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Fermentation Kinetics of Ethanol Production from Glucose and Xylose by Recombinant *Saccharomyces* 1400(pLNH33)

Mahesh S. Krishnan,* Nancy W. Y. Ho, and George T. Tsao

Laboratory of Renewable Resources Engineering, Purdue University, West Lafayette, IN 47907, E-mail: Krishnanms@ornl.gov

Abstract

Fermentation kinetics of ethanol production from glucose, xylose, and their mixtures using a recombinant *Saccharomyces* 1400(pLNH33) are reported. Single-substrate kinetics indicate that the specific growth rate of the yeast and the specific ethanol productivity on glucose as the substrate was greater than on xylose as a substrate. Ethanol yields from glucose and xylose fermentation were typically 95 and 80% of the theoretical yield, respectively. The effect of ethanol inhibition is more pronounced for xylose fermentation than for glucose fermentation. Studies on glucose-xylose mixtures indicate that the recombinant yeast co-ferments glucose and xylose. Fermentation of a 52.8 g/L glucose and 56.3 g/L xylose mixture gave an ethanol concentration of 47.9 g/L after 36 h. Based on a theoretical yield of 0.51 g ethanol/g sugars, the ethanol yield from this experiment (for data up to 24 h) was calculated to be 0.46 g ethanol/g sugar or 90% of the theoretical yield. The specific growth rate of the yeast on glucose-xylose mixtures was found to lie between the specific growth rate on glucose and the specific growth rate on xylose. Kinetic studies were used to develop a fermentation model incorporating the effects of substrate inhibition, product inhibition, and inoculum size. Good agreements were obtained between model predictions and experimental data from batch fermentation of glucose, xylose, and their mixtures.

Nomenclature: K_s, Monod constant, for growth on glucose or xylose (g/L); K_s', Monod constant, for product formation from glucose or xylose (g/L); K_i, Inhibition constant, for growth on glucose or xylose (g/L); K_i', Inhibition constant, for product formation from glucose or xylose (g/L); m, maintenance coefficient (/h); P, ethanol concentration (g/L); P_m, ethanol concentration above which cells do not grow (g/L), P_m', ethanol concentration above which cells do not produce ethanol (g/L); S, substrate concentration (g/L);

*Author to whom all correspondence and reprint requests should be addressed.

X, cell dry weight (g/L); $Y_{P/S}$, product yield constant (g-product/g-substrate); $Y_{X/glu}$, cell yield constant from glucose (g-cells/g-substrate); $Y_{X/xyl}$, cell yield constant from xylose (g-cells/g-substrate).

Greek Letters: μ, specific growth rate (/h); ν, specific rate of product formation (/h); μ_m, maximum specific growth rate (/h); ν_m, maximum specific rate of product formation (/h); μ_0, specific growth rate when no initial ethanol is present (/h); ν_0, specific rate of product formation when no initial ethanol is present (/h); β,γ, constants in product inhibition model (dimensionless).

Subscripts: *g*, glucose; *x*, xylose.

Index Entries: Recombinant *Saccharomyces* 1400(pLNH33); ethanol; xylose fermentation; kinetic model.

Introduction

Ethanol from renewable resources has received considerable attention over the years as a transportation fuel. The economics of fuel-ethanol production are significantly influenced by the cost of raw materials used in the production process. Lignocellulosic biomass, such as agricultural and forestry residues, has been identified as a potential feedstock in view of its ready availability and low cost *(1)*. Lignocellulosic hydrolyzates produced either chemically or enzymatically contain both pentoses and hexoses. The pentoses are comprised of D-xylose and L-arabinose whereas the major hexose is D-glucose *(2)*. *Saccharomyces* yeasts have been traditionally used in the ethanol fermentation industry and readily use glucose as a carbon source. However, these yeasts are unable to ferment xylose that is the major component of the pentose fraction. Since pentoses can account for 8–28% of the raw material *(3)*, it is necessary to convert xylose to ethanol as well for improving the process economics.

In recent years, advances in genetic engineering have led to the construction of xylose-fermenting microorganisms. Recombinant bacterial strains of *Escherichia coli (4–6)* and *Klebsiella oxytoca (7,8)* were developed first, followed by *Zymomonas mobilis (9)*. In addition, several species of naturally occurring yeasts such as *Pichia stipitis* and *Candida shehatae* are also able to ferment xylose to ethanol. However, the use of *Saccharomyces* yeasts is highly favored in commercial biomass to ethanol conversion processes owing to their traditional use in ethanol production, their tolerance to ethanol and other inhibitors, GRAS (Generally Regarded As Safe) status, and their use as nutrient enhancers in animal feeds. Attempts have been made to develop recombinant *Saccharomyces* yeasts that can ferment xylose *(10,11)*. These yeasts exhibit low ethanol yields and productivities and therefore are not effective xylose fermentors. Recently, Ho et al. *(12)* reported the development of much more effective recombinant *Saccharomyces* yeasts such as 1400(pLNH33) that can efficiently coferment glucose and xylose present in the same medium simultaneously to ethanol. These recombinant yeasts were developed by transforming high ethanol-tolerant *Saccharomyces* yeasts such as *Saccharomyces* sp. strain 1400 *(13)*, with a group of 2μ-based high

copy-number plasmids, collectively designated the pLNH plasmids. These group of plasmids contain the three xylose metabolizing genes i.e., xylose reductase gene (XR), xylitol dehydrogenase gene (XD), and xylulokinase gene (XK). Because the recombinant *Saccharomyces* 1400 yeasts, such as 1400(pLNH33) are plasmid-bearing transformants, they are not stable and lose their xylose-fermenting ability after being cultured in non-selective medium for numerous generations (for example, more than 10 generations). However, this problem has been overcome by the development of a stable recombinant *Saccharomyces* strain 1400-LNH-ST *(14,15)*, that contains multiple copies of the xylose metabolism genes integrated into the chromosome of the host strain 1400.

