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Unstructured Modeling of a Synthetic Microbial Consortium for Consolidated Production of Ethanol

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Abstract: A defined mixed culture of specialized microbes that exploits the native capabilities of each member species is a promising alternative to use of a single engineered microbe for cellulosic biofuels production. We explored such a synthetic consortium that couples the high cellulolytic activity of the filamentous fungus *Trichoderma reesei* with the ability of the yeasts *Saccharomyces cerevisiae* and *Scheffersomyces stipitis* to ferment hexose and pentose sugars to ethanol. Consortium stability was demonstrated by culturing the three microbes on a mixture of cellulose and xylan. As a first step towards understanding and manipulating this consortium, we developed a simple dynamic model with unstructured descriptions of enzyme synthesis, cellulose and hemicellulose degradation, sugar uptake, cell growth, and ethanol production. The batch culture model contained 10 ordinary differential equations with parameters obtained from the literature and experiment to the extent possible. The dynamic model was used to predict initial concentration of each cell type that maximized ethanol productivity for a fixed total inoculum concentration. The simulated ratio of cellulose to hemicellulose in the feedstock was varied to determine the effects on the optimal inoculum and ethanol productivity.

Keywords: dynamic modeling, microbial biotechnology, biofuels, bioreactors, process optimization

1. INTRODUCTION

The production of liquid fuels from biomass currently occurs in four major steps: pretreatment to make the feedstock more amenable to enzymatic degradation, hydrolysis of cellulose and hemicellulose to fermentable sugar monomers and oligomers, fermentation of simple sugars to fuels, and the recovery of the fuel from the reactor bulk. The high costs of both biomass pretreatment and purified cellulolytic enzymes are impediments to commercial development of this technology. Consolidated bioprocessing, which combines the saccharification and fermentation steps into one reactor, is a major goal of biofuels research (Lynd et al., 2005).

A majority of research efforts have focused on engineering multiple metabolic functionalities into a single organism. However, this approach often results in conversion inefficiencies due to bottlenecks in metabolic pathways and may place a heavy metabolic burden on the microbe. In recent years, research has increasingly focused on the use of defined microbial consortia for biotechnology applications (Brenner et al., 2008). Using consortia allows for the selection of microbes that are best suited for performing one step of a larger process and moves the engineering focus from introducing new functionalities to improving existing pathways. Other benefits of mixed culture systems include tunability and increased resistance to environmental stress.

The aerobic, filamentous fungi *Trichoderma reesei* is used commercially to produce purified cellulases for biotechnology applications. *T. reesei* has also been the subject of several efforts to model cell growth, enzyme synthesis, and biomass hydrolysis (Tholudur et al., 1999; Velkovska et al., 1997). However, this organism is unsuitable for ethanol production because it preferentially expresses the genes for acetate synthesis.

Saccharomyces cerevisiae is a robust, budding yeast that has been widely used for fermentation of refined corn starch to fuel ethanol. *T. reesei* has been successfully co-cultured with *S. cerevisiae* for the production of ethanol from cellulose (Hahn-Hägerdal and Häggström, 1985). However, *S. cerevisiae* is unable to utilize pentose sugars, such as xylose, that result from the hydrolysis of hemicellulose. Another species of yeast, *Scheffersomyces stipitis*, can natively ferment xylose to ethanol, but it retains a preference for glucose as a carbon source. *S. stipitis* is a Crabtree-negative yeast that produces ethanol under oxygen-limited culture conditions. *S. cerevisiae* and *S. stipitis* have been co-cultured for the production of ethanol from glucose and xylose mixtures (Delgenes et al., 1998; Taniguchi et al., 1997). In these studies, a respiratory-deficient strain of *S. cerevisiae* was used so the dissolved oxygen concentration could be more easily controlled at a level that was favorable for ethanol production by *S. stipitis*. Respiratory-deficient *S. cerevisiae* cannot utilize non-fermentable carbon sources, such as ethanol, once glucose has been exhausted (Goldring et al., 1971).

