Continuous Fermentation of Feed Streams Containing D-Glucose and D-Xylose in a Two-Stage Process Utilizing Immobilized Saccharomyces cerevisiae and Pachysolen tannophilus

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In the U.S., forest and crop residues contain enough glucose and xylose to supply 10 times the country's usage of ethanol and ethylene, but an efficient fermentation scheme is lacking. To develop a strategy for process design, specific ethanol productivities and yields of Pachysolen tannophilus NRRL Y-2460 and Saccharomyces cerevisiae NRRL Y-2235 were compared. Batch cultures and continuous stirred reactors (CSTR) loaded with immobilized cells were fed glucose and xylose. As expected from previous reports, Y-2235 fermented glucose but not xylose. Y-2460 consumed both sugars but fermented glucose inefficiently relative to Y-2235, and it suffered a diauxic lag lasting 10-20 h when given a sugar mixture. Immobilized Y-2235 exhibited increasing productivity but constant yield with increasing glucose concentration. In contrast, Y-2460 exhibited an optimum productivity at 30-40 g/L xylose and a declining yield with increasing xylose concentration. Immobilized Y-2235 tolerated more than 100 g/L ethanol while the productivity and yield of Y-2460 fell by 80 and 58%, respectively, as ethanol reached 50 g/L. A 38.8-g/L ethanol stream could be produced as 103 g/L xylose was continuously fed to Y-2460. If it was blended with a 274 g/L glucose stream to give a composite of 23.7 g/L ethanol and 107 g/L glucose, Y-2235 could enrich the ethanol to 75 g/L. Taken together these results suggest use of a two-stage continuous reactor for processing xylose and glucose from lignocellulose. An immobilized Y-2460 CSTR (or cascade) would convert the hemicellulose hydrolyzate. Then downstream, an immobilized Y-2235 plug flow reactor would enrich the hemicellulose-derived ethanol to more than 70 g/L upon addition of cellulose hydrolyzate.

INTRODUCTION

Wheat straw is a common lignocellulosic residue whose composition and hydrolysis products are known. For each 100 g straw, hemicellulose contains 16-21 g xylose, 3-6 g arabinose (unfermentable), and small amounts of other sugars (glucose, galactose, and mannose). Low-temperature hydrolysis with dilute sulfuric acid releases 85-95% of these sugars. Their extraction prior to cellulose hydrolysis is preferred in order to minimize decomposition products, including furfural and trimethylfurfural, which reduce sugar yield and inhibit subsequent fermentation. Cellulose contains 32-40 g glucose/100 g straw. From 40 to 80% of this sugar is extractable following harsh treatment with sulfuric acid as exemplified by Peoria, Madison, or Purdue LORRE processes. Higher sugar yields and uninhibited fermentation rates are promised by enzymatic methods which minimize decomposition products common to acid-catalyzed methods. The wheat straw example illustrates relative amounts of xylose and glucose typically available as separate streams for fermentation.

An efficient fermentation scheme for processing lignocellulose hydrolyzates has not been developed. The objective of this research was to provide data useful for process design. P. tannophilus was chosen for study because of its well-known ability to ferment xylose. S. cerevisiae was also of interest here because of its traditional role in glucose fermentation. Batch and/or immobilized-cell continuous cultures were used to characterize these yeasts. Experiments were intended to elucidate substrate preference, the severity of diauxic lag, culturing techniques for preventing diauxy, and the fermentation kinetics of each strain on glucose and/or xylose. This information was used to make practical decisions about the process design, including: whether to ferment both sugars with P. tannophilus or in two stages — glucose by S. cerevisiae and xylose by P. tannophilus; whether to ferment xylose before glucose or vice versa; whether to utilize xylose and glucose streams mixed or separately; and which reactor type to use [batch, continuous stirred tank (CSTR), or plug flow (PFR)].
EXPERIMENTAL METHODS

Organisms

Stock cultures of *P. tannophilus* NRRL Y-2460 and *S. cerevisiae* NRRL Y-2235 were maintained at 32°C on YM slants containing 3.0 g/L yeast extract, 3.0 g/L malt extract, 5.0 g/L peptone, 10.0 g/L D-glucose, and 20.0 g/L agar.

Growth Medium

The growth medium used in seed and batch cultures was the same as that previously described by Slininger et al. Either D-glucose or D-xylose, or both, served as carbon source. The medium pH was initially 4.5 ± 0.2.