In this paper, the kinetic studies of ethanol production from glucose, xylose, and their mixtures using strain 1400(pLNH33) are reported. These results were used to develop a fermentation model that incorporates the effects of substrate inhibition, product inhibition, and inoculum size.

Materials and Methods

Microorganisms

Saccharomyces yeasts 1400 and genetically engineered 1400(pLNH33) were used in the experimental work. The yeast 1400 (pLNH33) was obtained from Dr. Nancy Ho at the Laboratory of Renewable Resources Engineering, Purdue University.

The yeast strain 1400 *(13)* is a protoplast fusion product of *Saccharomyces diastaticus* and *Saccharomyces uvarum.*

Culture Conditions

The recombinant yeast 1400 (pLNH33) was maintained on YEPX seed cultures. The composition of the seed culture media/L of distilled water is as follows: 20 g D-xylose, 10 g yeast extract, 20 g Bactopeptone. The yeast was grown to an optical density (OD) of 400–450 Klett Unit (measured by a Klett-Summerson colorimeter) and then maintained at 4°C. The medium for the preparation of the inoculum was the YEPX medium previously described. One mL of the seed culture was added to a sterilized 250 mL Erlenmeyer flask with silicone sponge closure, containing 50 mL of medium. The inoculum was incubated at 30°C in a floor shaker at 150–200 rpm for 18–20 h (when the cells were in the late exponential phase) before being used to inoculate the fermentation medium.

The parent yeast strain 1400 was maintained on YEPD agar plates. The composition of the plate media per liter of distilled water is as follows: 20 g glucose, 10 g yeast extract, 20 g Bactopeptone, and 20 g agar. The yeast was inoculated on the plate medium at 30°C for 48 h and then maintained at 4°C. For the preparation of the inoculum, yeast extract-Bactopeptone medium with 20 g/L glucose was used. A loopful of yeast cells were transferred from the agar plate into 50 mL sterilized medium in a 250-mL Erlenmeyer flask.

Fermentation Conditions

The fermentation was performed in 250-mL Erlenmeyer flasks with silicone sponge closures, containing 100 mL sterilized medium. The fermentation medium consisted of 20 g/L Bactopeptone, 10 g/L yeast extract, and appropriate concentrations of glucose and/or xylose. The inoculum sizes used were in the range of 0.1–2.5 g/L. The fermentation conditions were same as those indicated earlier for the inoculum preparation.

Analytical Methods

Cell dry weight was determined by using a spectrophotometer (Coleman model 55, Perkin-Elmer, Maywood, IL) that measured the absorbance of the samples at a wavelength of 600 nm. Samples were diluted as required to assure absorbances of less than 0.5. In this region, the calibration curve was linear with a slope of 0.65 g dry weight/U absorbance.

High-performance liquid chromatography (HPLC) (Hitachi Ltd., Tokyo, Japan) with refractive index (RI) detector was used to analyze the concentrations of glucose, xylose, xylitol, ethanol and glycerol. A Bio-Rad (Hercules, CA) HPX-87H Ion-Exclusion column was used. The mobile phase was 0.005 *M* H_2SO_4 at a flow rate of 0.4 mL/min. YSI 2700 Select Biochemistry Analyzer (YSI Inc., Yellow Springs, OH) equipped with glucose membrane was used for rapid analysis of glucose concentration in the fermentation media.

Model Development

Substrate Inhibition Kinetics

Models for substrate inhibition kinetics are typically modified Monod form expressions *(16)*. In this work, the following expressions are used to account for the effect of substrate inhibition on cell growth and ethanol production using glucose and xylose as substrates:

$$\mu = \frac{1}{X}\frac{dX}{dt} = \frac{\mu_m S}{K_s + S + S^2/K_i} \tag{1}$$

$$\nu = \frac{1}{X}\frac{dP}{dt} = \frac{\nu_m S}{K_s' + S + S^2/K_i'} \tag{2}$$

Product Inhibition Kinetics

A number of correlations have been proposed in the literature for modeling the inhibitory effects of ethanol on cell growth and fermentation. These include exponential, linear, hyperbolic, parabolic, and nonlinear models *(17)*. In this work, a two-constant model *(18)* is used to describe the

kinetic pattern of ethanol inhibition on glucose and xylose fermentation. The model consists of the following expressions:

$$\frac{\mu}{\mu_0} = 1 - \left(\frac{P}{P_m}\right)^{\beta} \tag{3}$$

$$\frac{\nu}{\nu_0} = 1 - \left(\frac{P}{P'_m}\right)^{\gamma} \tag{4}$$

The magnitude of the constant β indicates the relationship between μ and *P*, whereas the magnitude of the constant γ indicates the relationship between ν and *P*.

