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ORIGINAL PAPER

Microbial removal of acetate selectively from sugar mixtures

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Abstract Acetic acid is an unavoidable constituent of the biomass hydrolysates generated from acetylated hemicellulose and lignin, and acetate affects the performance of microbes used to convert these hydrolysates into biofuels or other biochemicals. In this study, acetate was selectively removed from synthetic mixtures of glucose and xylose using metabolically engineered *Escherichia coli* strains having mutations in the glucose phosphotransferase system (PTS) genes (*ptsG*, *manZ*, *crr*), glucokinase (*glk*), and xylose (*xylA*). In batch culture, ALS1060 (*ptsG manZ glk xylA*) consumed exclusively acetate to depletion, and then consumed the two sugars only at a very slow rate (a growth rate of about 0.01 h^{-1}). We also examined the effects of an additional knockout of either *malX*, *fruA*, *fruB*, *bglF*, or *crr*, genes that are involved in other PTSs, and a batch process using KD840 (*ptsG manZ glk crr xylA*) demonstrated a further reduction in glucose or xylose consumption by *E. coli*. These results demonstrate the feasibility of using a substrate-selective approach for the pre-treatment of biomass hydrolysate for microbial processes.

Keywords Biomass hydrolysate · Xylose · Glucose · Ethanol · Acetate

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Introduction

Conversion of lignocellulosic biomass to fuels and chemicals by microbial fermentation is a promising alternative to petroleum-based processes [35]. Lignocellulosic materials are inexpensive and readily available, and are primarily cellulose and hemicellulose which can be hydrolyzed into a mixture of monosaccharides including glucose and xylose [19]. However, several challenges remain which limit the wide use of lignocellulosic biomass as a substrate for fermentation. One challenge is that biomass hydrolysates also contain inhibitors such as acetic acid (acetate). Acetate is an unavoidable product of hemicellulose depolymerization because arabinoxylans are acetylated [6, 27, 29]. Acetate strongly affects the microbial conversion of xylose, with only 1.5 g/L, for example, reducing the yield of ethanol using *Saccharomyces cerevisiae* 259ST by 50% at a pH of 5.0 [14]. The membrane of *S. cerevisiae* is readily permeable to acetate, and therefore this yeast is particularly susceptible to acetate inhibition [4]. Acetate also exacerbates other inhibitory effects: for example, the inhibition of ethanol formation by *Escherichia coli* due to furfuryl alcohol and 2-furfural is worse in the presence of acetate [32–34]. Although the generation of some inhibitors might be reduced by judicious design of the hydrolysis process or by genetic improvements in the biomass itself, elimination of all acetate in a lignocellulosic hydrolysate does not currently seem feasible.

A wide variety of strategies have been proposed to reduce the inhibitory effect of acetate on fermentation. One method is merely to find production organisms which are more tolerant to acetate [20]. Alternatively, acetate could be removed from the sugar mixture. For example, ion exchange [5, 16] or activated carbon [2] can remove acetate from solutions. Similarly, extraction with ethyl acetate

reduces acetic acid (and furfural, vanillin, and 4-hydroxybenzoic acid) and results in a 93% improvement in ethanol yield using *Pichia stipitis* [31]. These approaches involve an additional processing step which significantly affects overall costs [30]. Preferably, an approach should not only benefit the production microorganisms by removing acetate but also should be integrated into the bioprocess and be accomplished at very low cost.

