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# Optimizing the enzyme loading and incubation time in enzymatic hydrolysis of lignocellulosic substrates

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# HIGHLIGHTS

- ► A simple model gives estimated costs of lignocellulosic sugars.
- ▶ Variables include enzyme loading and incubation time.
- ► Those variables can be optimized for lowest sugar cost.
- ▶ Optimized values can be expressed in terms of three unit costs.
- ▶ Steam-exploded pine feedstock provided data for a worked example.

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# ABSTRACT

A mathematical model for costing enzymatic hydrolysis of lignocellulosics is presented. This model is based on three variable parameters describing substrate characteristics and three unit costs for substrate, enzymes and incubation. The model is used to minimize the cost of fermentable sugars, as intermediate products on the route to ethanol or other biorefinery products, by calculating optimized values of enzyme loading and incubation time. This approach allows comparisons between substrates, with processing conditions optimized independently for each substrate. Steam-exploded pine wood was hydrolyzed in order to test the theoretical relationship between sugar yield and processing conditions.

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#### 1. Introduction

Global production of ethanol almost doubled over a period of six years, from 12.1 billion gallons in 2005 to 22.9 billion gallons in 2011 (Renewable Fuels Association, 2006, 2012). Most of this ethanol was produced by fermentation of sugars sourced from grains in North America or sugarcane in South America, and most of it was used for transport fuels. Large annual increases will become increasingly difficult to repeat in the future, at least while ethanol production is based on feedstocks that are also used for food production. Lignocellulosic feedstocks have greater potential for expansion of the bio-based ethanol industry (Van Dyk and Pletschke, 2012). Several recent techno-economic studies have discussed the obstacles involved in the development of lignocellulosic ethanol (Aden and Foust, 2009; Piccolo and Bezzo, 2009; Huang et al., 2009; Gnansounou and Dauriat, 2010; Humbird et al., 2010; Stephenson et al., 2010; Gonzalez et al., 2011; Ljunggren et al., 2011). Two of the outstanding obstacles identified in those studies are the slow rate of enzymatic hydrolysis of cellulose and the cost of the enzymes. Those two obstacles are, to some extent, interdependent. This paper explores the interdependence and identifies the general principles involved in optimizing the balance to minimize production costs.

While enzyme consumption contributes just US\$0.05 to the cost of producing a gallon of corn ethanol, it might add as much as US\$0.34 to the cost of producing a gallon of lignocellulosic ethanol (Humbird et al., 2011). A common approach to reducing enzyme costs per unit of ethanol has been to increase the incubation time so as to increase the yield of sugar per unit of enzyme. This approach has limitations as increasing the incubation time leads to increased capital expenditure to cover the additional storage capacity. For example, Humbird et al. (2011) designed a plant to process corn stover for annual production of 80 million gallons of ethanol. In their design, incubation of the suspended solids over a period of 84 h, through enzymatic hydrolysis and fermentation,

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would require 20 reactors. The total installed cost of those reactors was estimated as US\$31 million, out of an installed cost of US\$232 million for all equipment in the plant.

It is intuitively obvious that the trade-off between enzyme loading and incubation time must depend on the kinetics of enzymatic hydrolysis. The trade-off is first considered in terms of a reasonably general mathematical theory, using dimensionless parameters to represent enzyme loading and incubation time. The consequences of the trade-off are then considered in more detail, using experimental data for a pretreated softwood substrate.

# 2. Methods

#### 2.1. Theory

The conditions for enzymatic hydrolysis were optimized by minimizing the cost, *Z*, of producing one tonne of fermentable sugars as an aqueous solution. An aqueous solution of fermentable sugars is an intermediate product on the separate hydrolysis and fermentation (SHF) route to bioethanol, biobutanol or other biorefinery products, and knowing its production cost is important in comparing alternative feedstocks and pretreatment processes (Humbird et al., 2011). Just three component costs were considered: the cost  $Z_s$  of the substrate, the cost  $Z_e$  of the enzymes, and the cost  $Z_i$  of incubation.