Model Incorporating Substrate and Product Inhibition Kinetics

$$\mu = \frac{1}{X}\frac{dX}{dt} = \frac{\mu_m S}{K_s + S + S^2 / K_i}\left\{1 - \left(\frac{P}{P_m}\right)^{\beta}\right\} \tag{5}$$

$$\nu = \frac{1}{X}\frac{dP}{dt} = \frac{\nu_m S}{K_s' + S + S^2 / K_i'}\left\{1 - \left(\frac{P}{P_m'}\right)^{\gamma}\right\} \tag{6}$$

$$-\frac{dS}{dt} = \frac{1}{Y_{P/S}}\frac{dP}{dt} = \frac{1}{Y_{X/S}}\frac{dX}{dt} + mX \tag{7}$$

The above model equations are applicable for both glucose (using subscript *g*) and xylose (using subscript *x*) as substrates. The model parameters in the substrate and product inhibition expressions were determined from single substrate experiments.

Inoculum Size Effects

In the range of 0.1–5 g/L, the inoculum size was found to have a significant effect on the model parameters μ_m and ν_m for both glucose and xylose fermentation. Because these are the key model parameters, it was necessary to account for the inoculum size effect on these parameters in the fermentation model. Empirical equations were used and are given below:

Glucose fermentation: $\mu_{m,g} = 0.152 * X^{-0.461}$ $\nu_{m,g} = 1.887 * X^{-0.434}$ (8)

Xylose fermentation: $\mu_{m,x} = 0.075 * X^{-0.438}$ $\nu_{m,x} = 0.16 * X^{-0.233}$ (9)

Cell Growth on Glucose and Xylose Mixtures

The model proposed for cell growth and fermentation on sugar mixtures is:

$$\mu_{\text{mixture}} = \frac{[\text{Glucose}]}{[\text{Glucose}]+[\text{Xylose}]}\mu_{\text{glucose}} + \frac{[\text{Xylose}]}{[\text{Glucose}]+[\text{Xylose}]}\mu_{\text{xylose}} \quad (10)$$

$$\frac{dP}{dt} = (\nu_{\text{glucose}} + \nu_{\text{xylose}})\, X \quad (11)$$

where $\mu_{glucose}$ and μ_{xylose} represent the specific growth rates on glucose and xylose respectively, and $\nu_{glucose}$ and ν_{xylose} represent the specific productivities on glucose and xylose, respectively. Equations 10 and 11 along with equations 5, 6, 7, 8, and 9 extend the model to take into account mixed glucose and xylose fermentation.

Parameter Estimation and Solution of Model Equations

The parameters in the model were evaluated by using a dynamic simulation package SPEEDUP (licensed by Aspen Tech, Inc., Cambridge, MA). Its parameter estimation feature seeks to minimize the residual sum of squares between the model predicted values and the experimental values. After estimation of the parameters, the model equations were solved by SPEEDUP to generate time profiles of the cell density, substrate (glucose, xylose), and ethanol concentrations.

Results

Kinetics of Glucose and Xylose Fermentation

Previous studies *(19)* have indicated that the fermentation performance of the parent *Saccharomyces* yeast 1400 and the recombinant *Saccharomyces* yeast 1400(pLNH33) are identical when glucose is used as the carbon source. Typical ethanol yields were in the range of 0.46–0.48 g ethanol/g glucose, that corresponds to 90–94% of the theoretical yield (0.51 g ethanol/g glucose). When xylose is used as the carbon source, the parent yeast is unable to grow and ferment xylose to ethanol. On the other hand, the recombinant yeast effectively ferments xylose and gives ethanol yields of 0.40 g ethanol/g xylose, that corresponds to 78.4% of the theoretical yield (0.51 g ethanol/g xylose).

The kinetics of glucose fermentation using the parent *Saccharomyces* yeast 1400 have been reported *(20)*. These data were used to model the glucose fermentation by recombinant yeast 1400(pLNH33) as well, because both the yeasts show identical growth and fermentation performance when glucose is the substrate. Figure 1 shows the substrate, product, and cell-density profiles during batch fermentation for various initial glucose concentrations. For glucose fermentation, significant lag times are observed in experiments with an initial concentration greater than 200 g/L. This can be