In this study, we propose a mixed-culture consisting of the cellulolytic fungus *T. reesei* and the two fermentative yeasts *S. cerevisiae* and *S. stipitis* such that the saccharification and fermentation steps are consolidated into a single reactor. A schematic diagram of the synthetic consortium is shown in Figure 1. The goal of the present study is three-fold: (1) experimentally demonstrate that the three-member consortium can be stably maintained when grown on the insoluble substrates cellulose and xylan; (2) develop and

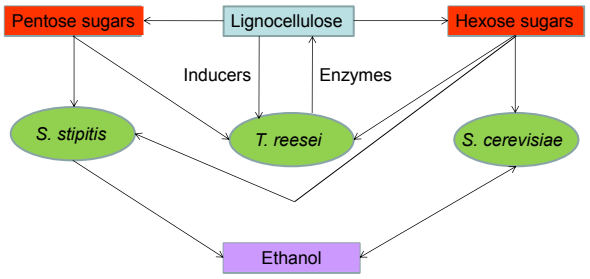

Fig. 1. Proposed synthetic consortium for consolidated production of ethanol.

parameterize an unstructured model of the consortium using previous studies and our preliminary experiments; and (3) utilize the model to predict optimal process strategies for ethanol production. This study represents a first step towards the development of predictive models for consolidated biofuels production with microbial consortia.

2. CONSORTIUM FEASIBILITY AND STABILITY

Feasibility of the consortium was evaluated using *S. cerevisiae* strain H1022 (ATCC 32167), *S. stipitis* NRRL Y-7124 (ATCC 58376), and *T. reesei* RUT-C30 (ATCC 56765). The consortium was cultured in a yeast synthetic medium supplemented with trace elements from *T. reesei* media. *S. cerevisiae* and *S. stipitis* were pre-cultured individually in shake flasks for 36 hours and *T. reesei* was precultured in a shake flask for 72 hours before inoculation into the reactor. Microcrystalline cellulose and xylan at concentrations of 16 g/L and 8 g/L, respectively, were used as carbon sources. Fermentations were conducted in an HEL BioX array of 4 parallel 250 mL stirred-tank bioreactors situated in a shared heating block (HEL Group Ltd., Barnet, UK). Bioreactor cultivations were performed at a temperature of 30°C and pH 5, the common optimal growth conditions for each organism. Each reactor was sparged at an air flow rate of 250 cc/min and stirred at 1000 RPM.

Analysis was performed by removing 2 mL samples from each reactor at least every 12 hours. Ethanol, glucose, and xylose concentrations were measured by two YSI 2700 SELECT biochemistry analyzers (YSI Inc., Yellow Springs, OH). Each sample was passed through a 40 µm cell strainer to separate *T. reesei* mycellium and insoluble substrates from the yeasts. The *T. reesei* concentration was determined by correlating dry cell weight of the retained solids to total cell protein concentration with a Bradford assay. The feedstock concentration was determined by subtracting the *T. reesei* concentration from the total solids concentration. Cell counts of *S. cerevisiae* and *S. stipitis* were performed on a hemacytometer in triplicate and averaged. The cell counts were converted to dry cell weight by drying counted samples.

Feasibility of the three-member consortium with wild-type *S. cerevisiae* was demonstrated through mixed-culture bioreactor experiments. Measured feedstock, microbe, and metabolite concentrations over the course of a 7 day fermentation are shown in Figure 2. The feedstock concentration decreased from its initial value of 24 g/L (16 g/L cellulose and 8 g/L xylan) due to the cellulytic activity of