The cost  $U_s$  of one tonne (dry mass) of substrate includes the cost of feedstock, pretreatment, disposal of any waste generated during pretreatment, attrition of the solid matter, and any neutralization and dilution steps up to the point at which the substrate enters the first saccharification reactor. If one tonne of substrate contains sufficient polysaccharides to produce *X* tonnes of fermentable sugars, and if the saccharification stage results in a mass fraction *Y* of those polysaccharides actually being converted to sugars, then the cost of substrate required to produce one tonne of fermentable sugars is:

$$Z_{\rm s} = U_{\rm s}/(XY) \tag{1}$$

An indicative value  $U_s$  is useful, in order to focus the discussion on relevant ranges of costs. Stephen et al. (2012) suggested a cost of US\$50/tonne dry weight for wood, including forest residues. Humbird et al. (2011) suggested a cost of US\$65/tonne dry weight for corn stover. The pretreatment process chosen by Humbird et al. (2011) was relatively mild, involving temperatures up to 158 °C and adding just 39% onto the cost of the feedstock. Greater severity was assumed in the present work, perhaps even doubling feedstock costs, so an indicative value of  $U_s$  = US\$100/tonne was used (Table 1).

The value of *X* was chosen to allow for incomplete hydrolysis, even at high enzyme loadings and long incubation times. An adjustable parameter  $\alpha$  was defined as the fraction of target polysaccharides accessible to enzymes, including polysaccharides that become accessible during the course of enzymatic hydrolysis, but excluding polysaccharides so thoroughly embedded in lignin that they never become accessible to enzymes. The value of *X* also allowed for one water molecule added to each sugar residue during hydrolysis so that, e.g., one tonne of cellulose is hydrolysed to

Tuble 1						
Indicative	unit	costs	discussed	in	Section	2.1

Table 1

Symbol	Association	Value
Us	Substrate	US\$100/tonne
Ue	Enzyme	US\$10/MFPU <sup>a</sup>
Ui	Incubation	US\$0.20/tonne/h

<sup>a</sup> MFPU = million filter paper units.

1.11 tonne glucose. The value of X was therefore  $1.11\alpha C$ , where C is the amount of the target polysaccharide, expressed as a fraction of the mass of the substrate. In some cases the target polysaccharide was cellulose only, in others the value of X was increased to include hemicelluloses.

The unit cost  $U_{\rm e}$  of the enzyme is expressed per MFPU, i.e., one million filter paper units of cellulase activity. The enzyme loading *E* is expressed as the MFPU of activity per tonne of dry mass of substrate. The cost of enzyme required to produce one – tonne of fermentable sugars is:

$$Z_{\rm e} = EU_{\rm e}/(XY) \tag{2}$$

Enzyme costs remain unclear, since there is currently no market for large-scale cellulase production, but a recent paper compared on-site and off-site production costs and concluded that likely costs were similar at approximately  $U_e = US$4/MFPU$  (Barta et al., 2010). Klein-Marcuschamer et al. (2012) suggested that enzyme costs might be as high as US\$20/MFPU. An indicative value of US\$10/MFPU was used in the present work (Table 1).

It is assumed that the cost of incubation is proportional to the incubation time t, expressed in hours. This assumption is valid if the suspended solids move through a train of identical stirred reactors, so that the number of reactors in a train is proportional to each of t and the flow rate. In practice, it might be convenient to lengthen incubation times by adding a relatively large reactor at the end of the train rather than adding additional reactors of the same volume. That complication is neglected here. The unit cost  $U_i$  of incubation is the sum of capital and operating costs associated with incubating one tonne of substrate for one hour.

$$Z_{\rm v} = t U_{\rm i} / (XY) \tag{3}$$

The unit cost depends on the solids content of the slurry. If the solids content is decreased by dilution with water, the total volume of the reactors increases and a larger capital investment is required. If the solids content is increased, agitation becomes more difficult and the reactor train might have to include a high-solids bioreactor or similar non-standard equipment (Larsen et al., 2008; Roche et al., 2009). Humbird et al. (2011) estimated costs of approximately \$18/tonne (dry weight) for incubation of a slurry of pretreated stover through 84 h of enzymatic hydrolysis and fermentation. That study led to an indicative unit cost of  $U_i = US$0.20/tonne/h for incubation (Table 1).$ 