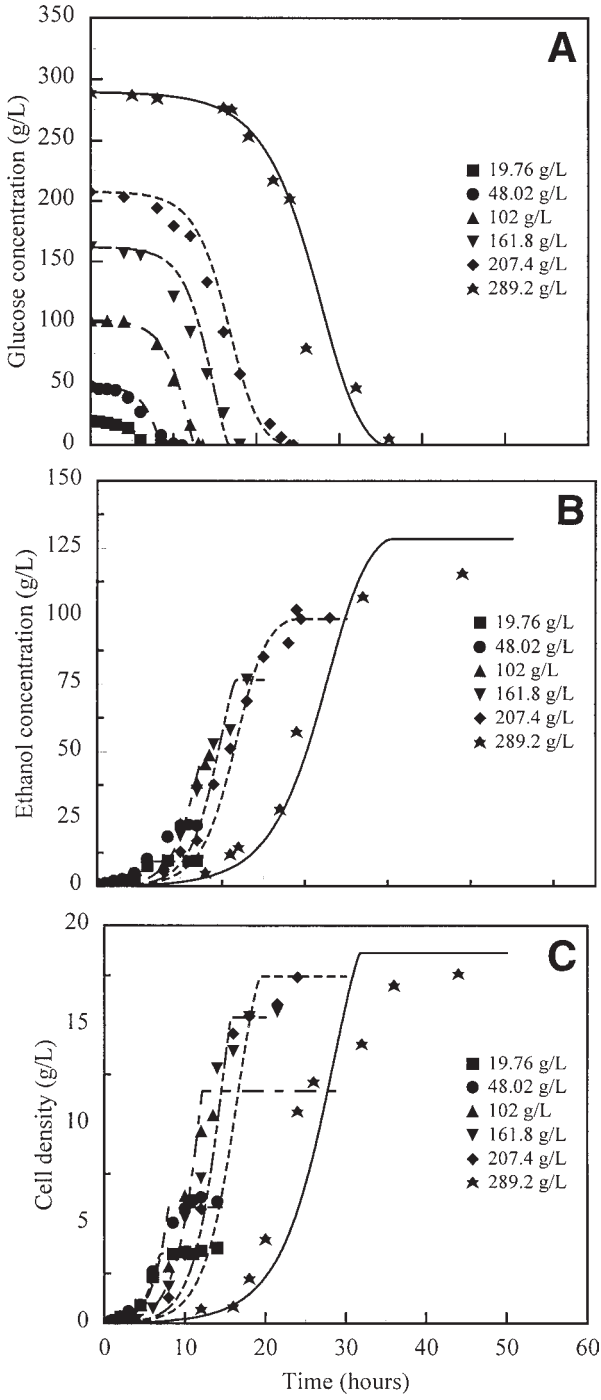

Fig. 1. Experimental and model profiles of **(A)** glucose utilization, **(B)** ethanol production, and **(C)** cell density at various initial glucose concentrations using *Saccharomyces* strain 1400. Lines represent model predictions and symbols represent experimental data. (- - -, 19.76 g/L; – – –, 48.02 g/L; – – -, 102 g/L; – - – 161.8 g/L; - - -, 207.4 g/L; —, 289.2 g/L).

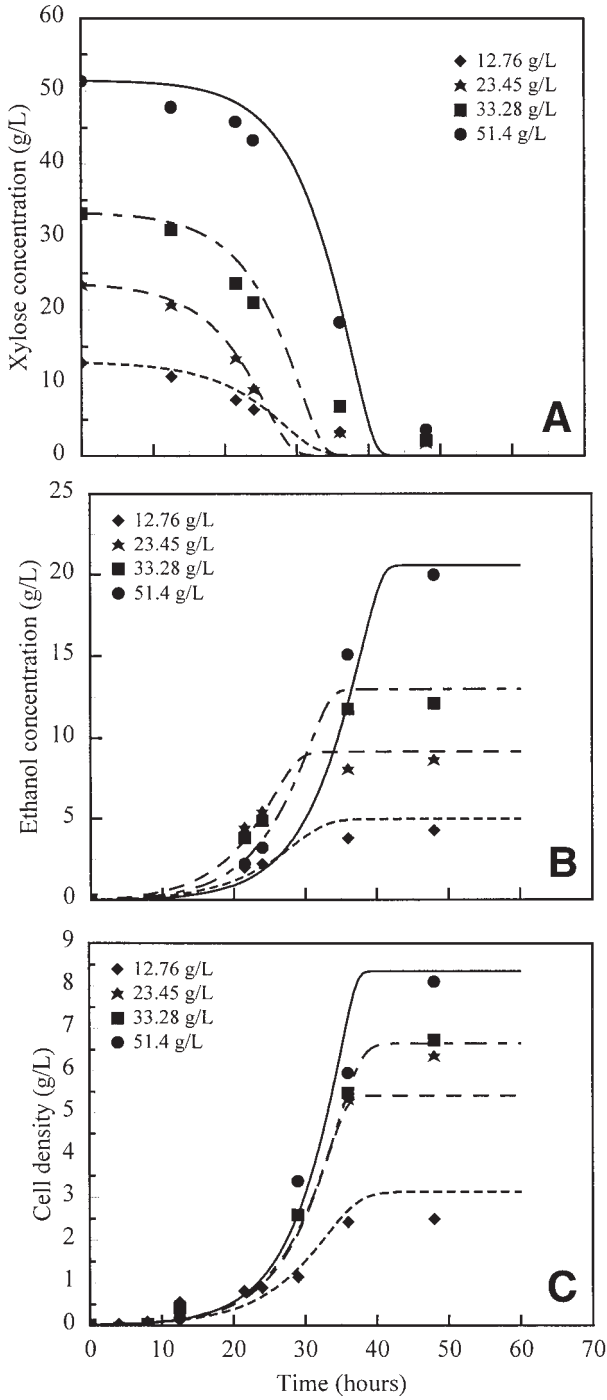

Fig. 2. Experimental and model profiles of **(A)** xylose utilization, **(B)** ethanol production, and **(C)** cell density at various initial xylose concentrations using recombinant *Saccharomyces* strain 1400(pLNH33). Lines represent model predictions and symbols represent experimental data. (- - - -, 12.76 g/L; – – – –, 23.45 g/L; – – – -, 33.28 g/L; —, 51.4 g/L).