We previously reported a biological strategy for selectively removing carbon sources from a mixture [10]. The approach involves the "design" of a single strain that will utilize *only* one component in a mixture. Because many organisms including *E. coli* readily consume acetate when this compound is the sole carbon source [15], acetate might be removed from a mixture of xylose, glucose, and acetate (for example) with a strain that has been genetically prevented from consuming xylose and glucose. Such an approach has been demonstrated to work in batch culture using *S. cerevisiae* mutants for the selective removal of acetate [28]. Similarly, wild-type *Issatchenkia occidentalis* can selectively remove furans from concentrated hydrolysate [12]. In *E. coli*, glucose uptake is primarily mediated by glucose phosphotransferase (EC 2.7.1.69) for which the glucose-specific membrane-bound $EIICB^{glc}$ component is encoded by *ptsG* [25] and the hydrophilic $EIIA^{glu}$ component is encoded by *crr* [21]. Glucose utilization is also mediated by mannose phosphotransferase (EC 2.7.1.69) for which the EII component is encoded by *manZ* [8, 17] and ATP-dependent glucokinase (EC 2.7.1.2) encoded by *glk* [8]. Xylose metabolism in *E. coli* begins with xylose isomerase, an enzyme encoded by *xylA*. Knocking out the *ptsG*, *manZ*, and *glk* genes prevents *E. coli* from consuming glucose in a short batch process, whereas a *xylA* mutant is unable to consume xylose [10]. Because these carbohydrate utilization genes are not known to affect acetate metabolism, a strain with these 4–5 knockouts (e.g., *ptsG manZ glk xylA* or *ptsG manZ glk crr xylA*) should prevent consumption of both glucose and xylose but allow normal acetate metabolism.

Using a mixture of xylose, glucose, and acetate as a model for biomass hydrolysates containing an inhibitor, this study aimed to construct an acetate-selective strain which does not consume xylose and glucose, and to demonstrate that this strain could effectively remove acetate from a mixture containing these sugars.

Materials and methods

Bacterial strains

The *Escherichia coli* strains studied are shown in Table 1. Strains containing Δ*ptsG*763::FRT, Δ*manZ*743::FRT, Δ*glk*-726::FRT, Δ*xylA*748::FRT, Δ*fruA*723::FRT, Δ*fruB*725::FRT, Δ*bglF*753::FRT, or Δ*crr*-746::FRT deletions were generated by transducing MG1655 with the corresponding Keio (FRT)Kan deletion [1] and then curing the Kan(R) using the pCP20 plasmid, which contains a temperature-inducible FLP recombinase as well as a temperature-sensitive replicon [9]. The Δ*xylA*748::(FRT)Kan and Δ*crr*-746::(FRT)Kan deletions that are contained in ALS1072 and KD840, respectively, are Keio (FRT)Kan deletions in which the Kan(R) was not cured. All cultures using ALS1072 or KD840 were supplemented with 100 mg/L kanamycin.

Shake flask growth conditions

Basal medium (BA) contained (per L): 13.3 g KH_2PO_4, 4.0 g $(NH_4)_2HPO_4$, 1.2 g $MgSO_4{\cdot}7H_2O$, 13.0 mg $Zn(CH_3COO)_2{\cdot}2H_2O$, 1.5 mg $CuCl_2{\cdot}2H_2O$, 15.0 mg $MnCl_2{\cdot}4H_2O$, 2.5 mg $CoCl_2{\cdot}6H_2O$, 3.0 mg H_3BO_3, 2.5 mg $Na_2MoO_4{\cdot}2H_2O$, 100 mg Fe(III)citrate, 8.4 mg $Na_2EDTA{\cdot}2H_2O$, 1.7 g citric acid, 0.0045 g thiamine·HCl, and acetate using $Na(CH_3COO){\cdot}3H_2O$. The value after "BA" indicates the concentration of acetate (for example, BA2 indicates medium having 2 g/L acetate). Media were supplemented with xylose and/or glucose as described in the text. Acetate, xylose, and glucose were autoclaved separately, sterilely combined, and pH was adjusted to 7.0 using NaOH. Concentrations are reported with respect to acetate as the monovalent anion.

Growth conditions

For shake flask experiments, 50 mL BA2 medium contained 2 g/L glucose in 250-mL baffled shake flasks cultured at 37°C and 350 rpm (19-mm pitch).

For bioreactor experiments, the selected strain was grown in a 5 mL pre-culture in a 30-mL shaking test tube containing 5 g/L tryptone, 2.5 g/L yeast extract, 5 g/L NaCl, and 2.5 g/L acetate, then transferred to a baffled 250-mL shake flask containing 50 mL BA10 medium incubated at 37°C and 250 rpm (19-mm pitch). When the optical density (OD) of the culture reached 2.0–2.5, the content of the shake flask was transferred to a bioreactor.

