0273-2289/94/4546—0367$04.00

Relative Rates of Sugar Utilization by an Ethanologenic Recombinant *Escherichia coli* Using Mixtures of Glucose, Mannose, and Xylose

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ABSTRACT

The volumetric rates of glucose (G), mannose (M), and xylose (X) utilization by recombinant *Escherichia coli* B (pLO1297) were compared in pH-stat batch fermentations with Luria broth containing various combinations of two of these sugars at differing mass ratios. Using single substrate media, the rates of glucose, mannose, and xylose utilization were 3.0, 0.8, and 1.5 g/L/h, respectively. With all two substrate media, hexose and pentose sugars were consumed simultaneously. At a mass ratio of 2:1 (M or X:G), the rate of glucose utilization was reduced to 1.7 and 1.2 g/L/h by mannose and xylose, respectively. In media containing glucose and xylose, the rate of xylose utilization was inhibited when the glucose component exceeded about 40% of the total sugar mass in the medium. At a mass ratio of 2M:1X, mannose did not inhibit the rate of xylose utilization. At a mass ratio of 1:2 (G or X:M), the rate of mannose utilization was unaffected by either glucose or xylose.

Synthetic media containing a mixture of hexose and pentose sugars were formulated to mimic different biomass hemicellulose hydrolysates. Relative to the rate in a single substrate medium, the respective rates of glucose and xylose utilization were 70% (2.1 g/L/h) and 40% (0.6 g/L/h) in a synthetic softwood prehydrolysate (SW) medium with a total reducing sugar (TRS) content of 45.7 g/L (20 wt% glucose, 30% xylose, and 50% mannose). However, the rate of mannose utilization in the SW medium was not inhibited. The respective rates of glucose and xylose utilization were 30% (0.9 g/L/h) and >90% (1.4 g/L/h) in a

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synthetic crop residue prehydrolysate (CR) medium with a TRS content of 46.9 g/L (10 wt% glucose, 73% xylose, and 17% arabinose).

Based on the results of this study, we suggest that the apparent "preference" for fermentation of hexose sugars by recombinant *E. coli* may be owing to the decreased rate of xylose transport caused by hexose sugars. Glucose is a more potent modulator of xylose utilization than mannose, but since xylose affects the rate of glucose utilization, this study also points to the importance of the concentration of the different sugars in terms of the relative rates of utilization by recombinant *E. coli*.

Index Entries: Fuel ethanol; recombinant *E. coli* B; sugar mixtures; xylose; mannose; arabinose; hemicellulose fermentation.

INTRODUCTION

The current practice of producing fermentation ethanol from sources of carbohydrate, principally starch and sucrose, which are valued as nutritional commodities (food or feed), results in a high-cost product that is seriously disadvantaged with respect to cost-effective competition in the transportation fuels marketplace (*1–3*). Although concerns centered on abating pollution and global warming are promoting interest in ethanol as an "environment-friendly" alternative transportation fuel, cost reduction is the prime driving force for R&D directed to bioconversion and fermentation technologies relating to the utilization of less expensive sources of fermentable carbohydrate (*4*).

Lignocellulosic biomass, including short rotation energy crops as well as agricultural, forestry, and municipal wastes, is considered an excellent alternative fermentation feedstock because it is inexpensive, plentiful, and renewable (*5,6*). The structure of lignocellulosic biomass is complex and consists primarily of three different polymeric substances: lignin, hemicellulose, and cellulose—the latter two being carbohydrates and the source of potentially fermentable sugars. Cellulose, which is a homopolymer of glucose and comprises about 50% of the dry mass, is strongly resistant to depolymerization unless it is pretreated (by a process known as prehydrolysis) to remove the impediments to enzymic digestion that are caused by lignin and the acetylated pentosan comprising the hemicellulose component of biomass (*7–9*). Unlike cellulose, hemicellulose is heterogeneous and thermochemical depolymerization is efficient and cost effective (*7,10–13*) producing mixtures of hexose and pentose sugars. Although the exact composition of a hemicellulose hydrolysate will depend on the nature of the feedstock, some generalizations are useful in formulating synthetic media with which to examine the fermentation performance of an ethanologenic microorganism in sugar mixtures. Hemicellulose in hardwood and agricultural crop residues in primarily composed of xylan,