Table 1
Estimated Fermentation Model Parameter Values

Parameter	Glucose fermentation	Xylose fermentation
μ_m (/h)	0.662	0.190
ν_m (/h)	2.005	0.250
K_s' (g/L)	0.565	3.400
K_s' (g/L)	1.342	3.400
K_i (g/L)	283.700	18.100
K_i' (g/L)	4890.000	81.300
P_m (g/L)	95.40 for $p \leq 95.4$ g/L	
	129.90 for $95.4 < p \leq 129.9$ g/L	59.040
P_m' (g/L)	103.00 for $p \leq 103$ g/L	
	136.40 for $103 < p \leq 136.4$ g/L	60.200
β	1.29 for $p \leq 95.4$ g/L	
	0.25 for $95.4 < p \leq 129.9$ g/L	1.036
γ	1.42 for $p \leq 95.4$ g/L	0.608
m (/h)	0.097	0.067
$Y_{P/S}$ (g/g)	0.470	0.400
$Y_{X/S}$ (g/g)	0.115	0.162

attributed to the effect of glucose inhibition on the yeast growth, as all other conditions such as temperature and nutrients are favorable. Figure 2 shows the corresponding results of batch xylose fermentation using recombinant *Saccharomyces* 1400(pLNH33) for various initial xylose concentrations. In the case of xylose fermentation, no substrate inhibition is observed till around 50 g/L but becomes significant above 70 g/L (data not shown). The data shown in Figs. 1 and 2 were used to estimate the parameters μ_m, ν_m, K_s, K_s', K_i, K_i' (Table 1) of the modified Monod model for glucose and xylose fermentation. The pronounced substrate inhibition effect for xylose fermentation is reflected by the relatively low magnitudes of K_i and K_i' compared to those for glucose fermentation. It should be noted that a low initial inoculum of 0.1 g dry weight/L cells were used in the above kinetic experiments. The substrate utilization rates can be improved by starting with a high inoculum.

In order to determine the effect of ethanol on the specific growth rates and the specific ethanol productivities, experiments with a range of initial ethanol concentrations in the fermentation media were performed. The initial sugar concentration in these experiments was 50 g/L, which is well in excess of the saturation constant. This sugar concentration was selected in order to separate ethanol inhibition effects from those of substrate or nutrient limitation. The effects of ethanol concentration as a single independent variable can be clearly discerned using this method as compared to batch studies with produced ethanol.

The experimental data of variation in specific growth rate of *Saccharomyces* 1400 and specific ethanol productivity on glucose as a function of the initial ethanol concentration are shown in Table 2. These data were fitted to

Table 2
Effect of Initial Ethanol Concentration on Specific Growth Rate and Specific Production Rate of *Saccharomyces* Strain 1400 (on Glucose)

μ/μ_0	ν/ν_0	P (g/L)
1.000	1.000	0.00
0.906	0.940	14.87
0.787	0.807	31.18
0.637	0.714	46.51
0.269	0.474	62.30
0.214	0.232	85.52
0.053	–	98.40
0.018	–	121.20
0.011	–	136.40

Luong's model (Fig. 3A). When there is no initial ethanol in the medium, the highest specific growth rate (μ_0) and specific ethanol-production rate (ν_0) are 0.6/h and 2.1/h, respectively. With increasing ethanol concentration, the magnitudes of μ and ν decline. Beyond an ethanol concentration of 100 g/L, there is a pronounced inhibition effect. Experimental data for recombinant *Saccharomyces* 1400(pLNH33) using xylose as the fermentation substrate are shown in Table 3. These data were also fitted to Luong's model (Fig. 3B). The corresponding values of μ_0 and ν_0 for xylose as the substrate are 0.19/h and 0.18/h, respectively. The data show that the effect of ethanol inhibition on μ and ν for xylose fermentation is stronger relative to glucose. This indicates that the ethanol inhibition on the specific growth rate and specific production rate is dependent on the substrate used in the fermentation medium. While performing experiments with added ethanol greater than 100 g/L, there was significant fluctuation in the experimental data. This may have been caused owing to evaporation of ethanol from the shake flasks. Consequently the model parameters for the specific production rate could not be accurately determined beyond an added initial ethanol concentration of 103 g/L. The estimated values of P_m, P_m', β, and γ for glucose and xylose fermentation are tabulated in Table 1. Again, a low initial inoculum of 0.1 g dry weight/L cells was used in the aforementioned experiments.

In both glucose and xylose fermentation, inoculum size was found to have a significant effect on the key model parameters μ_m and ν_m as shown in Fig. 4. Empirical Eqs. 8 and 9 were used to take this into consideration.