Combining Eqs. (1–3) gives the total cost of producing fermentable sugars:

$$Z = (U_{\rm s} + EU_{\rm e} + tU_{\rm i})/YX \tag{4}$$

This costing model neglects many of the costs and credits that would be considered in a detailed techno-economic model. In particular, enzymatic hydrolysis might produce a slurry of residual solids in a dilute solution of fermentable sugars. It is assumed that the costs of filtering out the residual solids and concentrating the sugar solution can be offset by credits for burning the residual solids to generate process heat.

The yield, *Y*, is a function of enzyme dose and incubation time, i.e., Y = Y(E,t). A simple equation for the yield is used here, based on an assumption that the conversion reaction follows fractal kinetics. Fractal kinetic theory has been applied to enzymatic hydrolysis of cellulose with some success (Väljamae et al., 2003; Wang and Feng, 2010; Wang et al., 2011). The label "fractal" refers to the non-integer exponent 1-h in an expression for conversion from cellulose to glucose (Väljamae et al., 2003):

$$Y = Y_{\infty}(E) \left( 1 - \exp\left( -kt^{1-h} \right) \right)$$
(5)

Here  $Y_{\infty}$  represents the ultimate yield in the limit  $t \to \infty$ , and depends on the enzyme loading. The rate constant k is a function of

the enzyme loading, perhaps also requiring a fractional exponent (Wang and Feng, 2010), so it was replaced by  $k = (E/\varepsilon)^m$  where  $\varepsilon$  is a measure of enzyme demand, to be determined as a characteristic of the substrate. Preliminary calculations indicated that a value of m = 1/2 was suitable in the present work, so no other value was assessed.

The exponent 1-h has been reported as showing a weak dependence on *E*, with a median value of 0.71 for enzymatic hydrolysis of 17 mixtures of cellulose, lignin and surfactant (Wang et al., 2011). The value was arbitrarily fixed at 1-h = 2/3 in the present work. This choice will be discussed further under Section 3. The incubation time was expressed as a dimensionless parameter  $t/\tau$ , where the time constant  $\tau$  is defined as the incubation time required to attain  $Y = 0.5 \exp(-1) = 0.347$ , in an experiment in which  $E/\varepsilon = 1$ .

Sattler et al. (1989) reported dose–response curves for an exceptionally wide range of values of *E*, and the shapes of those curves resembled exponential functions:

$$Y_{\infty} = 1 - \exp(-cE/\varepsilon) \tag{6}$$

Here  $c = \ln(2)$  so that  $\varepsilon$  is defined as the enzyme loading required for half maximum digestion of the substrate. Combining Eqs. (5) and (6) resulted in an expression containing just two adjustable parameters, i.e.,  $\varepsilon$  and  $\tau$ :

$$Y = (1 - \exp(-cE/\varepsilon)) \left(1 - \exp\left(-(E/\varepsilon)^{1/2}(t/\tau)^{2/3}\right)\right)$$
(7)

Our aim is to determine the values of  $E = E_{opt}$  and  $t = t_{opt}$  when the cost of fermentable sugar,  $Z = Z_{min}$ , expressed per tonne of dry weight, has been minimized. This occurs when two partial derivatives are both equal to zero, i.e.,  $\partial Z/\partial t = \partial Z/\partial E = 0$ . If *t* is kept constant and *E* is varied, differentiation of Eq. (4) with the condition  $\partial Z/\partial t = 0$  gives:

$$(\partial Y/\partial t)_{\rm opt} = U_{\rm i}/XZ \tag{8}$$

If *E* is kept constant and *t* is varied, differentiation of Eq. (4) with the condition  $\partial Z/\partial E = 0$  gives:

$$(\partial Y/\partial E)_{ont} = U_e/XZ \tag{9}$$

Differentiation of Eq. (7) and insertion of the results in Eqs. (8) and (9) gave transcendental equations, i.e., equations that lacked any exact algebraic solution. Approximate algebraic solutions were expressed in terms of dimensionless parameters  $E_{\text{opt}}/\varepsilon$ ,  $t_{\text{opt}}/\tau$ ,  $\tau U_i/U_s$  and  $\varepsilon U_e/U_s$ :

