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Research Article

Conversion of glucose-xylose mixtures to pyruvate using a consortium of metabolically engineered *Escherichia coli*

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Two strains of *Escherichia coli* were engineered to accumulate pyruvic acid from two sugars found in lignocellulosic hydrolysates by knockouts in the *aceE*, *ppsA*, *poxB*, and *ldhA* genes. Additionally, since glucose and xylose are typically consumed sequentially due to carbon catabolite repression in *E. coli*, one strain (MEC590) was engineered to grow only on glucose while a second strain (MEC589) grew only on xylose. On a single substrate, each strain generated pyruvate at a yield of about 0.60 g/g in both continuous culture and batch culture. In a glucose-xylose mixture under continuous culture, a consortium of both strains maintained a pyruvate yield greater than 0.60 g/g when three different concentrations of glucose and xylose were sequentially fed into the system. In a fed-batch process, both sugars in a glucose-xylose mixture were consumed simultaneously to accumulate 39 g/L pyruvate in less than 24 h at a yield of 0.59 g/g.

Keywords: Chemostat / Glucose / Lignocellulosic hydrolysate / Pyruvic acid / Xylose

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1 Introduction

Pyruvate (pyruvic acid) biochemically occupies the junction of glycolysis and the tricarboxylic acid cycle. The chemical is used directly as a food supplement and for the synthesis of several biochemicals such as L-DOPA [1] and N-acetyl-D-neuraminic acid [2]. Pyruvate is a useful metabolic precursor for a wide range of commercial products [3], and its microbial production has been recently reviewed [4,5]. For cells to accumulate significant pyruvate, deletions, or modifications of pathways leading from pyruvate are required [6]. Progress in metabolic engineering strategies, in conjunction with medium and bioprocess optimization, have led to greater than 70 g/L pyruvate from glucose using *Escherichia coli* [6,7], *Saccharomyces cerevisiae* [8], or *Candida glabrata* [9,10]. For example, previous studies demonstrated that pyruvate accumulation is encouraged in *E. coli* by four key gene deletions: *aceE* or another gene coding a component of the pyruvate dehydrogenase complex, *ldhA* coding lactate dehydrogenase, *pps* coding PEP synthase, and *poxB* coding pyruvate oxidase [7,11,12]. The *pflB* gene coding pyruvate formate lyase

can probably be retained because this gene is very sensitive to the aerobic conditions needed for pyruvate formation [13].

Lignocellulosic crops and forest residues represent a large and generally untapped source of carbohydrates that could be used to produce chemicals sustainably at lower cost [14]. Unfortunately, sugar liberated from lignocellulose is a mixture of hexoses and pentoses whose composition depends on the feedstock and the method of hydrolysis [15]. A single microbe has difficulty consuming the carbohydrates found in hydrolysates simultaneously because of differences in sugar transporters, ATP yield and the pathways used to catabolize these sugars [16]. Researchers have therefore strived to develop strains that metabolize glucose and xylose simultaneously [17], and typical strategies include supplementing the process with enzymes [18], slowing down glucose metabolism [19,20], disrupting glucose-mediated carbon catabolite repression [21], and overexpressing xylose transport and metabolic genes [22,23].

Another approach for the simultaneous conversion of sugar mixtures such as those found in lignocellulosic hydrolysates is to use a microbial consortium [24]. For example, one strain can be engineered to metabolize glucose (i.e., and not xylose), while a second strain of the same species can be engineered to metabolize only xylose [25]. An advantage of such a process is that each strain can be independently designed to carry out the one desired conversion optimally, xylose-to-product, or glucose-to-product. As a growth-associated product generated directly from glucose or xylose, pyruvate would appear to be an ideal

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Abbreviations: EMP, embden meyerhof parnas; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PTS, phosphotransferase system