but in softwood, the hemicellulose consists of a more complex mixture of sugars that is about 70% hexose (mannose, glucose, and galactose) and 30% pentose (xylose and arabinose) (*7*). The yeasts currently being used in starch and sucrose-based fermentations are unable to directly utilize pentose sugars (*14*) and although various pentose-utilizing ethanologenic organisms have been investigated, they all generally suffer from poor yield and productivity (*15,16*). Therefore, the cost of producing fuel ethanol from biomass and wastes can be significantly reduced by employing a biocatalyst capable of the efficient utilization of all the different sugars that are present in hemicellulose (*1–3*). Although pentose conversion is anticipated to have less economic impact when softwood is utilized as fermentation feedstock, it is important to test the fermentation performance of a potential process ethanologenic biocatalyst using softwood hydrolysates because coniferous woods are the main residues of forest industries including the potential opportunity to utilize the effluent streams from pulp mills that process primarily softwood feedstocks (*4*). Municipal wastes in the form of newspapers and cardboard packaging materials represent another softwood lignocellulosic resource for the production of fuel ethanol (*4*).

Recombinant DNA technology provides an opportunity to design and engineer a specific biocatalyst capable of the efficient fermentation of all the sugars present in lignocellulosic biomass (*17*). For the past three years, we have been conducting a systematic fermentation performance assessment of a patented (*18*), genetically engineered, *Escherichia coli* B (ATCC 11303 with the *pet* plasmid pLOI297) carrying the genes for both pyruvate decarboxylase and alcohol dehydrogenase II from *Zymomonas mobilis* (*19–22*) using both synthetic lab media (*23,24*) and biomass prehydrolysates prepared by different thermochemical processes from a variety of biomass/waste feedstocks, including both hardwood (aspen) (*25,26*) and softwood (pine) (*4*), newsprint (*4,27*), spent sulfite liquors (*28*), and corn crop residues (*29*).

Although fermentation performance testing of recombinant ethanologenic *E. coli* cultures has included hemicellulose hydrolysates from several different sources (*4,25–32*), the focus has been on yield, and the fermentation kinetics with respect to the individual component sugars has not been sytematically described. In addition to sugar-to-ethanol conversion efficiency (ethanol yield), productivity (i.e., the rate of fermentable sugar utilization) is included as one of the key techno-economic parameters used to assess fermentation performance of a candidate process biocatalyst (*1–4*).

The first step in sugar utilization is transport across the plasma membrane. Uptake is accomplished by various sugar-specific membrane proteins and the transport system can operate either in the direction of the concentration gradient (facilitated diffusion) or in opposition to the concentration gradient (active transport). To operate against the concentration

gradient requires energy, and depending on the mechanism and the form in which the energy is supplied, the membrane transport system is classified as either chemiosmotic or group translocation. Since chemiosmotic systems operate as H^+-sugar symporters (or OH^--sugar antiporters), they can be influenced by the pH of the medium. Since the operation of the sugar transport system can be rate limiting with respect to growth and metabolism, the regulation and kinetic characteristics of the various transport systems, the respect to both induction/repression and inhibition by other sugars, plays a very important role in determining the productivity of an ethanologenic biocatalyst in the presence of multiple fermentable substrates.

Under aerobic conditions, the order of preference with respect to the utilization of glucose and xylose by *E. coli* is well established whereby there is a sequential ordering (diauxie) such that glucose is used before xylose (*33*). However, under anaerobic conditions, the utilization of hexose (glucose and mannose) and pentose (xylose and arabinose) sugars by recombinant *E. coli* appears to be less tightly regulated (*31*) and the interactive relationship between hexose and pentose utilization is not understood. Using a buffered mineral salts medium that contained glucose and xylose in the mass ratio of 2.3:1, Lawford and Rousseau (*23*) demonstrated that the volumetric rate of xylose utilization was decreased by about 50%. Diauxie (biphasic growth) was not observed when recombinant *E. coli* was grown in Luria broth containing both glucose and xylose, and both sugars were used simultaneously although the rates of utilization relative to single substrate media were reduced (*24*). Perhaps the most convincing evidence regarding the ability of recombinant *E. coli* (pLOI297) to simultaneously utilize both glucose and xylose is provided by carbon-limited, steady-state continuous fermentations of a mixture of glucose (21.4 g/L) and xylose (12 g/L) (*24*).