Kinetics of Glucose-Xylose Mixture Fermentation

The growth behavior of recombinant *Saccharomyces* 1400(pLNH33) was studied on glucose-xylose mixtures whose compositions are given in Table 4. The cell-growth profiles (during the time period when both glu-

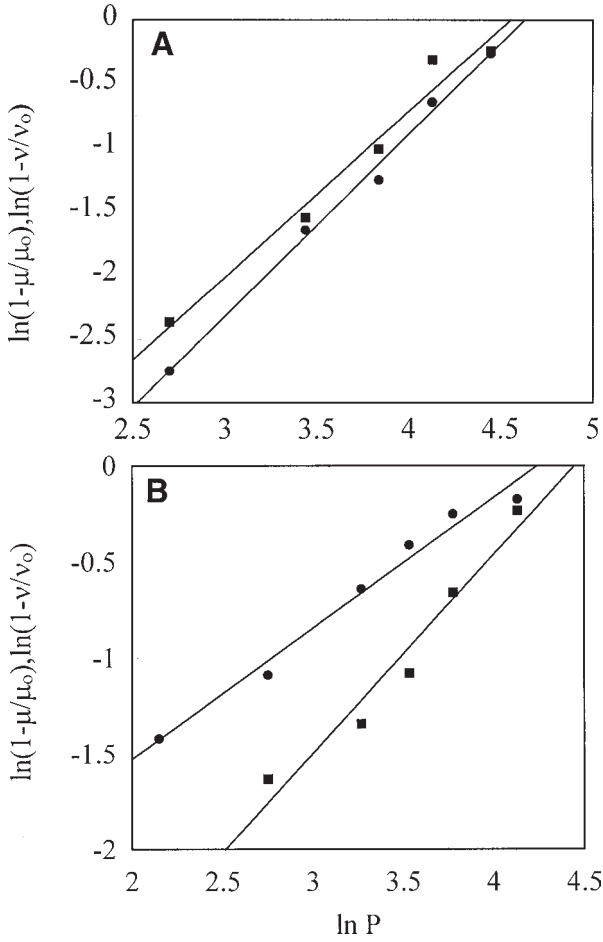

Fig. 3. Plot of ln (1-μ/μ_0) (■) and ln (1-ν/ν_0) (●) vs ln P for data of ethanol inhibition on: **(A)** glucose fermentation by *Saccharomyces* strain 1400 and **(B)** xylose fermentation by recombinant *Saccharomyces* strain 1400(pLNH33).

Table 3
Effect of Initial Ethanol Concentration on Specific Growth Rate and Specific Production Rate of Recombinant *Saccharomyces* Strain 1400(pLNH33) (on Xylose)

μ/μ_0	ν/ν_0	*P* (g/L)
1.000	1.000	0.00
0.989	0.758	8.58
0.804	0.663	15.69
0.739	0.472	26.34
0.660	0.337	34.33
0.482	0.219	43.79
0.205	0.157	62.49

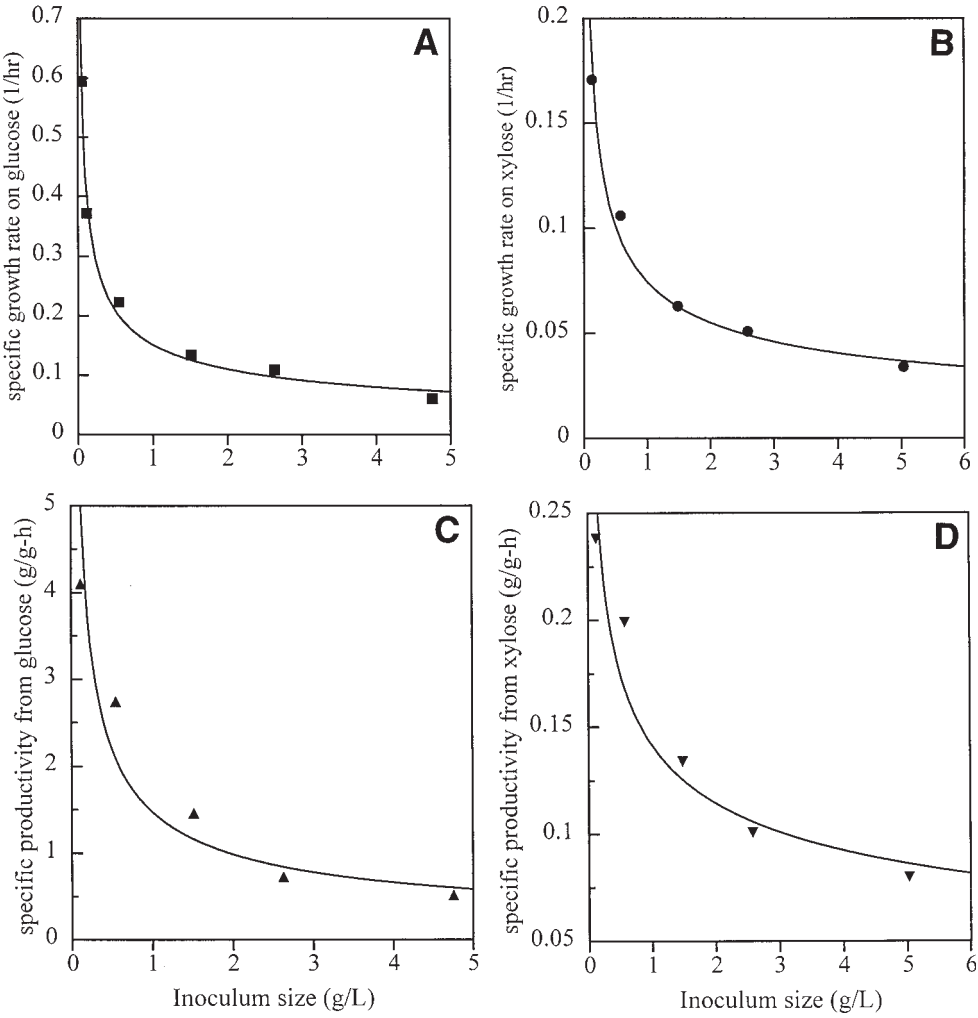

Fig. 4. Effect of inoculum size on specific growth rate and specific ethanol productivity of recombinant *Saccharomyces* 1400(pLNH33) on **(A)** and **(B)** glucose and **(C)** and **(D)** xylose as substrate.