$$(E/\varepsilon)_{\rm opt} = \left(1.27 + 1.31(\tau U_{\rm i}/U_{\rm s})^{0.38}\right) (\varepsilon U_{\rm e}/U_{\rm s})^{-0.36} \tag{10a}$$

$$(t/\tau)_{\rm opt} = 15.4 (\varepsilon U_{\rm e}/U_{\rm s})^{0.29} / \left(1 + 12.2 (\tau U_{\rm i}/U_{\rm s})^{0.53}\right)$$
(10b)

The factors and exponents in Eq. (10) were obtained by leastsquares fitting to numerical solutions. The approximate solutions differed from the numerical solutions by no more than  $\pm 4.0\%$  over the ranges  $0.1 < \varepsilon U_e/U_s < 2.0$  and  $0.02 < \tau U_i/U_s = 0.5$ . These ranges reflected indicative costs discussed above, along with values of  $\varepsilon$ and  $\tau$  for diverse substrates discussed in Section 3.

The approximate solutions illustrate general principles of optimization, e.g., neither  $(E/\varepsilon)_{opt}$  nor  $(t/\tau)_{opt}$  is sensitive to changes in the cost ratio  $\varepsilon U_e/U_s$ , and  $(t/\tau)_{opt}$  is more sensitive than  $(E/\varepsilon)_{opt}$ to changes in the cost ratio  $\tau U_i/U_s$ . These points are illustrated in Fig. 1, for five values of each of  $\varepsilon U_e/U_s$  and  $\tau U_i/U_s$ . The approximate solutions are also useful for spreadsheet evaluation of  $Z_{min}$ , given  $\varepsilon$ and  $\tau$  as input for a selected substrate.

Eq. (7) was used to calculate the yield,  $Y(E_{opt}, t_{opt})$ , for three values of the cost ratio  $\tau U_i/U_s$  and a range of values of the cost ratio  $\epsilon U_e/U_s$  (Fig. 2). For mid-range values of the cost ratios, Fig. 2 indicates  $Y(E_{opt}, t_{opt}) = 0.70$ . In other words, even if substrate costs are as high as US\$100/tonne, leaving approximately one-third of the cellulose in the residue is sometimes cheaper than adding



**Fig. 1.** Dimensionless enzyme loadings and incubation times optimized for five values of each of the cost ratios  $\varepsilon U_e/U_s$  (solid lines) and  $\tau U_i/U_s$  (broken lines).



**Fig. 2.** Fractional conversion of cellulose to glucose (*Y*) calculated for optimal values of enzyme loading and incubation, for three values of the cost ratio  $\tau U_i/U_s$ .

additional enzymes and installing additional incubation reactors to improve the yield of glucose.

#### 2.2. Substrate

Pine wood chips were used to produce steam-exploded pine wood (SEPW) for benchmarking. The conditions for steam explosion were 3% SO<sub>2</sub>/3 min/215 °C. The slurry was filtered and washed to give solids accounting for 66.9% of the mass of wood chips, on a dry basis.

Extractives were determined using a FOSS Soxtec System 1043 extraction unit with dichloromethane as the solvent. Lignin was determined using methods based on TAPPI Standard Method T 222 om-88 and TAPPI Useful Method UM 250. Fucose was added to the hydrolysate from lignin analysis, as an internal standard, and the carbohydrates were analyzed by ion chromatograph using a Dionex ICS 3000 instrument. The composition of the SEPW was: glucan 51.1%, other sugars 0.5%, Klason lignin 40.3%, acid-soluble lignin 0.4%, and extractives 7.1%.