Table 1. *Escherichia coli* strains used in this study

Strain	Relevant characteristics	Reference
ATCC8739	<i>Escherichia coli</i> C	Wild type
MEC589	ATCC8739 <i>ptsG763::</i> (FRT) <i>glk-726::</i> (FRT) <i>manZ743::</i> (FRT) <i>aceE732::</i> (FRT) <i>ldhA744::</i> (FRT) <i>poxB772::</i> (FRT) <i>pps-776::</i> Kan	This study
MEC590	ATCC8739 <i>xylA748::</i> (FRT) <i>aceE732::</i> (FRT) <i>ldhA744::</i> (FRT) <i>poxB772::</i> (FRT) <i>pps-776::</i> Kan	This study
MEC604	ATCC8739 <i>ptsG763::</i> (FRT) <i>glk-726::</i> (FRT) <i>manZ743::</i> (FRT) <i>aceE732::</i> (FRT) <i>ldhA744::</i> (FRT) <i>poxB772::</i> (FRT) <i>pps-776::</i> (FRT) <i>zwf-777::</i> Kan	This study
MEC605	ATCC8739 <i>xylA748::</i> (FRT) <i>aceE732::</i> (FRT) <i>ldhA744::</i> (FRT) <i>poxB772::</i> (FRT) <i>pps-776::</i> (FRT) <i>zwf-777::</i> Kan	This study

biochemical to generate from sugar mixtures using microbial consortia.

The vast majority of studies that focus on pyruvate generation have used glucose as the sole carbon source. The stoichiometric biochemical conversions of glucose and xylose to pyruvate (excluding water generated) are given by:

These balanced equations consider glucose to be taken up via the glucose phosphotransferase system, glucose to be metabolized by the embden meyerhof parnas (EMP) pathway, and xylose uptake to occur via the high-affinity ATP-dependent ABC transporter [26]. Although for both sugars the maximum theoretical yield is 0.966 g/g (as the pyruvate ion), the ATP yield from xylose is only one-third the ATP yield from glucose, while the NADH yield is much greater for the xylose-to-pyruvate conversion. Only recently has conversion of glucose-xylose mixtures to pyruvate been reported [27], with 29 g/L generated in 36 h by an engineered *Kluyveromyces marxianus*. However, xylose conversion was slower than glucose conversion, and redox-related by-products were formed: at the time the initial 40 g/L glucose was depleted (36 h), only about 13 g/L xylose was consumed, and 5 g/L xylitol had also accumulated [27].

For *E. coli*, xylose-selective strains require deletions in three genes involved in glucose uptake: *ptsG* codes the glucose-specific IIA component of the phosphotransferase system (PTS), *manZ* codes the mannose-specific IID component, *glk* codes glucokinase. These three genes are sufficient to prevent most glucose metabolism in *E. coli* [28]. Glucose-selective strains, unable to consume xylose, have a knockout in the *xylA* gene coding xylose isomerase [25, 26]. Into these parent strains, each selective for a single carbohydrate in a mixture, a pyruvate-generation “module” can be introduced.

In this study, *E. coli* strains were constructed to accumulate pyruvate at high yield and productivity using either glucose or xylose as carbon sources. Optimal strains were then used together as a consortium in continuous and fed-batch processes for the simultaneous conversion of glucose and xylose to pyruvate.

2 Materials and methods

2.1 Strains

Strains used in this study are shown in Table 1. Gene mutations were transduced into *E. coli* C (ATCC8739) from their respective strains in the Keio (FRT)Kan collection [29] by P1 phage transduction, and then selected on Kan plates. Forward primers external to the target gene and reverse primers within the kanamycin resistance cassette were used to check for proper chromosomal integration. The Kan antibiotic marker was removed using the pCP20 plasmid, which contains a temperature-inducible FLP recombinase as well as a temperature-sensitive replicon [30]. Gene knockouts and removal of the markers were verified by PCR. MEC589 and MEC604 are able to use xylose as a sole carbon source but the *ptsG*, *glk*, and *manZ* gene deletions prevents metabolism of glucose [31]. In contrast, MEC590 and MEC605 are able to use glucose as a sole carbon source but are unable to metabolize xylose.