Batch processes using 1.0 L BA10 medium were carried out in a 2.5-L bioreactor (Bioflo 2000, New Brunswick Scientific Co. Edison, NJ, USA). Air was sparged into the fermenter at a flow rate of 1.0 L/min, and the agitation was 500 rpm to prevent oxygen limitation. The pH was controlled at 7.0 using 20% (w/v) NaOH and 20% (v/v) H_2SO_4, and the temperature was controlled at 37°C. Antifoam C (Sigma) was used as necessary to control foaming.

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

Strain	Genotype
MG1655	F-λ- *ilvG*- *rfb*-50 *rph*-1 (K12 strain)
ALS1060	MG1655 Δ*ptsG*763::FRT Δ*manZ*743::FRT Δ*glk*-726::FRT Δ*xylA*748::FRT
ALS1072	MG1655 Δ*ptsG*763::FRT Δ*manZ*743::FRT Δ*glk*-726::FRT Δ*xylA*748::(FRT)Kan
ALS1122	MG1655 Δ*ptsG*763::FRT Δ*manZ*743::FRT Δ*glk*-726::FRT Δ*xylA*748::FRT Δ*crr*-746::FRT
ALS1123	MG1655 Δ*ptsG*763::FRT Δ*manZ*743::FRT Δ*glk*-726::FRT Δ*xylA*748::FRT Δ*fruA*723::FRT
ALS1124	MG1655 Δ*ptsG*763::FRT Δ*manZ*743::FRT Δ*glk*-726::FRT Δ*xylA*748::FRT Δ*fruB*725::FRT
ALS1125	MG1655 Δ*ptsG*763::FRT Δ*manZ*743::FRT Δ*glk*-726::FRT Δ*xylA*748::FRT Δ*bglF*753::FRT
KD840	MG1655 Δ*ptsG*763::FRT Δ*manZ*743::FRT Δ*glk*-726::FRT Δ*xylA*748::FRT Δ*crr*-746::(FRT)Kan

Fed-batch processes initially were operated in batch mode using BA5 medium. When the OD reached 3.0–3.5, BA10 medium supplemented with xylose and/or glucose was fed at an exponentially increasing rate pre-programmed to achieve a constant growth rate of 0.07 h^{-1} (Tandem 1081 pump, SciLog, Inc., Middleton, WI USA). Concentrated NH_4OH was used for base control.

Analyses

The optical density measured at 600-nm absorbance (UV-650 spectrophotometer, Beckman Instruments, San Jose, CA) was used to monitor cell growth. Glucose, xylose, acetate, and other organic by-products were quantified by using liquid chromatography with refractive index detection as previously described [11].

Results

Batch growth on acetate

Escherichia coli MG1655 is a common K12 strain with minimal alterations [18] able to consume acetate as the sole carbon source. Aerobic batch growth was measured on acetate in BA10 medium (i.e., initial concentration of 10 g/L acetate). After a lag of about 10 h, MG1655 was able to consume acetate at a growth rate of 0.23 h^{-1} and attain an OD of 7.5.

E. coli ALS1060 has four knockouts of genes coding for proteins involved in the utilization of xylose and glucose: *ptsG* encodes the $IICB^{Glc}$ domain of the phosphotransferase system (PTS) for carbohydrate transport [25], *manZ* encodes the IID^{Man} domain of the mannose PTS permease [17], *glk* encodes glucokinase [8], and *xylA* encodes xylose isomerase. These four mutations are hypothesized to prevent the utilization of both xylose and glucose. In order to determine whether these mutations had any effect on the growth on acetate, ALS1060 was grown in the same medium. Similar to MG1655, ALS1060 formed 2.5 g/L cells (OD = 7.7), and attained a specific growth rate of 0.22 h^{-1}.

Batch growth on acetate in the presence of sugars

The next objective was to determine whether acetate could be exclusively consumed from a mixture of sugars. Because ALS1060 contains knockouts involved in the consumption of xylose or glucose, the growth of this strain in a medium containing xylose, glucose, and acetate is expected to be identical to growth in a medium containing acetate alone. In order to test this expectation, ALS1060 was grown in batch culture over an extended period of time in BA10 medium with glucose alone, xylose alone, or in a mixture of xylose and glucose.