Recombinant *E. coli* grows on mannose as sole carbon source at a rate that is only about one-half of the rate with xylose and only about one-quarter of the rate of glucose (*32*). Using single substrate media, Beall et al. (*34*) reported that the volumetric ethanol productivities for glucose, mannose, and xylose for *E. coli* B(pLOI297) were 1.7, 1.0, and 0.9 g ethanol/L/h, respectively. In a recent study, Lawford and Rousseau (*32*) reported volumetric productivities of 1.7, 0.36, and 0.67 g ethanol/L/h for glucose, mannose, and xylose, respectively.

The purpose of this study was to quantitatively describe the behavior of recombinant *E. coli* B (pLOI297) in nutrient-rich lab media containing two different sugars (glucose, mannose, or xylose) with respect to the rate of utilization of each sugar separately. Synthetic prehydrolysate media that contained different mixtures of hexose and pentose sugars were used to explore the phenomenon of xylose sparing (*30*) that has been observed with recombinant *E. coli* and certain hemicellulose hydrolysates (*4,30,31*).

MATERIALS AND METHODS

Organism

Recombinant *E. coli* B (ATCC 11303 carrying the *pet* plasmid pLOI297) (*20*) was a gift from L. O. Ingram (University of Florida, Gainsville, FL) and was maintained in an antibiotic-supplemented medium as described previously (*23*). Batch fermentation media were inoculated at an initial cell density of 30–50 mg dry wt cells/L (OD_{550} approx 0.1–0.2).

Fermentation Media

The principle culture medium was Luria broth (*35*), which was supplemented with 5 m*M* $MgSO_4$ and 17 m*M* phosphate and designated as sLB. Sugar (glucose, xylose, or mannose) was added at the concentration specified. Antibiotics were not added to the sLB medium. All media were sterilized by autoclaving. The magnesium, phospate, and sugar supplements were autoclaved separately. The pH was controlled at 6.3 or 7.0 as specified for each fermentation experiment. The synthetic softwood prehydrolysate medium (SW) was formulated to mimic a softwood hemicellulose hydrolysate and consisted of Luria broth to which glucose (8.9 g/L), xylose (13.6 g/L), and mannose (23.2 g/L) were added. The synthetic crop residue prehydrolysate medium (CR) was formulated to mimic a corn cob hemicellulose hydrolysate and consisted of Luria broth to which glucose (4.8 g/L), xylose (34.3 g/L), and arabinose (7.8 g/L) were added. Acetic acid was not added to these synthetic prehydrolysate media.

Fermentation Equipment

pH-stat batch fermentations were conducted in a volume of 1500 mL in MultiGen™ (model F2000) stirred-tank bioreactors fitted with agitation, pH, and temperature control (30°C) (New Brunswick Scientific Co., Edison, NJ).

Analytical Procedures

Growth of precultures was measured turbidometrically at 550 nm (1 cm lightpath) and culture dry weight was measured by microfiltration. Compositional analyses of fermentation media and cell-free spent media were determined by HPLC analysis using HPX-P and HPX-H columns (Bio-Rad Labs, Richmond, CA) as described previously (*23,24*).

Determination of Fermentation Parameters

The volumetric rate of sugar utilization (Q_s^{max}) was estimated from the maximum slope in plots of sugar concentration vs elapsed fermentation

time. The process product yield ($Y_{p/s}$) was calculated as the mass of ethanol produced (final concentration) per mass of sugar added to the medium.

RESULTS AND DISCUSSION

Figure 1 shows fermentation time-courses for recombinant *E. coli* B (pLOI297) with respect to the utilization of three different sugars, namely glucose, xylose, and mannose, using Luria broth that contained different combinations of these sugars. Figure 1A illustrates the effect of adding either xylose or mannose on the rate glucose consumption. Similarly, Fig. 1B illustrates the effect of adding either glucose or mannose on xylose utilization and Fig. 1C shows the effect of adding either glucose or xylose on mannose utilization. Figure 1 shows that the rate at which each sugar disappears from the medium tends to be relatively constant during the latter stage of the batch fermentation. From the linear relationships illustrated in Fig. 1, the volumetric rate of sugar utilization (Q_s^{max}) was calculated for each sugar in all fermentations. The respective Q_s^{max} values are summarized in Table 1.

In all the fermentations that were a part of this study, the product yield was near the theoretical maximum of 0.51 g/g (Table 1). The lowest sugar-to-ethanol conversion efficiency of 92% was associated with mannose fermentations (Table 1). The high yield observed with all three sugars corroborates the reports of others (22,34) with the exception that Beall et al. (34) reported that the highest yield (0.49 g/g) was associated with mannose and the lowest (0.47 g/g) was for xylose fermentation.