2.2 Growth conditions

The defined basal medium to which carbon/energy sources were added contained (per L): 4.0 g NH_4Cl , 0.38 g KH_2PO_4 , 2.0 g K_2SO_4 , 0.15 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.25 mg $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.125 mg $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, 1.25 mg $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 0.875 mg $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.06 mg H_3BO_3 , 0.25 mg $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, 5.50 mg $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 20 mg $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$, 20 mg citric acid, 20 mg thiamine-HCl, 0.25 g L-leucine, and 100 mg kanamycin. The medium was adjusted to a pH of 7.0 with 20% w/v NaOH.

2.3 Batch, fed-batch, and chemostat processes

Each strain was first grown in 3 mL TYA medium [7], then after 10–14 h, 0.5 mL was used to inoculate 500 mL baffled shake flasks containing 50 mL of basal medium with 2.34 g/L $\text{Na}(\text{CH}_3\text{COO}) \cdot 3\text{H}_2\text{O}$ (1.0 g/L acetate) and 7 g/L D-(+)-xylose or 7 g/L D-(+)-glucose. After about 8 h when the OD reached 2, one or both of the shake flask contents were used to inoculate a 2.5 L bioreactor (Bioflo 2000, New Brunswick Scientific Co.,

Table 2. Comparison of strains during an acetate-limited chemostat at a dilution rate of 0.10–0.14 h^{−1} using a single strain and a single substrate

Strain	Substrate	Pyruvate yield (g/g)	Specific pyruvate productivity (g/gh)	Specific substrate consumption rate (g/gh)	Specific CO ₂ generation rate (mmol/gh)
MEC589	Xylose	0.62 ± 0.03	1.09 ± 0.13	1.74 ± 0.12	3.8 ± 0.2
MEC590	Glucose	0.60 ± 0.03	0.83 ± 0.08	1.39 ± 0.07	7.5 ± 2.7
MEC604	Xylose	0.55 ± 0.01	0.87 ± 0.13	1.58 ± 0.19	5.7 ± 0.9
MEC605	Glucose	0.64 ± 0.01	0.76 ± 0.05	1.19 ± 0.10	4.7 ± 1.0

New Brunswick, NJ, USA) containing 1.0 L basal medium with (1) 2.0 g/L acetate and either 15 g/L xylose or 15 g/L glucose (one strain), (2) 2.0 g/L acetate and 15 g/L of each sugar (one strain), or (3) 4.0 g/L acetate and 15 g/L of each sugar (two strains). Duplicate batch processes were performed.

A fed-batch study started as a batch processes as described above, with 1.0 L basal medium containing 4.0 g/L acetate, 15 g/L xylose, and 15 g/L glucose. When glucose and xylose were depleted, a 40 mL solution was added into the culture containing 15 g xylose, 15 g glucose and 3 g acetate. Batch and fed-batch studies were conducted with a constant agitation of 400 rpm and at 37°C. Air or oxygen-supplemented air was sparged at 1.0 L/min to maintain a dissolved oxygen above 40% of saturation. The pH was controlled at 7.0 using 25% w/v KOH/5% NH₄OH, and antifoam C (Sigma) was used as necessary to control foaming.

Duplicate continuous processes were conducted as chemostats and started as a 1.0 L batch process as described above except using 7.5 g/L sugar and 1.0 g/L acetate. After growth to an OD of 5, the chemostat was initiated at dilution rates of 0.08–0.15 h^{−1} with a feed of 30 g/L total sugar and 1 g/L acetate. For continuous processes using a consortium of two strains, the feed contained 1 g/L acetate, and three different (sequentially added) ratios of glucose:xylose: 15 g/L glucose + 15 g/L xylose (15:15), 20 g/L glucose + 10 g/L xylose (20:10), and 10 g/L glucose + 20 g/L xylose (10:20). A steady-state condition was assumed after five residence times at which time the oxygen and CO₂ concentrations in the effluent gas remained unchanged. The process operated at 37°C using 400 rpm agitation and 0.5 L/min air. The pH was controlled at 7.0 with 20% w/v NaOH, and antifoam C used as needed.

