulose conversion of 70%; and (4) conversion of C5 and C6 sugars expected from a typical process (see main text).

production facility, even discounting all other costs.

Next, and with the aim of exploring the effect of parameter choices on the cost of enzymes, we performed a sensitivity analysis based on the key parameters noted above. This is particularly valuable when studying uncertain parameters, such as the cost of poplar and the fermentation residence time, which we present as examples. It is natural to expect shorter fermentation residence times to be achievable with carbon sources simpler than poplar, such as amorphous cellulose (Ike et al., 2010). Furthermore, it could be possible, at least in principle, that *Trichoderma* could be engineered for faster growth rates, decreasing the residence time in the fermentation section. As for raw materials, the chosen baseline cost for poplar, at $60/MT, is in line with small-scale studies (Perrin et al., 2008) but is low compared to projections of the cost of biomass as the lignocellulosic biofuel market expands, which place it at ~$140/MT (Khanna et al., 2011). More importantly, the choices of carbon sources and inducers used commercially are not publicly discussed, making the cost of raw materials particularly uncertain (Gusakov, 2011).

Figures 3 and 4 show the sensitivity of the contribution of enzyme costs to poplar price and fermentation residence time, respectively, using the ethanol yield scenarios. Coincidentally, both graphs look strikingly similar, and parallel conclusions can be drawn from both. The results of Figure 3 show that, even if the poplar were freely available, enzymes would contribute between $0.60/gal and $1.30/gal, considerably higher than most literature values. The average for all values in all scenarios, a number that smoothes out the effects of assumptions at the optimistic or conservative extremes, is $1.05/gal. The results of Figure 4, on the other hand, show that decreasing the growth and fermentation time would help, in particular through lowering the capital cost associated with fermentation. However, as for the case of poplar feedstock prices, these changes would not, by themselves, alleviate the high cost of enzymes. For scenarios (2) and (4), in both analyses the contributions are all greater than $1/gal, implying that improving the economics of enzyme production is a significant hurdle. Lowering enzyme loadings required for saccharification would be an obvious target for improvement and one already being tackled by industrial and other research groups (Kim et al., 2011; Li et al., 2011; Yang et al., 2010). Examples include cellulases that are more stable, better pretreatment technologies that enable high saccharification yields at lower enzyme loadings, and methods to reduce the presence of phenolic compounds, which inhibit and deactivate cellulases. If enzyme loading could be lowered, for instance, to 5 FPU/g cellulose, the cost contribution of enzymes would be (1) $0.34/gal, (2) $0.55/gal, (3) $0.42/gal, and (4) $0.73/gal. This would have to be achieved at 20% solids loading achieving 70% conversion in 5 days, unless changes in the saccharification process cause an overall increase in ethanol production costs.

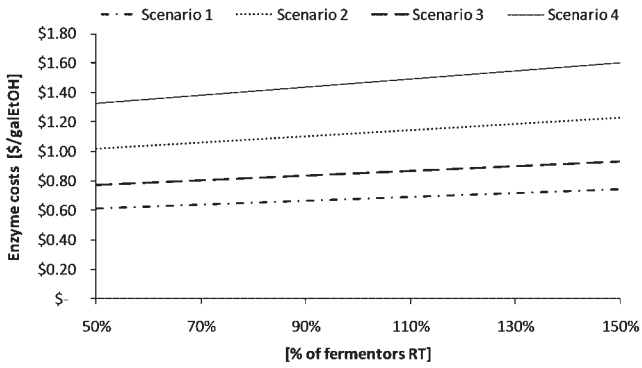

Figure 4. Sensitivity of the contribution of enzyme costs to fermentation residence times using the ethanol yield scenarios (same as in Fig. 3). The residence times for all seed and main fermentors were changed to the fraction of the baseline value that is indicated on the abscissa.

Our analysis shows that, in general, the vast majority of the literature to date has significantly underestimated the contribution of enzyme costs to biofuel production. The contribution can be lowered by shifting to lower cost feedstocks, reducing the fermentation times, and reducing the complexity of the process to drive down capital costs, among others. The analysis points to the importance of changes that occur at the biorefinery, rather than at the enzyme production facility: Much is to be gained by achieving high overall biofuel yields at low enzyme loadings. This fact places, indirectly, emphasis on the need for development of improved pretreatment and enzyme technologies. Without recognizing the challenge presented by high enzyme costs, it is hard to justify devoting time and resources to this issue. While improving enzyme activity has been the focus of a significant body or research, these improvements only influence biorefinery capital costs by reducing saccharification residence times, and do not address the enzyme production costs, which are reflected as operating costs. Techno-economic analysis of enzyme production, such as that here presented, can aid in directing efforts in this area.

Materials and Methods

A process model was constructed in SuperPro Designer (Intelligen, New Jersey), and is available for non-commercial use and review at http://econ.jbei.org. Assumptions for individual unit operations (utility use, throughput rates, sizing, etc.) were taken, when possible, from our previous study (Kazi et al., 2010; Wooley et al., 1999), a description of these is available in the aforementioned website. Equipment and raw material costs (with the exception of poplar and glucose costs) were from (Aden et al., 2002; Kazi et al., 2010; Wooley et al., 1999), updated to 2007 dollars using the CEPCI and ICI indices, respectively for equipment and materials. Updates from 2007 to 2010 dollars were also from the same indices, when available, or using the consumer price index as a measure of inflation. Poplar price at the farm gate was assumed to be $60/MT in accordance to a study by Perrin et al. (2008), and glucose price, used for fungal seed growth, was taken as the 2010 average market prices for US sugar ($0.79/kg (USDA). While sugar prices are expected to be lower than glucose prices (e.g., in the form of glucose syrup), US sugar prices are significantly higher than world market prices. We expect these trends to counteract each other and for the chosen value to serve as a reasonable estimate for a simple carbon source. Costs of wastewater treatment were from (Merrick & Co., 1998) based on biological oxygen demand (BOD), total Kjeldahl nitrogen (TKN), and total suspended solids (TSS).

Steam explosion of poplar was designed in accordance to Boussaid et al. (2000), Kovacs et al. (2009), and Negro et al. (2003). Poplar was pretreated for 4 min at 210°C with high-pressure steam (13 bars, 268°C). The pressure was then released and the content of the reactor cooled down, moisture of the pretreated poplar was approximately 50%. The stoichiometry and residence times of fungal growth and cellulase production were taken from Tholudur et al. (1999), Velkovska et al. (1997), and Wooley et al. (1999). Briefly, trains of three seed fermentors each were simulated, each providing 5% inocula to the next fermentor in the train. Fermentors were batched with corn steep liquor, ammonium hydroxide, and nutrients. The residence time of each seed fermentor was 96 h in the baseline case. All reactors were aerated at 0.577 vvm. The main fermentation process consisted of 11 bioreactors with residence time of 192 h in the baseline case. Finally, fungal biomass was filtered in a rotary vacuum filter and the cellulase in the supernatant was concentrated using an ultrafiltration unit according to Mores et al. (2001) and Roseiro et al. (1993) to produce a product stream with ~150 g/L enzyme. The separated biomass was incinerated as a biosafety measure.

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