Table 4
Composition of Glucose-Xylose Mixtures
Used for Growth Studies
with *Saccharomyces* 1400(pLNH33)

Mixture	Glucose (g/L)	Xylose (g/L)
1	59.3	0.0
2	44.6	16.4
3	30.1	30.5
4	15.4	43.2
5	0.0	53.8

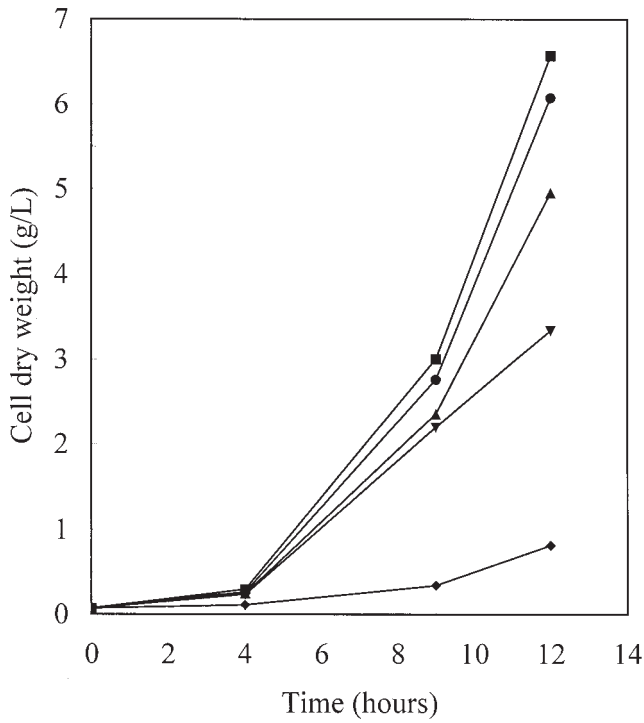

Fig. 5. Cell-growth profiles of recombinant *Saccharomyces* strain 1400(pLNH33) during fermentation of glucose-xylose mixtures. —■—, mixture 1; —●—, mixture 2; —▲—, mixture 3; —▼—, mixture 4; —◆—, mixture 5.

cose and xylose are present in the medium) are shown in Fig. 5. These results indicate that the growth rates of the yeast on Mixtures 2, 3, and 4 lie between two bounds: the upper bound (Mixture 1) representing the growth rate on glucose, and the lower bound (Mixture 5) representing the growth rate on xylose. Thus, mixtures containing a greater proportion of glucose support a higher growth rate than mixtures containing a greater proportion of xylose.

Typically, both glucose and xylose are present in lignocellulosic hydrolyzates. This requires that the fermentative microorganism be able to ferment xylose in presence of glucose. The recombinant yeast 1400 (pLNH33) has been genetically designed to ferment both glucose and xylose present in the same medium. To demonstrate this, fermentation of glucose-xylose mixtures (in 1:1 and 1:2 ratio) using this yeast were performed. Figure 6A shows the fermentation of a sugar mixture having a composition of 52.8 g/L glucose and 56.3 g/L xylose with a higher initial cell density of 2.3 g/L. Clearly, the simultaneous fermentation pattern of glucose and xylose is exhibited. The glucose utilization rate at 8.6 g/L-h is higher than the xylose utilization rate at 1.94 g/L-h. Ethanol concentrations of 44.3 and 47.9 g/L were achieved after 24 and 36 h, respectively. The final cell density was 11.5 g/L. Experimental data up to 24 h were used to calculate the yield,