#### 2.3. Enzymatic hydrolysis

Hydrolysis was performed in 20-ml capacity screw-capped glass tubes using 5 ml of 0.05 M sodium citrate buffer containing 0.01% w/v sodium azide, at 50 °C and pH 4.8. The tubes were agitated at 180 rpm in an inclined vibratory shaker. Never-dried

SEPW was added at concentration of 1.5% on a dry basis. Celluclast 1.5L was supplemented with Novozym 188 in activity ratios of 1 FPU to 1.25 IU respectively. Enzyme loadings corresponded to 5, 10, 20, 40, 80 and 100 MFPU/tonne, and incubation times were 1, 3, 5 and 24 h. For each sample, enzymatic hydrolysis was stopped by plunging the tube into boiling water for 5 min and then cooling it to room temperature in water. The mixture was then centrifuged at 4000 rpm for 10 min at 25 °C and the supernatant was collected for glucose analysis by a YSI-2700 glucose analyzer. Results were corrected for small amounts of glucose introduced along with the enzyme solution. The enzymatic hydrolysis experiments were duplicated, and the standard deviation was used to calculate the 95% confidence interval for each mean value. The median value of the 95% confidence interval on conversion of cellulose was ±0.015. Four data points were rejected because the 95% confidence intervals were more than ±0.075. The remaining 20 data points are shown in Fig. 3.

#### 3. Results and discussion

#### 3.1. SEPW characteristics

Steam exploded pine wood was chosen as a substrate because of its relatively high lignin content, so that enzymatic hydrolysis was relatively challenging. The experimental data points in Fig. 3 were plotted as cellulose converted to glucose, expressed as a fraction of all cellulose in the substrate. The solid curves were generated using Eq. (7) to calculate *Y* for least-squares best-fit values of  $\varepsilon$  = 7.1 MFPU/tonne substrate and  $\tau$  = 7.3 h, then multiplying *Y* by the accessibility parameter  $\alpha$  because not all of the cellulose was accessible to enzymes. The best-fit value of  $\alpha$  was 0.84, so the value of *X* for SEPW was 1.11 × 0.84 × 0.51 = 0.48.

The root-mean-square deviation from experimental values of  $\alpha Y$  was 0.026. This was close to the median value of 0.015 for the 95% confidence intervals on  $\alpha Y$ , so it was not considered worth-while to modify Eq. (7) to include any additional adjustable parameters. In particular, the experimental results were consistent with values of 1/2 and 2/3 for the exponents on  $E/\varepsilon$  and  $t/\tau$  in Eq. (7).

The best-fit value of  $\varepsilon$  = 7.1 MFPU/tonne was used to calculate a cost ratio  $\varepsilon U_e/U_s$  = 0.71, and the best fit value of  $\tau$  = 7.3 h was used to calculate a cost ratio  $\tau U_i/U_s$  = 0.015, for which Eq. (10) indicated  $E_{opt}$  = 12.3 MFPU/tonne and  $t_{opt}$  = 22 h. The three best-fit parameters for SEPW are listed in Table 2.

For the optimized processing conditions, Eq. (7) indicated a fractional conversion of Y = 0.66, corresponding to a glucose yield



**Fig. 3.** Experimental values of the fractional conversion of cellulose in SEPW to glucose ( $\alpha$ *Y*) plotted against the enzyme loading (*E*) for values of the incubation time *t* (h) shown alongside each curve. Error bars indicate 95% confidence intervals.

of *XY* = 0.31 tonne from one tonne of substrate, and leaving 0.72 tonne of the substrate as an insoluble residue. In other words, enzymatic hydrolysis under optimized conditions would leave more than 2 tonnes of residue, on a dry basis, for every tonne of glucose produced. It was assumed, in Section 2.1, that process heat gained from burning the residue would offset the costs of filtering and perhaps washing the residue. That assumption might not be valid at a high ratio of residue to glucose, so the absolute values of costs returned by the costing model should be regarded with caution.

# 3.2. Production costs

Inserting the optimized values  $E_{opt}$  and  $t_{opt}$  into Eq. (4), along with indicative unit costs from Table 1, led to a projected cost of US\$728/dry tonne of glucose produced from SEPW. The projected cost is based on the glucose content of an aqueous solution. Hemicelluloses accounted for a negligible portion of the SEPW, so glucose was expected to account for at least 99% of the sugars in the aqueous solution. Impurities such as furfural and phenolics substances might detract from the value of the glucose solution.