Batch culture using ALS1060 in BA10 medium containing 20 g/L glucose resulted in exclusive acetate consumption during the first 20 h of the process (Fig. 1). Moreover, during growth on acetate the specific growth rate was 0.21 h^{-1}, demonstrating that the presence of glucose did not affect this strain's growth on acetate. Interestingly, slow glucose consumption commenced about the time acetate was exhausted (Fig. 1), and the specific

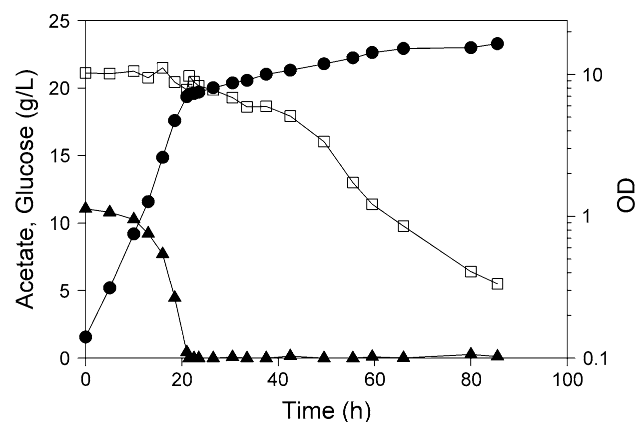

Fig. 1 Aerobic batch culture of *E. coli* ALS1060 using BA10 medium plus 20 g/L glucose. Glucose (*open squares*), acetate (*filled triangles*), and OD (*filled circles*) were measured over the course of the process

growth rate of ALS1060 after acetate was exhausted was 0.014 h^{-1}.

During batch culture using BA10 medium containing 10 g/L xylose ALS1060 consumed exclusively acetate, and the concentration of xylose remained unaltered throughout the process (Fig. 2). During the 24 h of acetate consumption, the specific growth rate was approximately 0.15 h^{-1}. Although xylose was not consumed, the presence of this sugar did reduce the growth rate on acetate by about 25%.

In order to study how the presence of both sugars influenced acetate utilization, ALS1060 was inoculated into BA10 medium with 20 g/L glucose and 10 g/L xylose. In this case, ALS1060 consumed the acetate in 30 h, during which time less than 2 g/L glucose and 0.6 g/L xylose was consumed. During this initial period, the cell growth rate was approximately 0.13 h^{-1}, approximately equal to the growth rate on acetate in the presence of xylose. Over the next 40 h, about 7 g/L glucose and 3 g/L xylose were slowly consumed (Fig. 3).

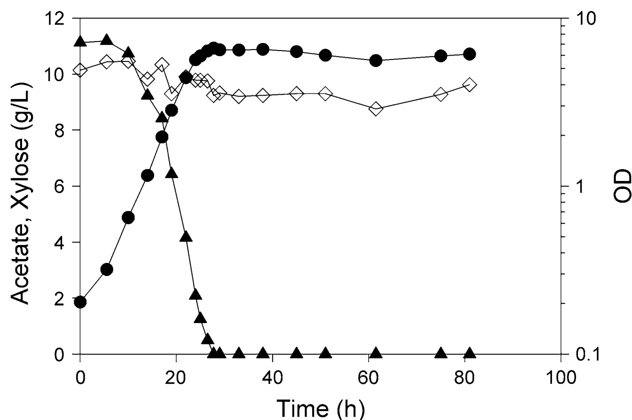

Fig. 2 Aerobic batch culture of *E. coli* ALS1060 using BA10 medium plus 10 g/L xylose. Xylose (*open diamonds*), acetate (*filled triangles*), and OD (*filled circles*) were measured over the course of the process

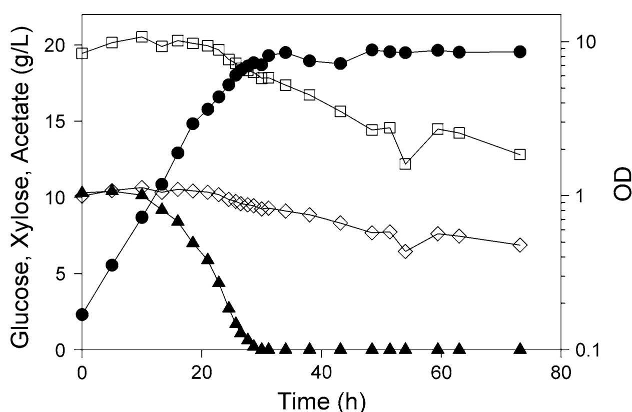

Fig. 3 Aerobic batch culture of *E. coli* ALS1060 using BA10 medium plus 20 g/L glucose and 10 g/L xylose. Glucose (*open squares*), xylose (*open diamonds*), acetate (*filled triangles*), and OD (*filled circles*) were measured over the course of the process