Seed Cultures

Fifty-milliliter seed cultures inoculated from slant were shaken in 125-mL flasks at 150 rpm and 28°C for 48 h prior to use. The growth medium for seed cultures contained 50 g/L glucose. Seed cultures were used to inoculate subsequent cultures to an initial absorbance of 0.1 at 620 nm.

Batch Cultures

Duplicate 400-mL cultures were shaken at 200 rpm and 32°C in 500-mL flasks plugged with cotton. Batch culture evaluations were done on four different substrate compositions: 50 g/L glucose, 40 g/L glucose plus 10 g/L xylose, 25 g/L glucose plus 25 g/L xylose, and 10 g/L glucose plus 40 g/L xylose.

Specific growth rates were determined from initial exponential growth phase and were calculated from the slope of the semilogarithmic plot of culture absorbance versus time. Ethanol concentrations were linearly correlated with time at confidence levels exceeding 95%. Consequently, average volumetric ethanol productivities (g/L/h) on each substrate were calculated by linear regression analysis of ethanol concentration versus time data. The average dry cell mass concentration over the ethanol evolution period was taken to be the ratio of the area under the volumetric productivity to average dry cell mass concentration. The ethanol yield on each substrate was taken to be the ratio of the maximum ethanol concentration attained to the substrate concentration consumed.

Batch Cultures with Large Inocula Pregrown on Xylose or Glucose

*P. tannophilus* was pregrown on 50 g/L xylose or glucose. Cultures of 1500 mL were inoculated with seed cultures and incubated in gauze-covered Fernbach flasks for 72 h at 28°C and 150 rpm. A Millipore Pellicon cassette system was used to concentrate and wash the cells with 12.6 g/L KH₂PO₄; 1.4 g/L K₂HPO₄ buffer. Buffered cell suspension was centrifuged (20 min, 7500 rpm) and resuspended to A₆₀₀ = 40 in growth medium with 26 g/L each of xylose and glucose. Duplicate 400-mL cultures were prepared in this way and incubated in 500-mL flasks with Bellco sponge caps. Agitation and temperature control was accomplished by a New Brunswick Aquatherm water bath shaker at 200 rpm and 32°C.

Preparation of Immobilized Cell Beads

*P. tannophilus* or *S. cerevisiae* were grown on media containing 50 g/L xylose or 50 g/L glucose, respectively. Twenty-liter cultures were carried out at 32°C in a New Brunswick 50-L fermentor aerated at 0.15 min⁻¹ and agitated at 200 rpm. After 72 h, the culture absorbance had increased from 0.1 to 30-40, and cells were harvested on a Sharpe’s continuous centrifuge.

For each continuous fermentor, wet cell paste equivalent to 6 g dry cell mass was diluted to 100 mL with 10 g/L Kelco Gel Algin HV and blended with a 1-mL Eppendorf pipette tip attached to form droplets. The flow rate was ca. 5 mL/min as droplets fell into a 1-L bath containing 1.2 g/L KH₂PO₄, 0.5 g/L MgSO₄, 20 g/L CaCl₂ and either 50 g/L xylose for *P. tannophilus* or 50 g/L glucose for *S. cerevisiae*. Upon solidifying, the calcium alginate beads averaged 3.4 mm diameter.

Single-Stage Continuous Cultures

Each batch of immobilized cell beads was transferred to a jacketed Bellco spinner flask of 250-mL working volume. The drycell mass (b) present in each spinner flask was 6 g. Enough fresh feed medium was added to the vessel to bring the total culture volume to 200 mL (ca. 120 mL broth plus 80 mL of beads). This volume was maintained throughout the course of each fermentation as a peristaltic pump continuously delivered fresh feed medium (1.2 g/L KH₂PO₄, 0.5 g/L MgSO₄, 3.0 g/L CaCl₂, and xylose (X) or glucose (G) as specified) at a volumetric rate (Q) while drawing off spent medium at an equal rate. Specific xylose and glucose supply rates were calculated as QX/b and QG/b, respectively. A uniform distribution of beads and beer constituents in the spinner flask was maintained by a magnetic stirrer. Water was circulated from a Hotpack bath through the vessel jacket to control temperature at 32°C.

The fermentor was not sparged since cell growth was limited by the nitrogen-free feed medium used. The only oxygen available diffused through a 13-mm-diameter Millipore Swinnex filter used as a vent.