2.4 Analytical methods

The optical density at 600 nm (OD) (UV-650 spectrophotometer, Beckman Instruments, San Jose, CA, USA) was used to monitor cell growth. For dry cell weight measurement, three 20.0 mL samples were centrifuged (3300 × g, 10 min), the pellets washed by vortex mixing with 20 mL DI water and then centrifuged again. The cell pellet was then dried in an oven at 60°C for 24 h and weighed. Samples were routinely frozen at −20°C for further analysis, and thawed samples were centrifuged (4°C, 10 000 × g for 10 min), and filtered (0.45 μm nylon, Acrodisc, Pall Corporation, Port Washington, NY). Liquid chromatography was used to quantify xylose, glucose, and organic products using RI detection as previously described [32]. In chemostat studies, concentrations of oxygen and CO₂ in the off-gas were measured

(Innova 1313 gas monitor, Lumasense Technologies, Ballerup, Denmark).

3 Results

3.1 Single strain steady-state processes

The overall aim of this study is the simultaneous conversion of xylose and glucose into pyruvate by a consortium of two *E. coli* strains. Deletions in the *ptsG glk manZ* genes greatly curtail glucose uptake, while a *xylA* deletion prevents xylose uptake [25]. Deletions in the *aceE ldhA poxB pps* genes promote pyruvate formation [7, 11]. Since acetate limitation provides the highest rate of glycolysis and pyruvate formation [7], acetate-limited chemostats at dilution rates of 0.10–0.14 h^{−1} were first conducted using single strains MEC589 or MEC590 on their respective carbon sources (Table 2). Both MEC589 on xylose and MEC590 on glucose attained pyruvate yields of about 0.60 g/g, slightly lower than the 0.70 g/g observed with an *E. coli* K-12 strain having deletions in *poxB*, *aceEF*, *pps*, *pflB*, and *ldhA* [7]. In comparing MEC589 and MEC590, the specific sugar consumption and pyruvate production rates were slightly greater in the conversion of xylose to pyruvate, while the specific CO₂ generation rate was about twice as high during the conversion of glucose compared to xylose (Table 2).

Previous studies have demonstrated that under steady-state conditions using wild-type *E. coli*, 20% of glucose flux enters the pentose phosphate pathway (PPP) [33]. The much greater CO₂ generation rate observed for MEC590 growing on glucose compared to MEC589 growing on xylose supports the hypothesis that for MEC590 a significant fraction of glucose enters the PPP. Therefore, the xylose-selective MEC604 (MEC589 *zwf*) and glucose-selective MEC605 (MEC590 *zwf*) were constructed which each additionally contained a deletion of the *zwf* gene coding glucose-6-phosphate 1-dehydrogenase, the first step in the PPP. Although the CO₂ generation rate did indeed decrease significantly for MEC605 growing on glucose, the pyruvate yield on glucose only increased slightly (Table 2). For MEC604 converting xylose to pyruvate, minor decreases in yield and specific productivity and no change CO₂ generation rate were observed. Moreover, for both MEC604 and MEC605 a dilution rate above 0.15 h^{−1} could not be sustained, in contrast to a maximum dilution rate of about 0.22 h^{−1} for MEC589 and MEC590 (data not shown), suggesting a decrease in the maximum-specific growth rate as a result of the *zwf* knockouts. These chemostat studies establish that the *zwf* deletion does not provide a benefit to

pyruvate formation under acetate-limited conditions, and therefore MEC589 and MEC590 were selected for further studies.

3.2 Consortium steady-state processes

In continuous culture, the strains MEC589 or MEC590 were each able to metabolize a feed containing 30 g/L xylose or 30 g/L glucose, respectively, and accumulate pyruvate at a 0.60 g/g yield. The next study examined how the two strains would perform together as a consortium in a glucose-xylose mixture containing a sugar mixture at a total concentration of 30 g/L, shifting between three different glucose-to-xylose ratios. The concentrations were selected to simulate potential varying sugar concentrations present in real lignocellulosic hydrolysate while still being comparable to the single-strain chemostats (i.e., Table 2). This system can assess the stability of the process as the microbes respond to shifting feed concentrations, presumably by rebalancing their relative populations to match the change in carbon source availability [25].