Additional knockouts to eliminate glucose consumption

The observation that ALS1060 grew slowly in the presence of glucose suggests that *E. coli* may have another means to transport and utilize (i.e., phosphorylate) glucose, and that mutations in the *ptsG manZ glk* genes are insufficient to prevent glucose consumption. With the goal of completely excluding glucose consumption, several other strains were next examined for growth on BA2 medium with 2 g/L glucose. These new strains had additional mutations in one of several other genes encoding other PTS proteins: *fruA* or *fruB* encoding proteins of the fructose-specific PTS (ALS1123 and ALS1124, respectively); the *bglF* gene involved in the PTS of β-glucosides (ALS1125), or *crr* which encodes the $EIIA^{glu}$ (ALS1122). To compare these strains, the rate of glucose uptake and the change in the optical density for approximately 30 h beyond the time that acetate was exhausted were measured. Although in each case the changes in optical density and glucose concentration over the course of 30 h were small, compared with ALS1060, only ALS1122 (with the *crr* mutation) showed a significantly reduced rate of glucose consumption, and this strain also showed the lowest change in the optical density (data not shown).

Batch growth of *crr* knockout on acetate in the presence of sugars

The next goal was to determine if KD840 (*ptsG manZ glk crr xylA*), containing the *crr* knockout additionally, would show reduced glucose consumption compared with ALS1060 (*ptsG manZ glk*). In BA10 medium with only acetate, KD840 formed approximately 2.5 g/L cells (OD = 7.1) at a specific growth rate of 0.23 h^{-1}, identical to ALS1060 and MG1655. In BA10 with 20 g/L glucose, KD840 completely consumed acetate during the first 35 h (Fig. 4), and cells attained a specific growth rate of

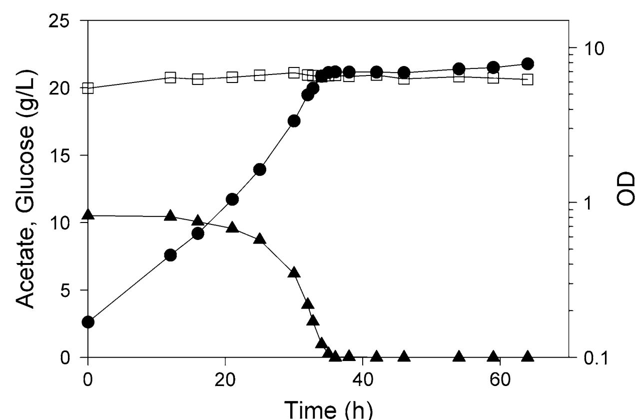

Fig. 4 Aerobic batch culture of *E. coli* KD840 on BA10 medium with 20 g/L glucose. Glucose (*open squares*), acetate (*filled triangles*), and OD (*filled circles*) were measured over the course of the process

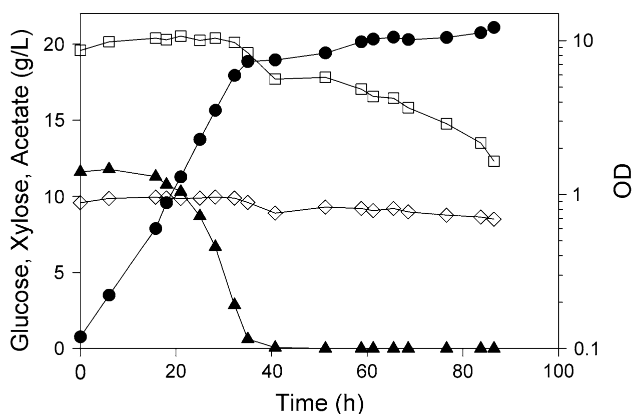

Fig. 5 Aerobic batch culture of *E. coli* KD840 on BA10 medium with 20 g/L glucose and 10 g/L xylose. Glucose (*open squares*), xylose (*open diamonds*), acetate (*filled triangles*), and OD (*filled circles*) were measured over the course of the process