Reactor performance was analyzed in terms of the effluent ethanol (E) and glucose (G) or xylose (X) concentra-
Two-Stage Cultures

Y-2235 Preceding Y-2460

Stage 1 was charged with immobilized S. cerevisiae ($b_1 = 6$ g) prepared as described above and was operated as specified for the single-stage case. Stage 2 was charged with a double batch of immobilized P. tannophilus ($b_2 = 12$ g) and was double the volume of stage 1, requiring a 500-mL spinner flask with a 400-mL culture volume (ca. 240 mL of broth plus 160 mL of beads). The feed medium, containing both glucose ($G_f = 5-100$ g/L) and xylose ($X_f = 50$ g/L), entered the first stage at $Q = 17 \pm 2$ mL/h where glucose was converted to ethanol. The specific glucose supply rate ($0.014-0.28$ g/L/h) was slow enough to limit the productivity of the first stage. Thus, effluent glucose concentration ($G_1$) was 0 and the ethanol concentration ($E_1$) was $0.5G_f$. The effluent from the first stage was fed to the second where the remaining xylose was fermented by P. tannophilus. The specific xylose supply rate to the second stage ($0.071$ g/g/h) did not limit the fermentation rate because 2-28 g/L xylose was observed in the stage 2 effluent.

The specific ethanol productivity was calculated as $QE_1/b_1$ in stage 1 and as $Q(E_2 - E_1)/b_2$ in stage 2. Ethanol yields were calculated based on glucose consumed,

$$Y_{EG} = E_1/(G_f - G_1)$$

for xylose consumed,

$$Y_{EX} = (E_2 - E_1)/(X_f - X_1)$$

and total sugar fed,

$$Y_{EGX} = E_2/(G_f + X_f)$$

Y-2460 Preceding Y-2235

This two-stage fermentation was set up according to the flow diagram in Figure 1 for wheat straw hydrolysates. Stage 1 was charged with $b_1 = 6$ g immobilized P. tannophilus and operated as for the single-stage case ($V_{R1} = 200$ mL). It was fed a xylose solution of $X_f = 103$ g/L at $Q_x = 1.91$ mL/h. Stage 2 had half the volume of stage 1 ($V_{R2} = 100$ mL, ca. 60 mL of broth plus 40 mL of beads), and the beads contained $b_2 = 1.1$ g of S. cerevisiae instead of 6. The feed to stage 2 was the effluent from stage 1 plus a glucose stream of $G_f = 274$ g/L flowing at a rate of $Q_G = 1.22$ mL/h. Thus the total flow through stage 2 was $Q_x + Q_G$, or 3.13 mL/h. With values of $X_f$, $G_f$, $V_{R1}$, $V_{R2}$, and $b_1$ given, values for $Q_x$, $Q_G$, and $b_2$ were chosen to provide complete substrate conversion.

Continuous Reactor Sizing

An objective of reactor design is to minimize $V_R$ for a required fraction conversion and substrate supply rate. Useful design equations can be derived that express reactor volume ($V_R$) as a function of volumetric flow of feed solution into the reactor ($Q$), feed xylose ($X_f$) or glucose ($G_f$) concentration, specific sugar consumption rate ($q$), fraction sugar conversion ($f = (X_f - X)/X_f$ or $= (G_f - G)/G_f$), and biomass per reactor volume ($B = b/V_R$).

For a plug flow reactor (PFR) fed xylose, the design equation is

$$V_R = QX_f / B \int df / q$$

This equation indicates that PFR volume required is proportional to the area under the curve of $1/q$ vs. $f$ between the desired conversion limits. For a continuous stirred tank reactor (CSTR), the equation reduces to

$$V_R = QX_f(f_{out} - f_{in}) / Bq_{out}$$

Figure 1. Scheme for fermenting xylose-rich and glucose-rich hydrolysate streams. $X_f$ and $G_f$ are concentrations of xylose and glucose feed streams. $Q_x$ and $Q_G$ are volumetric flow rates. $E_1$ and $E_2$ are ethanol concentrations leaving the P. tannophilus and S. cerevisiae reactors. $Y_{EGX}$ and $Y_{EG}$ are ethanol yields. $G_f$ and $E_f$ indicate resultant glucose and ethanol concentrations feeding the second reactor. Complete sugar conversion is assumed for each stage. For wheat straw, $Q_G = 1.5X_fQ_x/G_f$ is also assumed.
Thus the required CSTR volume is proportional to the product of inverse outlet reaction rate ($1/q_{out}$) and desired conversion between inlet and outlet ($f_{out} - f_{in}$).