For comparison, the wholesale price of glucose syrup was US\$0.307/dry lb in 2011 (USDA, 2012), corresponding to US\$676/ dry tonne. Glucose syrup is a liquid hydrolysate made from starch, and the price is based on dry weight. Obviously lignocellulosic sugars cannot be regarded as a competitive fermentation feedstock unless the projected production cost can be lowered to a value far below the wholesale price for corn syrup.

Humbird et al. (2011) published a projected cost of US\$0.12/ dry lb for sugars in a dilute aqueous solution envisaged as a notional product of enzymatic hydrolysis of pretreated corn stover. That cost corresponds to US\$264/dry tonne. They used a technoeconomic model that was based on enzymatic hydrolysis of all pretreated biomass, not just the solid component, so glucose was expected to account for just 60% of the sugars in their notional solution. Xylose was expected to account for 33% of the sugars, so the options for bioethanol production would be restricted to those organisms that are capable of fermenting xylose. Most current bioethanol producers use brewers' yeast, which is incapable of fermenting xylose. This restriction would detract from the market value of the product.

Roche et al. (2009) reported experimental data for enzymatic hydrolysis of dilute-acid pretreated corn stover at initial reactor loadings of 20% solids, three different enzyme loadings and incubation times up to two weeks. The long incubation times were needed because of slow hydrolysis in high-solids processing. The substrate contained 59.1% glucan and 5.1% xylan, so the xylose would have been a minor component of the sugar solution. Eq. (7) was used to interpret the published data, leading to the best-fit substrate characteristics listed in Table 2. Optimization according to Eq. (10) led to an enzyme loading of 4.6 MFPU/ tonne and incubation time of 191 h. Inserting those values into Eq. (4), with indicative unit costs from Table 1, gave a projected production cost of US\$350/dry tonne of sugars. While the projected production cost was higher than that calculated by Humbird et al. (2011) for a similar substrate, the reason for the difference is clear. Humbird et al. (2011) assumed that a sugar yield of Y = 0.90 could be achieved for an incubation time of 84 h. Using the aspirational value of t = 84 h as input brought the projected production cost down to US\$309/dry tonne sugars. This is close to the result reported by Humbird et al. (2011), showing that the two costing models can give similar results for similar input. The relatively high projected cost for sugars from SEPW was therefore attributed to substrate recalcitrancy, and in particular the relatively high enzyme demand, rather than the use of a novel costing model.

Table 2		
Best-fit parameters	for	substrates.

Substrate <sup>a</sup>	Temperature (°C)	$\alpha^{\rm b}$	ε <sup>b</sup> (MFPU/ tonne)	τ <sup>b</sup> (h)	Reference
SEPW	50	0.84	7.1	7.3	This work
CS	48	0.90	0.8	300	Roche et al. (2009)
CS	50	1.00	2.3	34	Ouyang et al. (2011)
CS + PEG	50	1.00	1.4	49	Ouyang et al. (2011)
SPS	40	(1.00) <sup>c</sup>	10.4	22	Börjesson et al. (2007)
SPS + PEG	40	(1.00) <sup>c</sup>	5.2	36	Börjesson et al. (2007)
SPS	50	(1.00) <sup>c</sup>	16.4	5.2	Börjesson et al. (2007)
SPS + PEG	50	(1.00) <sup>c</sup>	5.7	14	Börjesson et al. (2007)

<sup>a</sup> SEPW = steam exploded pine, CS = pretreated corn stover, SPS = steam-pretreated spruce.

<sup>b</sup>  $\alpha$  = Accessibility parameter,  $\varepsilon$  = characteristic enzyme demand,  $\tau$  = characteristic incubation time.

<sup>c</sup> Assumed value.