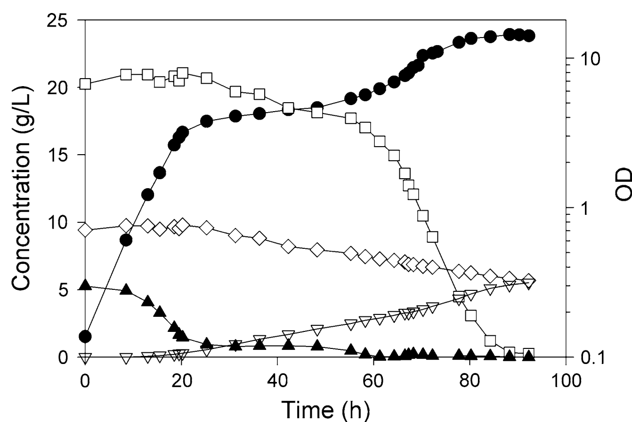

Fig. 6 Aerobic fed-batch culture of *E. coli* ALS1072 using a mixture of glucose (*open squares*), xylose (*open diamonds*), and acetate (*filled triangles*). OD (*filled circles*) and succinate (*inverted filled triangles*) were also measured over the course of the process

0.13 h^{-1} on acetate. During the 35 h of acetate consumption and for another 35 h after acetate consumption, glucose was not consumed. In BA10 20 g/L glucose and 10 g/L xylose, similar to results in the mixture of acetate and glucose, acetate was completely consumed by 40 h with a specific growth rate of 0.12 h^{-1}. However, over the course of the subsequent 40–50 h, about 7 g/L glucose and 1 g/L xylose were consumed (Fig. 5), similar to the previous observations for ALS1060. During this period the OD also increased slowly at a growth rate less than 0.01 h^{-1}.

These batch processes using KD840 or ALS1060 were duplicated with the same overall results: glucose was consumed slowly after acetate was exhausted, xylose was only consumed in the presence of glucose, and KD840 showed slower glucose consumption than ALS1060.

Fed-batch growth on acetate in the presence of sugars

To determine whether the acetate-selective strains could continuously remove acetate, fed-batch processes were carried out using ALS1072 and KD840. ALS1072 is identical to ALS1060, but also is resistant to kanamycin, which permitted this antibiotic to be included in the medium to reduce the probability of contamination. During a batch process the cell growth rate is maximal because the cells are not limited by the availability of the carbon source acetate. In contrast, an acetate-limited fed-batch process controls the growth rate below its maximum rate and maintains the substrate (acetate) concentration near zero throughout the process. After an initial batch phase of about 20 h, xylose, glucose, and acetate were fed at a slow exponential rate for an additional 70 h.

ALS1072 consumed acetate during the batch phase and attained a specific growth rate of 0.13 h^{-1}. Glucose and xylose were not consumed during the batch phase (20 h), but were consumed slowly during the fed-batch phase (Fig. 6). Interestingly, succinate, which was not detected during batch growth, accumulated to a concentration of 5.5 g/L.

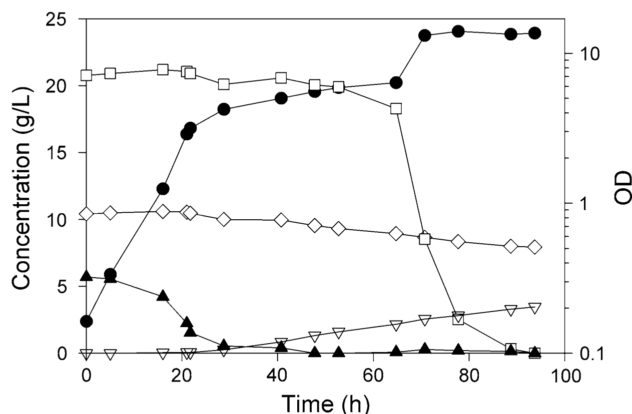

Fig. 7 Aerobic fed-batch culture of *E. coli* KD840 using a mixture of glucose (*open squares*), xylose (*open diamonds*), and acetate (*filled triangles*). OD (*filled circles*) and succinate (*inverted filled triangles*) were also measured over the course of the process

KD840 also consumed acetate during the initial batch phase and attained a specific growth rate of 0.13 h^{-1}. The concentrations of both sugars were unchanged during the batch phase, and also during the first 31 h of the fed-batch process (Fig. 7). The glucose concentration decreased rather quickly after about 62 h, becoming exhausted at about 95 h. Succinate again accumulated ultimately to a concentration of 3.5 g/L.