**Analyses**

**Biomass**

Biomass concentration was directly proportional to the batch culture absorbance $A(620)$ and equal to $kA$ where $k$ was 0.136 g/L. A Bausch & Lomb spectrophotometer was used for measurements. Samples were diluted so that the absorbance fell between 0.05 and 0.4 where Beer's law was a linear function of concentration.

**Ethanol and Sugars**

Samples (ca. 3 mL) taken from batch and continuous culture vessels were centrifuged and forced through a 0.45-μm syringe filter to remove cells and particulates prior to freezer storage. Ethanol concentration was assayed on a Varian 3700 gas chromatograph equipped with a 6-ft Porapak Q column operated at 190°C. D-Xylose and D-glucose concentrations were measured with a Waters high performance liquid chromatograph (HPLC) equipped with a BioRad HPX-87H ion exclusion column and a refractive index detector. The column was operated at 28°C with 0.0017N H₂SO₄ as the mobile phase.

**RESULTS AND DISCUSSION**

**Catabolite Repression of Xylose Uptake by P. tannophilus: Avoiding Diauxic Lag**

Figure 2 shows *Pachysolen*’s behavior on various mixtures of glucose and xylose. Glucose was consumed exclusively, but once it was gone, xylose consumption began. A significant 10–20-h diauxic lag in ethanol and biomass production curves was evident as metabolism switched from glucose to xylose utilization. When cells were pregrown (preinduced) on xylose, diauxy was eliminated (Fig. 3). This behavior is characteristic of catabolite repression in which glucose represses induction of enzymes for xylose conversion.

While this phenomenon has been noted by previous workers, the length of diauxic lag, as reported here, was of primary interest. Though hemicellulose hydrolyzates may be xylose rich, they are actually sugar mixtures that may exhibit diauxy. Continuous fermentations catalyzed by immobilized populations of xylose-induced cells promise to minimize time lost to diauxic lags, cell production, and reactor maintenance.

**S. cerevisiae — More Efficient at Fermenting Glucose than P. tannophilus**

Batch cultures of *P. tannophilus* and *S. cerevisiae* given 50 g/L glucose were compared. As shown in Table I, *S. cerevisiae* NRRL Y-2235 grew 1.5 times as fast, and produced ethanol at 80–100% theoretical yield (0.51 g/g).

<table>
<thead>
<tr>
<th>Yeast</th>
<th>Specific growth rate (g/g/h)</th>
<th>Average ethanol productivity (g/L/h)</th>
<th>Volumetric Specific yield (g/g/h)</th>
<th>Ethanol yield (g/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. tannophilus</em></td>
<td>0.30</td>
<td>1.11</td>
<td>0.65</td>
<td>0.38</td>
</tr>
<tr>
<td>NRRL Y-2460</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. cerevisiae</em></td>
<td>0.45</td>
<td>2.47</td>
<td>1.59</td>
<td>0.45</td>
</tr>
<tr>
<td>NRRL Y-2235</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table I. Comparative performance of *P. tannophilus* and *S. cerevisiae* on growth medium containing 50 g/L glucose.**

Figure 2. Fermentation of glucose/xylose mixtures by *P. tannophilus.*
over twice as rapidly as \textit{P. tannophilus}. These data indicate that, although \textit{P. tannophilus} fermented glucose more rapidly than xylose,\textsuperscript{12} it was inferior to \textit{S. cerevisiae} on glucose. Thus, separate steps for glucose conversion by \textit{S. cerevisiae} and xylose conversion by \textit{P. tannophilus} are needed for most efficient fermentation of lignocellulosic sugar.

**Variation of Immobilized \textit{S. cerevisiae} Productivity with Glucose Feed Rate to the Continuous Culture**

\textit{Immobilized \textit{S. cerevisiae}} was evaluated in a stirred continuous reactor fed 100 g/L glucose solution over a range of flow rates. Figure 4 shows that increasing the flow rate resulted in increased effluent glucose concentra-