Figure 1 shows a result of this “shifting feed” chemostat using a consortium of MEC589 and MEC590. After 8.25 h of the batch process, the chemostat was initiated with a 15 g/L glucose and 15 g/L xylose (15:15) feed, corresponding to “time = 0 h” for the continuous process shown in Fig. 1. After 40 h the first steady-state was approached, and the overall pyruvate yield was 0.61 g/g, while the specific pyruvate productivity was 0.99 g/gh. Because the process operated under acetate-limited conditions, some residual sugars were anticipated, and at 40 h the effluent contained 1.1 g/L glucose and 1.1 g/L xylose. At 40 h the process step-change shifted to a 20:10 feed, and the glucose concentration in the effluent increased to 2 g/L in less than two residence times before decreasing to 0.1 g/L as the new steady-state was achieved at about 80 h. At this second steady-state, the overall pyruvate yield was 0.65 g/g, while the specific pyruvate productivity was 1.0 g/gh. The process was step-change shifted again to a 10:20 feed, and immediately the xylose concentration increased

Figure 1. Growth and pyruvate production by a consortium of *E. coli* MEC589 and MEC590 in a chemostat sequentially fed different glucose-xylose mixtures. From 0–40 h the feed contained approximately 15 g/L glucose/15 g/L xylose (15:15), from 40–80 h the feed contained a 20:10 mixture, and after 80 h the feed contained 10:20 mixture. OD (○), pyruvate (●), xylose (▲), glucose (■), yield (✕). The dilution rate through the process was 0.14 h^{-1} .

to 1.7 g/L before decreasing to 0.5 g/L. At the final steady-state that was taken to occur at 114 h, the overall pyruvate yield was 0.68 g/g and the pyruvate productivity was 1.2 g/gh. The pyruvate concentration decreased slightly during each transition to a new steady-state in response to a step-change in feed composition. Despite shifting the feed composition, though, the system was able to maintain the pyruvate yield above 0.60 g/g from the three different glucose-xylose mixtures, corresponding to a pyruvate concentration above 18 g/L for the 30 g/L total sugar feed. At the constant dilution rate of 0.14 h^{-1} used, the volumetric productivity of pyruvate was 2.5–2.7 g/Lh throughout the process regardless of the glucose-xylose feed composition. This “shifting feed” chemostat was conducted twice with essentially identical results (data not shown). Potential *E. coli* by-products such as lactate, succinate, formate, and ethanol were not detected ($<0.1 \text{ g/L}$) in any chemostat study. Throughout the processes the acetate concentration in the effluent remained less than 0.1 g/L.

3.3 Batch and fed-batch processes

Continuous processes provide quality steady-state yield and rate data and provide high volumetric rates. Batch or fed-batch processes are typically preferred because these operational modes allow a greater product accumulation. Thus, the performance of each strain in a batch process using a single sugar was next examined (Fig. 2). MEC589 converted nominally 15 g/L xylose into over 10 g/L pyruvate for a yield of 0.67 g/g (Fig. 2A), while MEC590 converted about 15 g/L glucose into 9.2 g/L pyruvate for a yield of 0.59 g/g (Fig. 2B). Each single-strain process also contained initially 2 g/L acetate, which was exhausted in 8–10 h, with cell growth ceasing and pyruvate production continuing after acetate depletion. The performance of each strain alone was then examined using both sugars at an initial concentration of 15 g/L (Fig. 3). As expected, MEC589 converted xylose into pyruvate with a yield of 0.65 g/g without metabolizing glucose (Fig. 3A) while MEC590 converted glucose into pyruvate for a yield of 0.58 g/g without metabolizing the xylose (Fig. 3B). Each strain was largely unaffected by the presence of the other sugar. The results demonstrate that under batch conditions the two strains act selectively and independently in their metabolism of only one sugar.

Both strains MEC589 and MEC590 were also introduced together into the glucose-xylose mixture. As shown in Fig. 4, the two strain consortium performed essentially as each one had behaved when growing on its one specific substrate. The final pyruvate concentration achieved from the glucose-xylose mixture was nearly 19 g/L (overall yield of 0.61 g/g). Moreover, xylose and glucose were consumed in parallel, with the mixed-sugar process (i.e. Fig. 4) occurring in essentially the same time as each individual sugar process (i.e. Figs. 2 and 3). The volumetric pyruvate productivity was 1.44 g/Lh, about half of the productivity obtained in the chemostat processes.