Discussion

Substrate-selective degradation of acetate from mixed sugars using metabolically engineered *E. coli* is a novel

strategy that could potentially be applied to the removal of this compound from lignocellulosic hydrolysates. *E. coli* ALS1060 with mutations in the phosphotransferase system (PTS) genes of glucose (*ptsG*, *manZ*), glucokinase (*glk*), and xylose (*xylA*) did preferably degrade acetate, though this strain consumed glucose slowly after acetate was exhausted. Furthermore, ALS1060 consumed xylose, but only in the presence of glucose.

With the goal of eliminating the consumption of glucose, strains containing an additional mutation in the *crr*, *malX*, *fruA*, *fruB*, or *bglF* gene were compared, and KD840 (*ptsG manZ glk xylA crr*) showed the greatest potential for eliminating glucose consumption. The *crr* gene encodes the $EIIA^{glu}$ protein involved in the PEP-phosphotransfer of glucose. The $EIIA^{glu}$ protein is also required for the transport of sucrose or maltose [21], and therefore the absence of the *crr* gene should prevent not only glucose but also sucrose and maltose uptake by the cells. The additional knockout of the *crr* gene did reduce but not eliminate the cells' ability to consume glucose, particular when this sugar was present alone. Considering that KD840 has knockouts in all the known genes involved in glucose uptake, it remains unclear how this organism is able to consume glucose at all.

The PTS plays a complex role in metabolism. For example the $EIIA^{glu}$ protein is non-phosphorylated when glucose is present in the medium, and in this state binds to non-PTS permeases and other enzymes such as glycerol kinase [23]. Similarly, non-phosphorylated $EIIB^{Glc}$ binds the Mlc repressor protein, relieving its repression from genes *ptsHI*, *ptsG*, *mlc*, *manXYZ*, and *malT* [24]. Non-phosphorylated EI binds to the chemotaxis protein CheA, inhibiting its autophosphorylation and thus causing smooth swimming [22]. In addition, protein IIA^{Glc} exerts negative control of expression for the gene encoding the σS subunit of RNA polymerase [22]. In summary, the PTS forms a complex regulatory network involved in coordinating cellular processes related to the cell's capacity to find, select, transport, and metabolize various carbon sources. Therefore, genetic alterations to PTS components can have wide-ranging effects on cell physiology. In the context of this current study, the central issues are whether the deletion of *xylA* and the genes in the glucose PTS prevent the consumption of these sugars and whether these deletions have little impact on acetate consumption. The results suggest that some of these gene deletions in the presence of sugars do reduce acetate consumption.

In batch experiments with ALS1060, the presence of xylose reduced the growth rate on acetate by 40% even though xylose was not significantly consumed. This result suggests that xylose interacts with acetate transport or metabolism in *E. coli*, perhaps by binding to a transport protein. Also, the addition of the *crr* knockout consistently reduced by 40% growth rate on acetate only in the presence of glucose (i.e., KD840 versus ALS1060), implying that the *crr* gene is involved with acetate metabolism in the presence of glucose. Although numerous PTS gene mutations are known to affect the consumption of glucose, acetate utilization has not been previously related to xylose uptake or to the PTSs in *E. coli* [25]. Although an acetate permease has been identified [13], acetate also freely diffuses across the *E. coli* cell membrane, is converted to acetyl CoA by either the phosphotransacetylase-acetate kinase or acetyl CoA synthetase pathways [3, 7, 26], and metabolized through the glyoxylate shunt, all processes which are not known to be affected by the presence of glucose or xylose.