**Figure 4.** Continuous fermentation of (a) 100 g/L glucose by \textit{S. cerevisiae} and (b) 100 g/L xylose by \textit{P. tannophilus} immobilized in 3.4-mm calcium alginate beads suspended in a stirred reactor. Effluent concentrations of ethanol (E), glucose (G), and xylose (X), specific ethanol productivity (QE/b), and ethanol yield (\(Y_{E,G} \) or \(Y_{E,X} \)) are plotted as functions of volumetric feed rate (Q) and specific substrate feed rates (\(Q_{G,b} \) or \(Q_{X,b} \)).
tion and specific ethanol productivity, but decreased reactor ethanol concentration. It also indicates that productivity was limited by specific glucose supply rate less than or equal to 0.39 gj gjh, making \( G = 0 \). Above this critical supply rate, \( G \) exceeded 0; and ethanol productivity appeared to be a linear function of \( G \) in the concentration range tested (Fig. 5). It is assumed that a saturating glucose concentration exists, giving a Monod-type substrate dependence. Regardless of flow rate, the ethanol yield remained ca. 0.50 g/g, 98% of the theoretical yield.

### Ethanol Tolerance of Immobilized \textit{S. cerevisiae} Fed Concentrated Glucose Solutions

As indicated in Figure 4, 50 g/L was the maximum ethanol concentration attained from our 100 g/L glucose feed. The yeast’s tolerance of higher ethanol concentrations was tested by feeding more concentrated glucose solutions (150-300 g/L) at glucose supply rates (0.24–0.47 gj gjh) which, at low ethanol concentrations, would be rate limiting and result in zero effluent sugar. The maximum ethanol concentration allowing fermentation was ca. 111 g/L (Table II). At this concentration, specific productivity was less than 30% of that noted (Figs. 4 and 5) for a 100 g/L glucose-fed reactor operating with only 20 g/L glucose and 40 g/L ethanol in the effluent.

Luong’s model\(^{13}\) of ethanol productivity as a function of ethanol concentration was modified to allow the uninhibited productivity to vary with glucose concentration in accordance with our data (Fig. 5). The modified form accurately predicted productivity at various glucose and ethanol concentrations as shown in Table II. Taking \( Y_{EG} = 0.51 \), \( G = G_f - fG_r \), and \( E = E_f + Y_{EG}fG_r \), Fig. 6 was generated to show the expected decline in productivity with reaction progress. The PFRs are expected to minimize reactor volume when production obeys kinetics of this sort because the reaction proceeds at top speed near the inlet to compensate for slow rates occurring near the outlet.

### Variation of Immobilized \textit{P. tannophilus} Productivity with Xylose Feed Rate to the Continuous Culture

Ethanol production by \textit{P. tannophilus} on xylose was slower and less efficient than that by \textit{S. cerevisiae} on glucose (Figs. 4 and 5). As feed rate was increased, effluent xylose concentration and specific productivity increased, even though ethanol yield and concentration declined. At specific xylose supply rates less than or equal to 0.034 gj gjh, sugar feed rate limited specific ethanol productivity, but at higher supply rates, productivity was a Monod function of reactor xylose concentrations up to 40 g/L (Fig. 5). Reactor xylose concentrations greater

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**Table II.** Ethanol productivity and yield of immobilized \textit{S. cerevisiae} NRRL Y-2235 in a single-stage continuous reactor fed various glucose solutions at flow rate of 0.0095 ±0.0005 L/h.

<table>
<thead>
<tr>
<th>Feed glucose (g/L)</th>
<th>Effluent Glucose (g/L)</th>
<th>Ethanol (g/L)</th>
<th>Specific ethanol productivity (g/g/h)</th>
<th>Ethanol yield (g/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>154</td>
<td>0</td>
<td>76.8</td>
<td>0.116</td>
<td>0.51</td>
</tr>
<tr>
<td>200</td>
<td>10</td>
<td>95.0</td>
<td>0.165</td>
<td>0.51</td>
</tr>
<tr>
<td>250</td>
<td>37</td>
<td>106.5</td>
<td>0.173</td>
<td>0.51</td>
</tr>
<tr>
<td>300</td>
<td>76</td>
<td>111.0</td>
<td>0.167</td>
<td>0.51</td>
</tr>
</tbody>
</table>

\( Y_{EG} = \frac{b}{a} \), \( Y_{E/G} = \frac{b}{a} \) as functions of effluent sugar concentration for (a) \textit{S. cerevisiae} and (b) \textit{P. tannophilus} immobilized in 3.4-mm calcium alginate beads and suspended in a stirred reactor fed 100 g/L sugar.
Figure 6. Plot of reciprocal specific glucose conversion rate ($q^{-1} = 0.51 / P_F$) vs. fraction glucose conversion showing the volume required for a plug flow reactor containing $S. \textit{cerevisiae}$ entrapped in 3.4-mm calcium alginate beads. Consistent with the Figure 1 process for wheat straw hydrolyzates, calculations assume that the feed contains ethanol at $E_f = 19$ g/L, that $G_f = 100$ g/L glucose, and that complete sugar conversion is required. The hatched area is proportional to reactor volume $V_R$ since area $= 0.59 = V_R/(QG_f)$. Note that the CSTR volume required for complete glucose conversion would be $3.4QG_f/B$, or 5.8 times the required PFR volume.