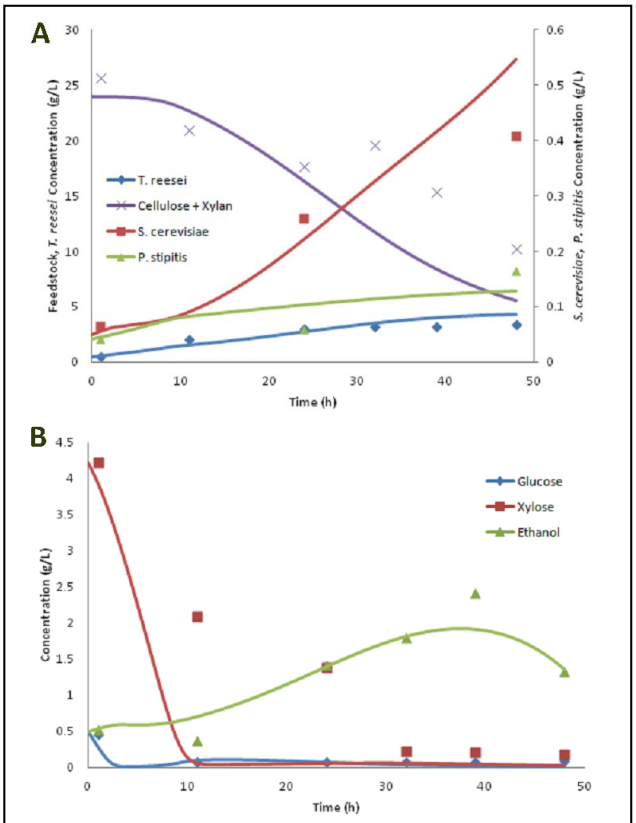

Fig. 2. Experimental data (points) and model predictions (lines) for consortium consisting of *T. reesei*, *S. cerevisiae*, and *S. stipitis* grown on 16 g/L cellulose and 8 g/L xylan.

T. reesei and degradation into fermentable sugars. Non-zero initial glucose, xylose, and ethanol concentrations were observed due to residual amounts present in the inocula. The glucose concentration rapidly dropped below 0.1 g/L, as the glucose produced from cellulose hydrolysis was quickly consumed by the three microbes. While xylose was initially present at much higher concentrations due to unconsumed sugar present in the *T. reesei* and *S. stipitis* inocula, the xylose concentration eventually dropped below 0.2 g/L due to consumption by *T. reesei* and *S. stipitis*. Increasing cell concentrations were observed for all three microbes, demonstrating that each microbe was actively participating in consortium metabolism despite substrate competition. The ethanol concentration increased due to synthesis by the two yeasts until approximately 48 hours, at which time the concentration dropped due to consumption by *S. cerevisiae* and possibly by *S. stipitis*. This undesirable behavior can be mitigated by the use of respiratory-deficient *S. cerevisiae*, which is incapable of growth on ethanol. To our knowledge, such a mutant does not exist for *S. stipitis*.

3. UNSTRUCTURED CONSORTIUM MODEL

The consortium model for conversion of cellulosic biomass to ethanol was developed by synthesizing elements of previously published models for each cell type. The unstructured model consisted of the following dynamic mass balances for batch fermentation:

$$\frac{dB_h}{dt} = -r_1 \quad (1)$$

$$\frac{dE_h}{dt} = \alpha_h X + \beta_h X - \gamma_h E_h \quad (2)$$

$$\frac{dX}{dt} = \mu_h X + \mu_p X - k_d X \quad (3)$$

$$\frac{dH}{dt} = r_1 - \mu_h \frac{X}{Y_{xh}} - \mu_{yh} \frac{Y}{Y_{yh}} - \mu_{zh} \frac{Z}{Y_{zh}} \quad (4)$$

$$\frac{dM}{dt} = \mu_h \frac{X}{Y_{xmh}} + \mu_p \frac{X}{Y_{xmp}} + \mu_{yh} \frac{Y}{Y_{ymh}} - \mu_{ym} \frac{Y}{Y_{ym}} + \mu_{zh} \frac{Z}{Y_{zmh}} + \mu_{zp} \frac{Z}{Y_{zmp}} \quad (5)$$

$$\frac{dB_p}{dt} = -r_2 \quad (6)$$

$$\frac{dEp}{dt} = \alpha_p X + \beta_p X - \gamma_p E_p \quad (7)$$

$$\frac{dP}{dt} = r_2 - \mu_p \frac{X}{Y_{xp}} - \mu_{zp} \frac{Z}{Y_{zp}} \quad (8)$$

$$\frac{dY}{dt} = \mu_{yh} Y + \mu_{ym} Y \quad (9)$$

$$\frac{dZ}{dt} = \mu_{zh} Z + \mu_{zp} Z \quad (10)$$

where B_h is the cellulose concentration, E_h is the cellulase concentration, X is the *T. reesei* biomass concentration, H is the glucose concentration, M is the ethanol concentration, B_p is the hemicellulose concentration, E_p is the hemicellulase concentration, P is the xylose concentration, Y is the *S. cerevisiae* biomass concentration, and Z is the *S. stipitis* biomass concentration. The feedstock was assumed to contain only cellulose (1) and hemicellulose (6), consisting of glucose and xylose monomers, respectively. *T. reesei* was assumed to produce only two cellulolytic enzymes, cellulase (2) and hemicellulase (7), with basal synthesis rates α_h and α_p and induced synthesis rates β_h and β_p. Enzyme degradation was assumed to follow first-order kinetics with cellulase and hemicellulase degradation rate constants γ_h and γ_p. The enzymes were assumed to hydrolyze the cellulose and hemicellulose polymers into glucose (4) and xylose (8) monomers, respectively. *T. reesei* (3) and *S. stipitis* (10) were modeled to grow on both glucose and pentose monomers, with *T. reesei* containing an additional term for cell death. *S. cerevisiae* (9) was allowed to grow on both glucose and ethanol, with ethanol consumption not possible for the respiratory-deficient mutant. Ethanol (5) was modeled to be produced only by *S. cerevisiae* and *S. stipitis*.