In order to provide a comparative reference point for describing the interactive effect of the different sugars on sugar transport and metabolism, control experiments were conducted and consisted of separate batch fermentations using individually each of the three sugars, glucose, xylose, or mannose (Fig. 1). The values for Q_s^{max} associated with glucose, xylose, and mannose utilization were 3.0, 1.5, and 0.8 g/L/h, respectively (Table 1). Previously, Beall et al. (34) reported that the same recombinant *E. coli* strain fermented mannose and xylose at the same rate, which was about one-half the rate of glucose utilization. Although Beall et al. (34) reported only volumetric rates of ethanol production associated with glucose, xylose, and mannose utilization, respective valuse for Q_s^{max} of 3.5, 1.9, and 2.0 g/L/h were derived from the reported product yield data.

Effect of Xylose and Mannose on Glucose Utilization

The effect of xylose on anaerobic glucose utilization by recombinant *E. coli* (pLOI297) was examined using Luria broth media in which the mass concentration ratio of xylose:glucose was either 1:2 or 2:1. The fermentation time-courses with respect to glucose consumption are shown in Fig. 1A (open square and triangle symbols) and the corresponding values for

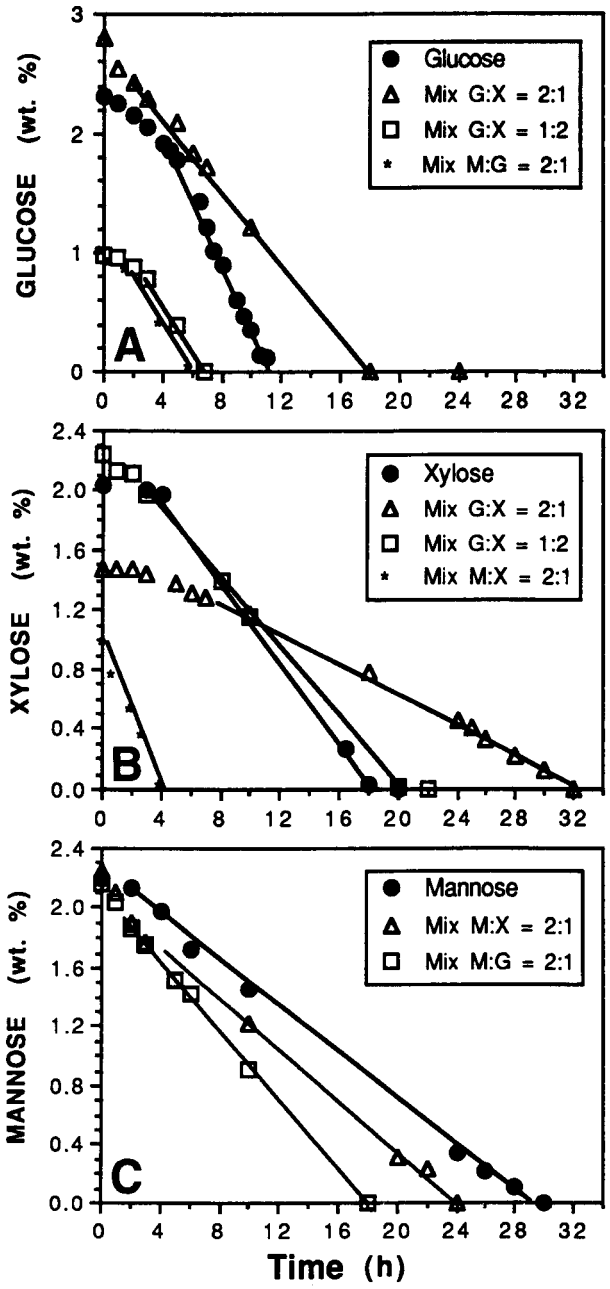

Fig. 1. Fermentation time-course with respect to sugar utilization by recombinant *E. coli* (**A**) glucose utilization; (**B**) xylose utilization; (**C**) mannose utilization. The mass ratios of the different sugars are indicated in the symbol legends. G = glucose; X = xylose; and M = mannose. The values for the volumetric and specific rates of sugar utilization are given in Table 1.