# 3.3. Lowering lignocellulosic sugar costs

The comparison between SEPW and corn stover as substrates confirmed that enzyme demand is a major contribution to production costs, accounting for 54% of costs for SEPW and between 25% and 38% for corn stover incubated at optimized enzyme loadings. The comparison also drew attention to the large change in enzyme demand required to achieve a small change in the projected costs of lignocellulosic sugars. In the previous section, decreasing enzyme demand by a factor of nine resulted in a decrease in sugar cost by a factor of two. This point is illustrated in Fig. 4. The solid curve represents sugar costs calculated for a constant value of  $\tau$  = 7.3 h, as for SEPW, but the value of  $\varepsilon$  was allowed to vary. The curve describes a near-linear function.

It is possible to decrease enzyme demand by using additives, e.g., poly(ethylene glycol), surfactants, proteins or peptides (Sewalt et al., 1997; Kaar and Holtzapple, 1998; Yang and Wyman, 2006; Börjesson et al., 2007). The experiments described in the literature were designed to demonstrate increases in sugar yield, rather than decreases in enzyme demand, so most of the published results were not ideal as input for the model used in this work. A literature search for relevant parameters led to just seven data points added in Fig. 4. With one exception, the published results could not be used without making assumptions as discussed below.

The exception was an experiment reported by Ouyang et al. (2011). Pretreated corn stover solids were mixed with poly(ethylene glycol) (PEG) and incubated for six different times at five different enzyme loadings. The best-fit substrate characteristics are



**Fig. 4.** Sensitivity of sugar production costs to changes in enzyme demand. Solid line and solid circle: calculated for  $\tau$  = 7.3 as for SEPW. Open symbols: costs based on literature data interpreted as described in the text. The dotted line marks the wholesale price for glucose syrup, averaged over 2011 (USDA, 2012). SEPW = steam-exploded pine, SPS = steam-pretreated spruce, CS = pretreated corn stover.

listed in Table 2. Ouyang et al. (2011) published a smaller data collection for the same substrate without PEG. That collection was interpreted with the value of  $\alpha$  = 1.00 fixed as for the substrate with PEG, so that the value of *X* remained unchanged while  $\varepsilon$  and  $\tau$  were selected for the best fit (Table 2). Eq. (10) was used to optimize enzyme loadings and incubation times, and the two costs from Eq. (4) are shown connected by an arrow in Fig. 4.

Results reported by Börjesson et al. (2007) indicated an even more dramatic improvement when PEG was added to steampretreated spruce (SPS). Those results were therefore used as input, despite the fact that they were obtained for a single value of the enzyme loading and the conclusions are therefore less reliable than those based on the published data discussed above. The value of the adjustable parameter  $\alpha$  was arbitrarily fixed at unity since it could not be determined by fitting. Best-fit values of  $\varepsilon$  and  $\tau$  are listed in Table 2 for SPS with and without PEG, at two different incubation temperatures. Eq. (10) was used to optimize enzyme loadings and incubation times, and pairs of costs from Eq. (4) are shown connected by arrows in Fig. 4. The plotted points lie below the solid curve because of the optimistic choice of  $\alpha$  = 1.00, compared with the experimental best-fit value of  $\alpha$  = 0.84 for SEPW.

While the plotted points in Fig. 4 lie close to the solid curve, it is important to note that the cost of adding PEG to the substrate was neglected. The published results were obtained for PEG loadings of 39 kg/tonne substrate (Ouyang et al., 2011) and 50 kg/tonne substrate (Börjesson et al., 2007). Those loadings would add a significant increment on the unit cost  $U_s$ . Additives can be effective at lower loadings (Kristensen et al., 2007), so the costs of an effective loading might be small relative to other costs associated with the substrate. Estimating the costs associated with using additives is beyond the scope of the current work.

# 4. Conclusions

A mathematical model, based on a study of underlying enzyme kinetics, enables adjustment of processing conditions to minimize the cost of enzymatic hydrolysis of lignocellulosic feedstocks. The model can be used to assess ideas for improving the economic viability of production of lignocellulosic biofuels. Targeting lower enzyme demand is a high priority, since a large change in enzyme demand is required to achieve a relatively small change in production costs. Enzyme demand can be lowered by using stover instead of softwood feedstocks, or by using processing additives. The latter approach shows potential for improving profits from lignocellulosic biofuels.

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