The cellulosic and hemicellulosic fractions were assumed to be independently degraded and to follow first-order enzyme kinetics coupled with a Langmuir adsorption isotherm (Kadam et al., 2004):

$$r_1 = \frac{k_1 E_{hm} K_{had} E_h \frac{{B_h}^3}{B_{h0}}}{1 + E_{hm} K_{had} B_h} \frac{1}{1 + \frac{H}{K_{i1h}} + \frac{P}{Ki1p}} \quad (11)$$

$$r_2 = \frac{k_2 E_{pm} K_{pad} E_p \frac{{B_p}^3}{B_{p0}}}{1 + E_{pm} K_{pad} B_p} \frac{1}{1 + \frac{H}{K_{i1h}} + \frac{P}{Ki2p}} \quad (12)$$

where r_1 and r_2 are the reaction rates of cellulose and hemicellulose hydrolysis, k_1 and k_2 are the reaction rate constants, E_{hm} and E_{pm} are the maximum concentrations of adsorbed enzymes, K_{had} and K_{pad} are dissociation constants, and B_{h0} and B_{p0} are initial feedstock concentrations. These rate equations were derived assuming that the reactions catalyzed by cellulase and hemicellulase were much slower than the hydrolysis of sugar oligomers to monomers by β-glucosidase and β-xylosidase. Therefore, the cellulolytic enzymes were assumed to convert the feedstock polymers directly into glucose and xylose monomers. Moreover, the cellulose and hemicellulose fractions were assumed to become increasingly recalcitrant to degradation. An inhibition term was included in each expression to model end product inhibition by glucose and xylose.

Induced enzyme expression by *T. reesei* followed previously published expressions for the synthesis of cellulase (Tholudur et al., 1999):

$$\beta_h = \frac{\beta_{hm} H}{K_{bh} + H} \frac{1}{1 + \frac{H}{K_{ibhh}}} \quad (13)$$

$$\beta_p = \frac{\beta_{pm} P}{K_{bp} + P} \frac{1}{1 + \frac{H}{K_{ibph}}} \frac{1}{1 + \frac{P}{K_{ibpp}}} \quad (14)$$

where β_h and β_p are the induced rates of cellulase and hemicellulase synthesis, β_{hm} and β_{pm} are the maximum rates of induced synthesis, and K_{bh} and K_{bp} are saturation constants of induction. Due to lack of data, the induced hemicellulase synthesis rate was assumed to follow the same saturation kinetics as cellulase. Inhibition terms were added to reflect the suppression of enzyme synthesis by sugars through end product inhibition.

The growth rates for the consortium microbes on each substrate were modeled as follows:

$$\mu_h = \frac{\mu_{hm} H}{K_{mh} + H} \frac{1}{1 + \frac{M}{K_{imhm}}} \quad (15)$$

$$\mu_p = \frac{\mu_{pm} P}{K_{mp} + P} \frac{1}{1 + \frac{H}{K_{imph}}} \frac{1}{1 + \frac{M}{K_{impm}}} \quad (16)$$

$$\mu_{yh} = \frac{\mu_{yhm} H}{K_{ymh} + H} \frac{1}{1 + \frac{M}{K_{imyhm}}} \quad (17)$$

$$\mu_{ym} = \frac{\mu_{ymm} M}{K_{ymm} + M} \frac{1}{1 + \frac{H}{K_{imymh}}} \quad (18)$$

$$\mu_{zh} = \frac{\mu_{zhm} H}{K_{zmh} + H} \frac{1}{1 + \frac{M}{K_{imzhm}}} \quad (19)$$

$$\mu_{zp} = \frac{\mu_{zpm} P}{K_{zmp}+P} \frac{1}{1+\frac{H}{K_{imzph}}} \frac{1}{1+\frac{M}{K_{imzpm}}} \quad (20)$$