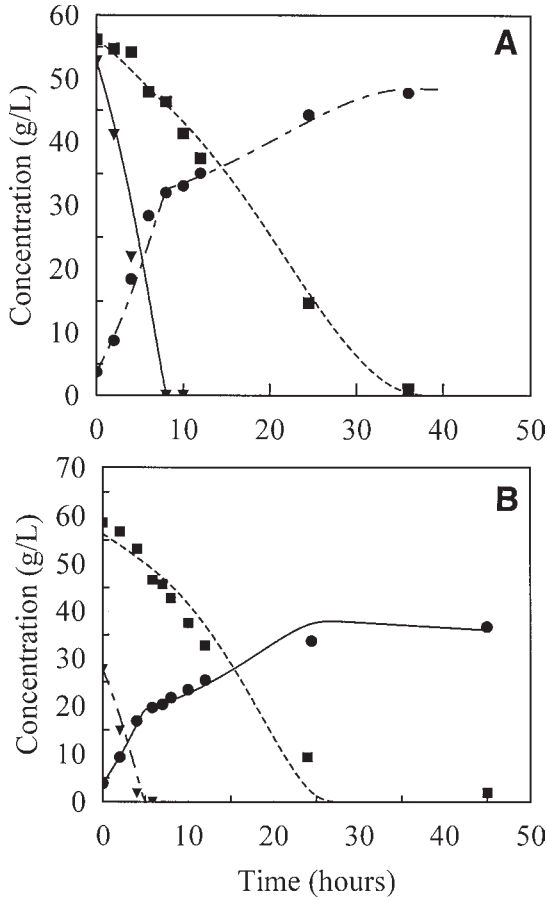

Fig. 6. Experimental and model predictions for batch fermentation of **(A)** 1:1 and **(B)** 2:1 glucose-xylose mixture using recombinant *Saccharomyces* strain 1400(pLNH33). Lines represent model predictions and symbols represent experimental data. (—, predicted glucose; - - - -, predicted xylose; – – – – predicted ethanol). (A) ▼, experimental glucose; ■, experimental xylose; ●, experimental ethanol. (B) ▼, experimental glucose; ■, experimental xylose; ●, experimental ethanol.

as the fermentation is nearing completion by this time. Based on a theoretical yield of 0.51 g ethanol/g sugars, the ethanol yield from this experiment was calculated to be 0.46 g ethanol/g sugar or 90% of the theoretical yield. Glycerol and xylitol were produced in minor amounts to the extent of 5.6 g/L and 3.2 g/L. Figure 6B shows the fermentation results obtained on a 27.7 g/L glucose and 58.6 g/L xylose mixture. It can be seen again that both sugars are consumed simultaneously although the rate of glucose utilization (6.49 g/L-h) is higher than the rate of xylose utilization (2.09 g/L-h). Ethanol concentrations of 33.73 g/L and 36.7 g/L were obtained at the end of 24 and 48 h, respectively. Because the fermentation is nearly complete at the end of 24 h, experimental data up to 24 h were used to calculate ethanol yield. The ethanol yield from this experiment is 0.44 g ethanol/g sugars that

corresponds to 86% of the theoretical yield. Glycerol and xylitol were produced in minor amounts to the extent of 5.1 g/L and 4.5 g/L, respectively. The initial and final cell densities were 2.3 g/L and 11 g/L, respectively.

The parameters estimated from single substrate experiments were used in the model for sugar-mixture fermentation. The initial cell density used for the fermentation of glucose-xylose mixtures was higher than those used in single substrate kinetic experiments. This was accounted for by incorporating the inoculum size effect into the model. As seen from Fig. 6A and B, the predicted results are in good agreement with the experimental data. The model captures the high ethanol-production rate during the initial phase of primarily glucose fermentation, followed by the slower production rate from xylose after glucose is consumed. The simultaneous utilization of glucose and xylose (as seen from the experimental data) is also predicted by the model.

Discussion

Results presented in this article show that recombinant *Saccharomyces* 1400(pLNH33) is an effective fermentor of xylose to ethanol. This strain also efficiently co-ferments glucose-xylose mixtures to ethanol. Kinetic studies show that the recombinant *Saccharomyces* 1400(pLNH33) is more osmo-tolerant and ethanol tolerant when glucose, rather than xylose, is used as the fermentation substrate. Ethanol tolerance of the fermenting microorganism is a critical factor in the design of an ethanol production process. It is clear from the product-inhibition studies that ethanol inhibition on the specific growth rate and specific productivity is dependent on the fermentation substrate. Although the parameters P_m and P_m' for glucose fermentation were determined to be 129.9 and 136.4 g/L, respectively, the corresponding parameter values for xylose fermentation were 59.04 and 60.4 g/L. The magnitude of these parameters were also in the range of the highest ethanol concentrations obtained by fed-batch fermentation studies *(19)*. This result has significant implications for process design because when the ethanol concentration in the fermentor approaches 60 g/L, xylose in the feed is fermented rather slowly. Simultaneous production and recovery of ethanol from the fermentation broth is one approach to overcome this process problem *(21)*. However, this problem can eventually be overcome by the development of improved recombinant xylose-fermenting *Saccharomyces*. It should be noted that at this ethanol concentration, the recombinant yeast can continue to use glucose for growth and ethanol production.

Xylose fermentation can be further improved through the development of improved xylose fermenting *Saccharomyces* yeasts. For example, the stability problem experienced with using 1400(pLNH33) is not a problem with the recent successful development of the 1400(LNH-ST) strain that contains multiple copies of integrated XR, XD, and XK genes in the chromosome of the 1400 yeast *(14)*. Furthermore, these recombinant yeasts were designed specially for fermenting mixed sugars present in the cellu-

losic biomass hydrolyzates, and to use glucose for growth and then to ferment glucose and xylose present in the hydrolyzates to ethanol. The concentration of xylose in hydrolyzates obtained from cellulosic feedstocks is usually lower than 4%. Therefore, growth inhibition at high concentration of xylose should not pose a process problem even with 1400(pLNH33) and related pLNH plasmid-mediated *Saccharomyces* transformants.

In the area of model development, the inclusion of the inoculum size effect on the parameters μ_m and ν_m (in addition to the substrate and product-inhibition effects) enabled a generalized model to be constructed. Thus, the model can be used for high cell-density batch fermentation or for continuous fermentation with high cell loading. The good agreement of the predicted model results with the experimental data indicate that this model can provide a basis for future fermentation optimization studies.

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