Table III. Ethanol yield expected for various reactors fed 100 g/L xylose. 

<table>
<thead>
<tr>
<th>Reactor configuration</th>
<th>Ethanol yield (g/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFR</td>
<td>0.18</td>
</tr>
<tr>
<td>CSTR-PFR</td>
<td>0.20</td>
</tr>
<tr>
<td>Two-CSTR series</td>
<td>0.28</td>
</tr>
<tr>
<td>CSTR</td>
<td>0.31</td>
</tr>
</tbody>
</table>

1 This refers to reactor configurations designated in Figure 7. 
2 Figure 5 data were used to calculate reactor ethanol yield $= [\int Y_{ex} \, df]/[\int df]$. 

must be weighed against the costs of building and maintaining larger reactors.

Ethanol Tolerance of Immobilized $P. \textit{tannophilus}$

Processes for producing ethanol from lignocellulose commonly combine hemicellulose and cellulose hydrolyzates so that one solution containing both glucose and xylose results. For example, the TVA process recycles the cellulose hydrolyzate to the hemicellulose hydrolysis reactor because it contains sulfuric acid, useful for catalyzing the next reaction.14 During our experimentation, similar glucose-xylose mixtures were fed to a two-stage fermentor containing immobilized yeast. Glucose was converted by $S. \textit{cerevisiae}$ in the first stage, and xylose by $P. \textit{tannophilus}$
in the second. This order prevented glucose uptake by the 
*P. tannophilus*, but it did not allow efficient xylose utilization
(Table 4). As stage 2 ethanol went from 19 to 53 g/L
(*G* from 12 to 100 g/L), the productivity and yield of
immobilized *P. tannophilus* on xylose dropped by 80 and
58%, respectively. Ethanol accumulation in the glucose
stage inhibited further accumulation by *P. tannophilus*
on xylose.

**Fermentation of Separate Xylose and Glucose Streams in a Two-Stage CSTR with Immobilized *P. tannophilus* Preceding *S. cerevisiae***

Figure 1 proposes a more efficient method of processing
xylose and glucose from lignocellulose that takes advantage
of the availability of xylose as a fermentable stream separate from the glucose. Once the xylose stream is converted to ethanol by *P. tannophilus*, it is enriched with glucose. Ethanol-tolerant *S. cerevisiae* is used to further augment the ethanol concentration. The flow rates (*Qx* and *Qg*) of xylose (*Xf*) and glucose (*Gf*) solutions are related by the mass ratio of xylose and glucose being processed. For example, about 3 mass units of glucose to 2 of xylose are recoverable from wheat straw, making *Qg* =
1.5*Xf*/*Gf*. Extracted xylose and glucose streams are expected to be little more concentrated than *Xf* = 100 g/L and *Gf* = 250 g/L; so assuming ethanol yields of *YE*/
*X ≤ 0.3 and *YEG ≤ 0.51, little more than 67 g/L ethanol will be accumulated on wheat straw-derived sugar. This figure could be raised if the sugar streams were concentrated or if starch hydrolysis was a coexisting process. In

![Figure 7. Reciprocal specific xylose conversion rate (qX = YeXb/(qF)) vs. fraction xylose conversion calculated from Figure 5 data. The plots compare reactor volumes required for plug flow (PFR) and continuous stirred tank (CSTR) reactor configurations using *P. tannophilus* entrapped in 3.4-mm calcium alginate beads. Complete conversion of a 100-g/L xylose feed is required. Hatched areas are proportional to reactor volume since area = *V* vs./(*QX*).](image)

### Table IV. Performance of a two-stage* immobilized cell CSTR fed mixtures of 50 ±2 g/L xylose and variable glucose at 17 ±2 mL/h.