where μ_h and μ_p are the growth rates of *T. reesei* on glucose and xylose, μ_{yh} and μ_{ym} are the growth rates of *S. cerevisiae* on glucose and ethanol, and μ_{zh} and μ_{zp} are the growth rates of *S. stipitis* on glucose and xylose. Cell growth was assumed to follow Monod kinetics. Because glucose is the preferred substrate for each microbe, growth on xylose was assumed to be repressed by the presence of glucose. These expressions were modified by the addition of terms that reflect the inhibitory effect of ethanol on cell growth. Because wild-type *S. cerevisiae* can grow aerobically on ethanol, this effect was included in the model for completeness even though respiratory-deficient *S. cerevisiae* cannot metabolize ethanol.

We are able to find values for 34 model parameters directly in the literature. Other parameter values were manually fit to experimental growth curves or simply specified. For example, 3 parameters were estimated from data available in the literature. An additional 6 parameters associated with the synthesis of hemicellulase and degradation of hemicellulose were simply chosen to be equal to the corresponding parameters for cellulase/cellulose. Parameter values for respiratory deficient *S. cerevisiae* growth were assumed to be equal to those of a respiratory competent cell growing under anaerobic conditions. For simplicity in this initial study, the remaining 10 parameters were adjusted by trial and error until the model predictions showed reasonable agreement with our data. Future work will involve more systematic parameter estimation using optimization methods.

The dynamic mass balances were solved in MATLAB (Mathworks, Natick, MA) with the ordinary differential equation solution code ode23. The batch time was defined as the time at which both cellulose and hemicellulose fell below 10% of their initial concentrations. The ethanol productivity was calculated by dividing the final ethanol concentration at by the batch time. The ethanol yield was calculated by dividing the final ethanol concentration by the total biomass consumed. The profit was calculated by subtracting the feedstock and pretreatment costs from the market value of the ethanol produced, and then dividing this value by the batch time to express a preference for shorter fermentations. Corn stover was used as a representative feedstock with a raw material cost of 83 $/Mg. The capitol cost required to perform AFEX pretreatment was estimated to be 18 $/ton and the market price for ethanol was taken as 2.657 $/gal.

4. MODELING RESULTS

4.1 Model Parameter Estimation

The experimental growth curves in Figure 2 were compared to a batch simulation of the consortium model using pure culture parameters extracted from the literature (not shown). The primary difference between the experimental and predicted results was the distribution of cell mass between the three microbes. A reported value for the yield of *T. reesei* on xylose (Tholudur et al., 1999) did not fit well with our data, so the yield coefficient was increased until better agreement was achieved. *T. reesei* still grew to a higher concentration than expected, indicating that the fungus was competitive for fermentable substrates with the two yeasts. Lower than expected cell mass concentrations of *S. cerevisiae* and *S. stipitis* could have been a result of this competition or the inhibitory effects of cellulase or acetate produced by *T. reesei*. In light of these observations, the model parameters were adjusted to better predict the behavior of the microbes in mixed culture. The more competitive nature of *T. reesei* for fermentable substrates was expressed by lowering the saturation constants for glucose and xylose uptake. Decreasing the yield coefficients of *S. cerevisiae* and *S. stipitis* resulted in improved agreement between experiment and model (Figure 2).

Consortium model predictions were further accessed by comparison to published growth curves for pure-culture and co-culture experiments involving the three microbes. An overlay of experimental and predicted batch culture results for *T. reesei* grown on a mixture of xylose and cellulose (Mohagheghi et al., 1988) is shown in Figure 3A. This comparison provided simultaneous verification of the model parameters involved in *T. reesei* metabolism of cellulose,