For a final assessment of the consortium process for the production of pyruvate, the batch experiment was repeated. However, in this case the culture was fed a mixture of 15 g xylose, 15 g glucose, and 2 g acetate (in 50 mL water) at the time that the two sugars were depleted (Fig. 5). In this fed-batch process, a final concentration of 39 g/L pyruvate was obtained in

Figure 2. Pyruvate production in single-strain batch processes: (A) *E. coli* MEC589 growing on xylose, (B) *E. coli* MEC 590 growing on glucose. OD (○), pyruvate (●), xylose (▲), glucose (■), acetate (▽).

24 h, corresponding to a yield of 0.59 g/g and a productivity of 1.65 g/Lh. The fed-batch process provides a greater productivity than the batch process because the average biomass concentration is greater in the fed-batch process. Importantly, the two-strain consortium, with each microbial strain designed to accomplish one conversion selectively, was able to convert both xylose and glucose simultaneously to the product pyruvate.

4 Discussion

In this study two *E. coli* strains were engineered to have specific characteristics: (1) deletions in the *ldhA*, *aceE*, *poxB*, and *pps* genes permitted them to accumulate pyruvate in the presence of acetate [7], and (2) each strain was able to metabolize only one sugar—either xylose or glucose. Thus, one strain contained a deletion in the *xylA* gene that prevented xylose metabolism, while the second strain contained deletions in the *ptsG*, *manZ*, and *glk* genes preventing glucose metabolism [28]. When introduced into a glucose-xylose mixture, these strains independently carried out the conversion of one sugar to pyruvate, resulting in a microbial system that simultaneously converted both sugars to the product pyruvate. The absence of a functional pyruvate dehydrogenase complex (one component coded by *aceE*) prevented

Figure 3. Pyruvate production in single-strain batch processes on glucose-xylose mixtures: (A) *E. coli* MEC589, (B) *E. coli* MEC 590. OD (○), pyruvate (●), xylose (▲), glucose (■), acetate (▽).

the intracellular formation of acetyl CoA, and therefore acetate was a required nutrient [7]. Because acetate was required, this biochemical could be used as a growth-limiting substrate, effectively insuring that a large fraction of the sugars are converted into pyruvate in preference to biomass [7].

Since carbon labeling studies showed that wild-type *E. coli* growing under nutrient-limited conditions metabolizes 20% of glucose through PPP [33], blocking the PPP might improve glycolytic flux and thus pyruvate yield and productivity. However, a deletion in the *zwf* gene coding glucose-6-phosphate 1-dehydrogenase, the first enzyme of the PPP, did not significantly increase pyruvate yield or productivity. Moreover, this deletion decreased slightly the maximum-specific growth rate since the *zwf* strains were not able to sustain a dilution rate of 0.15 h⁻¹ that was readily achieved in strains having intact glucose-6-phosphate 1-dehydrogenase. Although the *zwf* knockout did reduce the CO₂ generation significantly, since the CO₂ generation rate was already low as result of the *aceE* deletion, this additional available carbon only modestly (i.e. and not significantly) contributed to pyruvate yield. Blocking the PPP also has the consequence of preventing one route to NADPH production [33]. Thus, the absence of improvement in pyruvate yield in the *zwf* strains could merely be a consequence of cells redirecting carbon metabolism toward other NADPH-generating pathways. Future studies to explore this effect would be to provide cells with

Figure 4. Pyruvate production in two-strain batch process using *E. coli* strains MEC589 and MEC590 growing on a glucose-xylose mixture. OD (○), pyruvate (●), xylose (▲), glucose (■), acetate (▽).