<table>
<thead>
<tr>
<th>Feed glucose concentration (g/L)</th>
<th>Effluent ethanol concentration (g/L)</th>
<th>Specific ethanol productivity (g/g)</th>
<th>Ethanol yield (g/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>2</td>
<td>17(2)</td>
<td>0.005</td>
</tr>
<tr>
<td>12</td>
<td>5</td>
<td>19(4)</td>
<td>0.020</td>
</tr>
<tr>
<td>25</td>
<td>11</td>
<td>23(4)</td>
<td>0.026</td>
</tr>
<tr>
<td>40</td>
<td>18</td>
<td>28(4)</td>
<td>0.046</td>
</tr>
<tr>
<td>50</td>
<td>24</td>
<td>35(6)</td>
<td>0.066</td>
</tr>
<tr>
<td>60</td>
<td>30</td>
<td>36(14)</td>
<td>0.090</td>
</tr>
<tr>
<td>75</td>
<td>32</td>
<td>39(13)</td>
<td>0.078</td>
</tr>
<tr>
<td>100</td>
<td>50</td>
<td>53(28)</td>
<td>0.151</td>
</tr>
</tbody>
</table>

* Stage 1 contained *S. cerevisiae* and Stage 2 contained *P. tannophilus*. 
*Residual xylose concentration (g/L) is given in parenthesis.
*YE* refers to ethanol produced per total of glucose and xylose fed. This is different from *YEG* and *YEX* which are based on sugar consumed.

The latter case, higher glucose to xylose mass ratios would be possible.

Table V summarizes the performance of a two-stage fermentation designed to test the process outlined in Figure 1. Glucose and xylose feed concentrations were similar to those extractable from hydrolyzed wheat straw. Simple CSTR reactors were used for both *P. tannophilus* and *S. cerevisiae* stages although the PFR design would be preferred for the latter. Choice of reactor size, flow rates, and cell mass for each stage was based on the 1/q vs. f curves of Figures 6 and 7. Note that the calculations for Figure 6...
assumed an inlet ethanol concentration $E_r = 19$ g/L to coincide with the proposed feed stream to the $S. \text{cerevisiae}$ fermentor. The observed results were consistent with the performance predicted by the data for CSTR configurations (Table III and Figs. 6 and 7), except that $Y_{EX}$ was observed as 0.38 instead of 0.31. Thus, ethanol accumulation reached 75 g/L which was higher than the 73.9 g/L predicted. The higher productivities and yields of this process compared to those of the one summarized in Table IV are evidence of its utility. This processing scheme promises ethanol concentrations in the range optimizing total product cost as shown by Maiorella and co-workers.15

CONCLUSIONS

When pregrown on xylose, $P. \text{tannophilus}$ consumes glucose in preference to xylose and exhibits a 10–20-h diauxic lag as enzymes for xylose uptake are induced. However, $S. \text{cerevisiae}$ ferments glucose more rapidly and produces higher ethanol yields. Thus it appears that a process for efficient conversion of xylose and glucose from lignocellulose should consist of two steps—one using $P. \text{tannophilus}$ to ferment the xylose and the other using $S. \text{cerevisiae}$ to ferment the glucose. The xylose fermentor design requires special attention. Although the ethanol productivity goes through an optimum, the ethanol yield of $P. \text{tannophilus}$ decreases as xylose concentration increases. Consequently, a single continuous stirred tank reactor operating at 100% conversion promises the optimum yield. A PFR–CSTR series, which allows high sugar concentrations to occur, can provide 100% conversion at minimum reactor volumes; but low ethanol yields are expected to result. Economical compromises of yield and rate are possible. For example, a two-CSTR series could offer 90% of the optimum yield at one-third the reactor volume. Unlike $P. \text{tannophilus}$, the ethanol yield of $S. \text{cerevisiae}$ was independent of substrate concentration, and a PFR would minimize the glucose reactor volume without yield losses. Because $S. \text{cerevisiae}$ is more ethanol tolerant than $P. \text{tannophilus}$, rates and yields are maximum if the xylose fermentor precedes the glucose fermentor. This arrangement is feasible if lignocellulose hydrolysis is designed to produce separate xylose- and glucose-rich streams from hemicellulose and cellulose, respectively. The ethanol stream produced by $P. \text{tannophilus}$ during xylose fermentation can be blended with the glucose stream and then fed to the $S. \text{cerevisiae}$ fermentor to further enrich the ethanol concentration for cost effective recovery. The above conclusions drawn from our experimental results provide some useful guidelines for designing a fermentation scheme applicable to lignocellulose processing.

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References