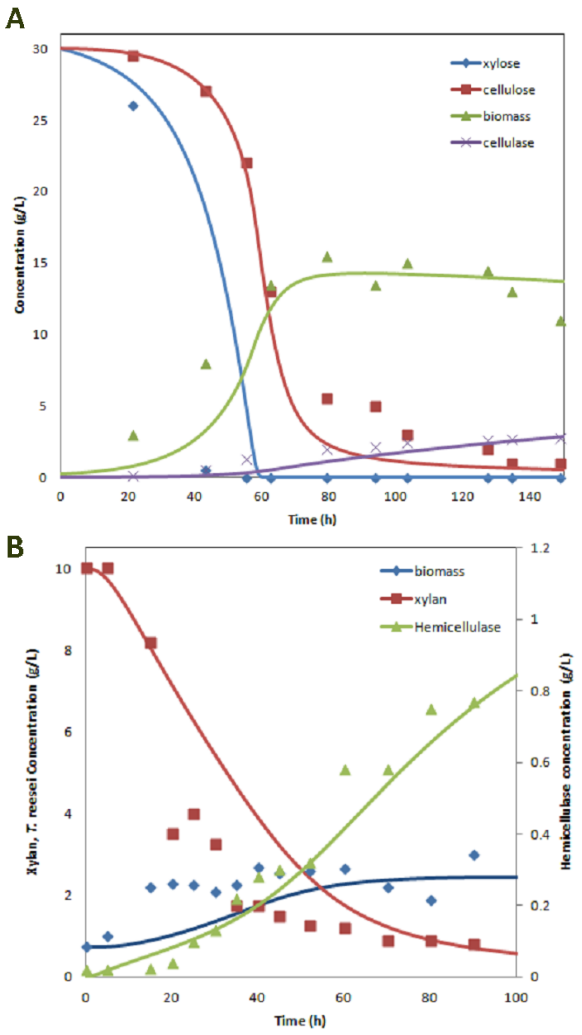

Fig. 3. Comparison of model predictions (solid lines) and experimental growth curves (data points) for (A) *T. reesei* growth on 30 g/L xylose and 30 g/L cellulose (Mohagheghi et al., 1988) and (B) *T. reesei* growth on 10 g/L xylan (Gamerith et al., 1992).

glucose, and xylose. Experimentally determined cellulase activity was converted to concentration using a conversion factor of 0.5 g/L/FPU. The model showed good agreement for cellulase synthesis, biomass degradation, and xylose consumption but cellulose was degraded at a faster rate than observed experimentally.

An overlay of experimental and predicted batch culture results for *T. reesei* grown on beechwood xylan (Gamerith et al., 1992) is shown in Figure 3B. In the original study, parameters for hemicellulase production and hydrolysis were fit using the experimental growth curves. We found that multiplying hemicellulase activity by conversion factor of 2 g/U resulted in improved model agreement. While the general trends of xylan, hemicellulase, and cell mass were captured by our model, the model predictions lagged behind experimental data for the first 40 hours. This discrepancy be attributed to the presence of residual xylose in the preculture medium that induced additional hemicellulase synthesis and produced unmodeled growth substrate.

The *S. cerevisiae* and *S. stipitis* models were assessed using co-culture data for growth on glucose and xylose (Taniguchi et al., 1997) shown in Figure 4. Because the biomass of each cell type was not measured, prediction accuracy with respect to the individual biomass concentrations could not be evaluated. The model qualitatively described the observed experimental rates of glucose and xylose consumption, biomass growth and ethanol production and satisfactorily predicted the final concentrations of biomass and ethanol. However, the dynamics associated with the diauxic shift from glucose to xylose as the growth substrate was not captured. Because the model was specifically designed to predict consortium dynamics with low concentrations of glucose and xylose, no attempt was made to model this effect. The results in Figures 2-4 suggest that the unstructured model provides reasonable predictions of consortium batch culture dynamics.

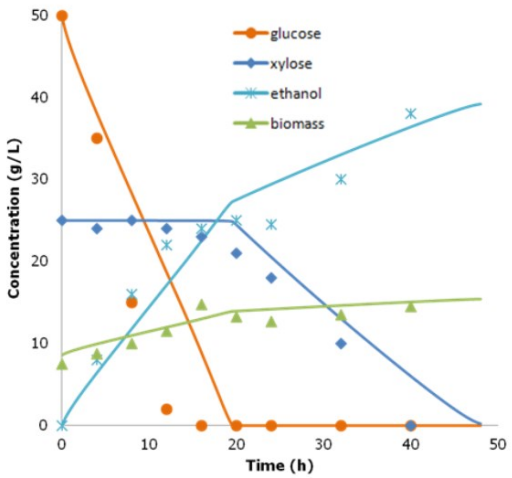

Fig. 4. Comparison of model predictions (solid lines) and experimental growth curves (data points) for respiratory-deficient *S. cerevisiae* and *S. stipitis* co-culture grown on 50 g/L glucose and 25 g/L xylose (Taniguchi et al., 1997).