Figure 5. Pyruvate production in two-strain fed-batch process *E. coli* strains MEC589 and MEC590 growing on a glucose-xylose mixture. OD (○), pyruvate (●), xylose (▲), glucose (■), acetate (▽). A mixture of 2 g acetate, 15 g xylose, and 15 g glucose in 50 mL was added at $t = 13$ h when the initial glucose and xylose was approximately depleted.

a NADPH-generating step within glycolysis, such as replacing the native NAD-dependent glyceraldehyde-3-phosphate dehydrogenase (GAPDH) with an NADP-dependent GAPDH [34]. Previous research demonstrated that this replacement increases glycolytic capacity [35]. For the xylose-selective strain, the lack of improvement resulting from the *zwf* knockout could be attributed to the low flux of carbon into the oxidative portion of the PPP during normal growth on xylose [33]. A potential advantage of the consortium approach in a system design to convert glucose and xylose simultaneously is that each strain can be designed independently [36]. So, for example, for products in which a *zwf* deletion did improve yield from glucose (but not

xylose), the glucose-selective strain could include that knockout that is not used in the xylose-selective strain.

In this study, the inoculum for the consortium batch processes contained each of the two strains at the same cell density, and this approach was satisfactory because the growth and productivity of the two strains on their respective sugar were similar (Table 2). If one of the strains grew much faster on its sugar than the other strain grew on its substrate, then other approaches could be used for a batch process such as delaying the inoculation of the faster growing strain or reducing its inoculation volume. Delaying inoculation has been used previously to align the two-strain conversion of xylose and glucose into lactate [37].

Under continuous fermentation conditions, three different ratios of glucose-xylose in the feed were examined. An important benefit of the consortium approach for the conversion of sugar mixtures is that the individual strains respond independently to changing feed conditions. Thus, under acetate-limited conditions, the xylose and glucose were both converted to pyruvate simultaneously at the maximum rates. In the chemostat processes, the cell population likely adjusted naturally to match the feed of each substrate. Similarly in a fed-batch process, the two strains each consume their one substrate according to that one substrate's availability. If both substrates are converted at similar rates, then the portion of the process that is unproductively converting only one substrate is small. If one substrate is consumed much more slowly than the other, though, then steps might have to be taken to insure that the population of that slower strain is relatively high at the onset of the process. Such an alignment is not possible in a one-strain conversion [38].

A microbial consortium approach for the conversion of lignocellulosic hydrolysates could theoretically use any microbe that is both able to accumulate pyruvate and is able to metabolize both xylose and glucose (and by extension additional sugars). The industrial strain used for pyruvate production, *C. glabrata*, does not naturally metabolize xylose, and thus significant additional work would be needed to incorporate xylose uptake and metabolic genes before this strain could be considered for this application. Of course, a microbe which better tolerates low pH and metabolizes both sugars might be superior to *E. coli* in a consortium approach to generate pyruvate. Incorporating xylose-consumption genes has been used in *K. marxianus* to allow single-strain production of pyruvate from mixtures of xylose and glucose (26). The consortium approach to convert sugar mixtures could also theoretically be applied toward the production of any biochemical product. Indeed, microbial consortia of the same species have previously been applied toward nongrowth associated products succinic acid (36) and lactic acid (37).

In this study, acetate was the growth-limiting nutrient, and the two strains of *E. coli* did not appear to differentiate their acetate metabolism. Moreover, in a fed-batch process in which a dose of acetate, glucose, and xylose was added during the process (Fig. 5), the acetate was consumed within 2 hours, and the cells generated pyruvate from glucose and xylose during a nongrowth phase. If the two strains comprising the consortium grew differentially on the growth-limiting substrate, then one strain might outcompete the other. In such a circumstance some other growth-limiting

substrate could potentially be used, or some additional genetic perturbations introduced to prevent their differential growth.

Practical application

Hydrolysis of lignocellulosic biomass generates a mixture of carbohydrates, principally glucose, and xylose. The simultaneous microbial conversion of these sugars to generate a product has long vexed bioprocess engineers. In this work, two *Escherichia coli* strains are introduced into a glucose-xylose mixture to convert these sugars simultaneously into pyruvic acid. Simultaneous conversion is accomplished because the strains have knockouts that lead to pyruvate accumulation and which allow each one to consume only one sugar. In batch, continuous as well as fed-batch processes, the effective formation of pyruvic acid is demonstrated.

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