Interestingly, succinate was observed in the fed-batch processes but not in the batch processes. A fed-batch process for the consumption of an inhibitor like acetate is an ideal operational mode because such a process can maintain a low concentration of inhibitor to avoid any toxicity. In each batch process the cells were not exposed to acetate again after the medium became exhausted with this substrate. In contrast, in the fed-batch process acetate was continually fed, so that the cells were also continuously metabolizing acetate at a rate equal to the rate acetate was being supplied. Thus, the practical difference between fed-batch and batch processes is the prolonged metabolism of acetate in the former, suggesting succinate is derived from acetate. Indeed, *E. coli* has been shown to synthesize succinate from acetate as a result of the glyoxylate shunt [36]. The presence of succinate does suggest that under these growth conditions *E. coli* is unable to oxidize NADH fast enough to prevent succinate from being transported out of the cell. Furthermore, the formation of succinate suggests that when acetate is present glucose and xylose remain the preferred substrates for biomass formation. That is, succinate accumulation may be explained by the presence of glycolysis in the consumption of glucose and xylose toward biomass precursors and the absence of gluconeogenesis in the utilization of acetate.

Although a batch process could be implemented to remove acetate selectively from sugar mixtures without significantly reducing the concentration of glucose or xylose, the results using a fed-batch process suggest that a prolonged continuous process might not be as successful. Glucose particularly was consumed at a higher rate in fed-batch after 60 h than was observed in any batch process. Microbial isolates withdrawn from the later stages of the fed-batch process were observed to metabolize glucose within 20 h when reinoculated into fresh acetate-glucose mixtures (results not shown), providing strong evidence that unknown genetic mutations occurred during the prolonged fed-batch process which reintroduced the ability to metabolize glucose. Additional research will be necessary

to characterize these mutations, and to determine whether additional knockouts can prevent the observed increase in glucose consumption these mutations cause.

Our work extends previous efforts to remove acetate and other inhibitors selectively from sugar mixtures. Schneider [28] used a similar approach with *S. cerevisiae*, an organism which would of course not naturally metabolize xylose anyway. In that study, 6.8 g/L acetate was nearly completely metabolized in 24 h with minimal sugar degradation. These authors used a high concentration of pre-grown cells as inoculum, and do not provide information on the rate of degradation or whether sugars were slowly consumed after acetate was depleted. A recent work of Fonseca et al. [12] also used hydrolysate, reporting 6.1% removal of 3.3 g/L acetate in 72 h, although the hydrolysate was concentrated by a factor of 5 in order to prevent significant sugar degradation. In the current study a synthetic medium containing a high concentration (10 g/L) acetate was inoculated with only 5% inoculum volume, attaining a high rate of degradation within 20 h.

Microbial acetate-selective removal from sugar mixtures compares favorably with other methods to remove acetate such as ion exchange or extraction. In contrast to ion exchange or extraction, this microbial process does not involve an additional unit operation or the associated costs for that equipment. Not only is this microbial method compatible with any subsequent microbial process to convert the sugar mixtures into a biochemical product, but the two envisioned microbial processes could also be conducted in a single vessel. Comparison of this method with a single-tolerant-microbe approach must be judged by the additional time necessary to remove the acetate (about 20 h for 10 g/L acetate in this unoptimized study) compared with any reduction in productivity caused by the presence of acetate for the single-organism approach (yeast without improved tolerance fails to grow in 10 g/L acetate [14]). Additionally, this method has the advantage of retaining a portion of the original carbon and other nutrients in the form of biomass which could serve as nutrients in a subsequent microbial process. The acetate has thus essentially been upgraded from an inhibitor to a nutrient. Neither methods which propose more acetate-tolerant organisms nor methods which remove acetate and other nutrients like extraction actually capture the microbial value of acetate. Finally, this method could be extended to other inhibitory compounds found in lignocellulosic hydrolysates.

The concept of using strains which can only metabolize a single carbon source in a mixture of carbon sources offers the prospect of selectively removing inhibitors such as acetate which are generated during a biomass hydrolysis process [10]. Our results demonstrate the feasibility of this approach using a batch process, in which the process would be halted after acetate exhaustion in order to prevent loss of xylose or glucose. Further study of the mechanisms of glucose and xylose metabolism in the mutant strains developed for this study may increase our understanding of sugar metabolism and lead to the development of a strain in which glucose and xylose consumption is completely eliminated and a more efficient fed-batch process.

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