4.2 In silico Inoculum Optimization

The initial feedstock concentration for the model consortium was chosen to be 75 g/L with cellulose and hemicellulose supplied at a 2:1 ratio. With the total inoculum concentration fixed at 6.0 g/L, we used the consortium model to predict the initial species concentrations that would maximize batch ethanol productivity. An equal inoculum with 2.0 g/L of each cell type was predicted to yield an ethanol productivity of 0.26 g/L/h. The initial cell concentrations were varied in increments of 0.1 g/L and batch simulations were performed until the inoculum with the highest ethanol productivity was found. Productivity increased with increasing *T. reesei* inoculum until a plateau was reached. At this point, increased enzyme concentration did not result in faster hydrolysis because of feedstock recalcitrance. The initial concentration of *T. reesei* was chosen to be 3.0 g/L, a value close to the start of the plateau. Therefore, the total yeast inoculum was chosen to be 3.0 g/L with varying amounts of *S. cerevisiae* and *S. stipitis* simulated to identify the optimal ratio.

The optimal inoculum of 2.3 g/L *S. cerevisiae* and 0.7 g/L *S. stipitis* yielded a productivity of 0.35 g/L/h, a 35% increase compared to the equal inoculum case. If *S. stipitis* produced ethanol strictly from xylose at a yield equal to that for *S. cerevisiae* to produce ethanol from glucose, we would expect the ratio of the two yeasts in the optimal inoculum to equal the ratio of cellulose to hemicellulose in the feedstock. Despite consuming both glucose and xylose, *S. stipitis* had lower ethanol yields that resulted in an optimal inoculum with a much larger concentration of *S. cerevisiae*. Predicted concentration profiles obtained with the optimal inoculum are shown in Figure 5. While requiring experimental validation, the predicted yield of 0.21 g ethanol/g feedstock compared favorably to yields obtained with other microbial systems grown on cellulosic feedstocks. The model predictions were

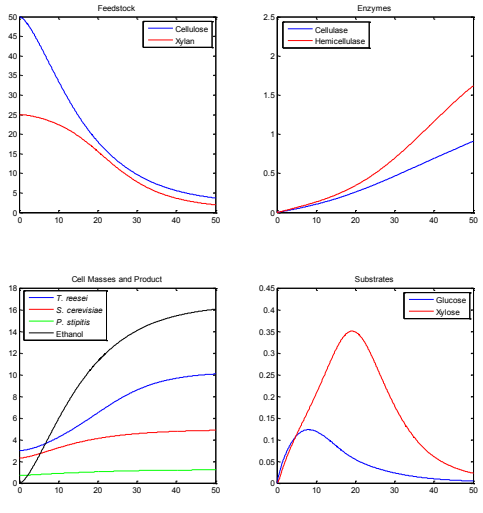

Fig. 5. Predicted concentration profiles for the synthetic consortium with an optimized inoculum of 3.0 g/L *T. reesei*, 2.3 g/L *S. cerevisiae*, and 0.7 g/L *S. stipitis* grown on 50 g/L cellulose and 25 g/L hemicellulose in batch culture.

difficult to compare to the experimental data in Figure 2 because the simulated inoculum concentration (6.0 g/L) was much greater than that used experimentally (1.0 g/L).

One benefit of mixed cultures compared to pure cultures is the ability to tune the inoculum of each cell type to the available carbon sources. The type of the lignocellulosic feedstock will dictate the amounts of cellulose and hemicellulose that are available for conversion. The effect of changing the feedstock composition was examined with the model by fixing the total feedstock concentration at 75 g/L and varying the amount of hemicellulose from 1 to 37 g/L (approximately 1-50% hemicellulose). For each feedstock ratio, the consortium was simulated for a inoculum composed of 3.0 g/L *T. reesei* and 3.0 g/L of the two yeasts. The amount of each yeast species was varied in increments of 0.1 g/L, and the inoculum that yielded the highest ethanol productivity for each feedstock ratio was used for analysis.

The impact of changing the feedstock composition on important consortium properties is shown in Figure 6. The optimal inoculum did not contain *S. stipitis* until there was at least 19 g/L hemicellulose in the feedstock. As the hemicellulose content increased beyond this point, the optimal yeast incoculum contained increasingly more *S. stipitis*. Because *S. cerevisiae* is more efficient at converting glucose to ethanol than *S. stipitis* is at converting xylose to ethanol, a cellulose-rich feedstock was predicted to produce more ethanol at a higher productivity. However, ethanol productivity reached a maximum of 0.41 g/L/h when the feedstock had a 67:8 ratio of cellulose to hemicellulose. For feedstocks with more cellulose, the high yield of ethanol from glucose was negated by the longer batch times that resulted from the recalcitrance of hemicellulose. A plot of profit per time illustrates that greater revenues were achieved with hemicellulose-poor feedstocks. For hemicellulose compositions below the profitability maximum, the profit curve follows the trend of ethanol productivity. For larger hemicellulose compositions, the profit has greater similarity to the ethanol titer curve. Using our definition of batch time that requires at least 90% consumption of hemicellulose,

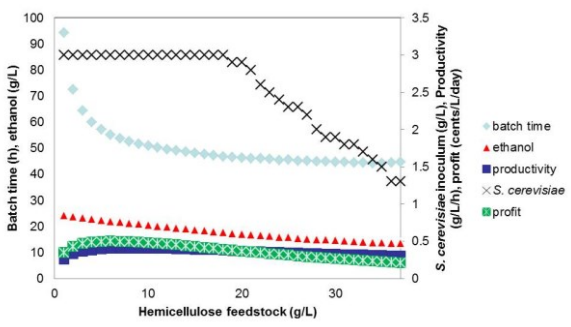

Fig. 6. Predicted effect of feedstock composition on the batch time, total ethanol produced, ethanol productivity, *S. cerevisiae* inoculum concentration, and profit of the synthetic consortium. The inoculum concentrations were optimized to produce the largest ethanol productivity for the given feedstock.

process economics were driven by ethanol productivity for hemicellulose-poor feedstocks and by ethanol titer for higher containing hemicellulose feedstocks.

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REFERENCES

Brenner, K.; You, L.; Arnold, F. H., Engineering microbial consortia: a new frontier in synthetic biology. *Trends in Biotechnology* **2008,** 26, (9), 483-489.

Delgenes, J. P.; Escare, M. C.; Laplace, J. M.; Moletta, R.; Navarro, J. M., Biological production of industrial chemicals, i.e. xylitol and ethanol, from lignocelluloses by controlled mixed culture systems. *Industrial Crops and Products* **1998,** 7, (2-3), 101-111.

Gamerith, G.; Groicher, R.; Zeilinger, S.; Herzog, P.; Kubicek, C. P., Cellulase-poor xylanases produced by Trichoderma reesei RUT C-30 on hemicellulose substrates. *Applied Microbiology and Biotechnology* **1992,** 38, (3), 315-322.

Goldring, E. S.; Grossman, L. I.; Marmur, J., Petite Mutation in Yeast. *J. Bacteriol.* **1971,** 107, (1), 377-381.

Hahn-Hägerdal, B.; Häggström, M., Production of ethanol from cellulose, Solka Floc BW 200, in a fedbatch mixed culture of *Trichoderma reesei* C 30, and *Saccharomyces cerevisiae*. *Applied Microbiology and Biotechnology* **1985,** 22, (3), 187-189.

Kadam, K. L.; Rydholm, E. C.; McMillan, J. D., Development and Validation of a Kinetic Model for Enzymatic Saccharification of Lignocellulosic Biomass. *Biotechnology Progress* **2004,** 20, (3), 698-705

Lynd, L. R.; Zyl, W. H. v.; McBride, J. E.; Laser, M., Consolidated bioprocessing of cellulosic biomass: an update. *Current Opinion in Biotechnology* **2005,** 16, (5), 577-583.

Mohagheghi, A.; Grohmann, K.; Wyman, C., Production of cellulase on mixtures of xylose and cellulose. *Applied Biochemistry and Biotechnology* **1988,** 17, (1), 263-277.

Taniguchi, M.; Tohma, T.; Itaya, T.; Fujii, M., Ethanol production from a mixture of glucose and xylose by co-culture of Pichia stipitis and a respiratory-deficient mutant of Saccharomyces cerevisiae. *Journal of Fermentation and Bioengineering* **1997,** 83, (4), 364-370.

Tholudur, A.; Ramirez, W. F.; McMillan, J. D., Mathematical modeling and optimization of cellulase protein production using Trichoderma reesei RL-P37. *Biotechnology and Bioengineering* **1999,** 66, (1), 1-16.

Velkovska, S.; Marten, M. R.; Ollis, D. F., Kinetic model for batch cellulase production by Trichoderma reesei RUT C30. *Journal of Biotechnology* **1997,** 54, (2), 83-94.