Table 1
Relative Rates of Glucose, Mannose, or Xylose Utilization
by Recombinant *E. coli* B (pLOI297) with Media Containing Various Sugar Mixtures

Medium composition	pH	Glucose, g/L	Xylose, g/L	Mannose, g/L	TRS, g/L	max. biomass, gDW/L	Q_s^{max}, g/L/h	$Y_{p/s}$ g/g
Luria broth supplemented with:								
Glucose	6.3	23.2	0	0	23.2	2.43	3.00	0.55
Glu:Xyl								
2:1	6.3	28.0					1.56	
			14.7		42.7	3.00	0.56	0.50
Xylose	6.3	0	20.4	0	20.4	1.49	1.54	0.49
Xyl:Glu								
2:1	7.0	9.9					1.18	
			22.4		32.7	2.36	2.00	0.50
Mannose	6.3	0	0	22.0	22.0	1.72	0.80	0.47
Man:Glu								
2:1	7.0	9.93					1.71	
				21.6	30.9	2.33	1.19	0.47
Man:Xyl								
2:1	7.0		9.9				2.17	
				22.4	32.3	2.01	0.90	0.48
Synthetic biomass prehydrolysates (supplemented Luria broth)								
Softwood	7.0	8.9					2.10	
			13.6				0.63	
				23.2	45.7	2.88	1.20	0.49
Crop Residue								
	7.0	4.8					0.92	
			34.3				1.42	
				7.8[a]	46.9	2.10	1.40[a]	0.49

TRS = Total reducing sugar (concentration); DW = dry weight.

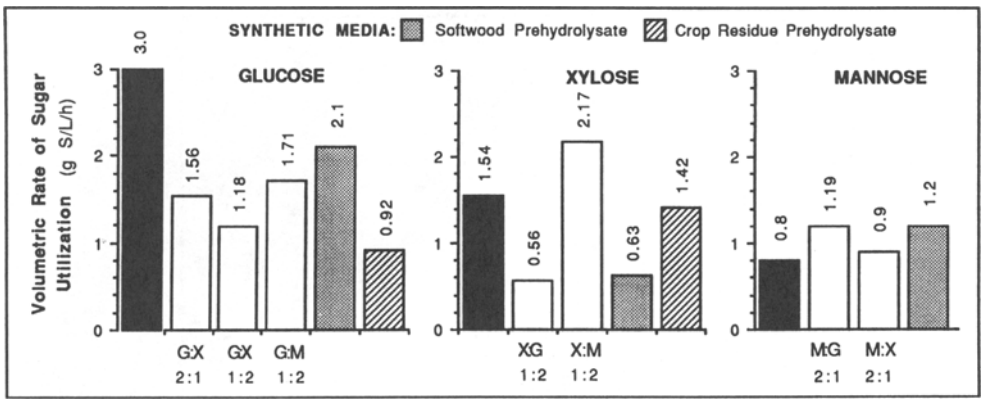

Fig. 2. Comparative volumetric rates of glucose, xylose, and mannose utilization by recombinant *E. coli*. The mass ratio of different sugars in media containing two sugars is indicated. The volumetric rate of utilization of the individual sugars for two different synthetic prehydrolysate media (softwood and crop residue) are included as per symbols indicated. G = glucose; X = xylose; and M = mannose.

Q_s^{max} are given in Table 1. The values for Q_s^{max} are compared graphically in Fig. 2. At both concentrations of xylose tested, the rate of glucose utilization (relative to the rate observed in the absence of added xylose) was decreased (Fig. 2).

In a similar fashion, the effect of mannose on glucose utilization was examined using a medium in which the mass concentration ratio of mannose:glucose was 2:1. The fermentation time-course with respect to glucose consumption is shown in Fig. 1A (star symbol), and the corresponding value for Q_s^{max} is given in Table 1 and plotted graphically in Fig. 2. Although the rate of glucose utilization is slowed in the presence of mannose, the degree of inhibition caused by mannose appears to be less severe than that caused by xylose at the same mass ratio (Fig. 2).

Effect of Glucose and Mannose on Xylose Utilization

A similar experimental design was employed to examine the effect of glucose and mannose on xylose utilization. The fermentation time-courses are shown in Fig. 1B and the values for Q_s^{max} are given in Table 1 and compared graphically in Fig. 2. At a mass ratio of glucose:xylose of 2:1 (Fig. 1B, open triange symbol), the rate of xylose utilization is only about 45% of the rate in the absence of added glucose (Fig. 2). This observation confirms earlier reports concerning the inhibitory effect of glucose on xylose utilization by this recombinant strain of *E. coli* (*23,24,30*).

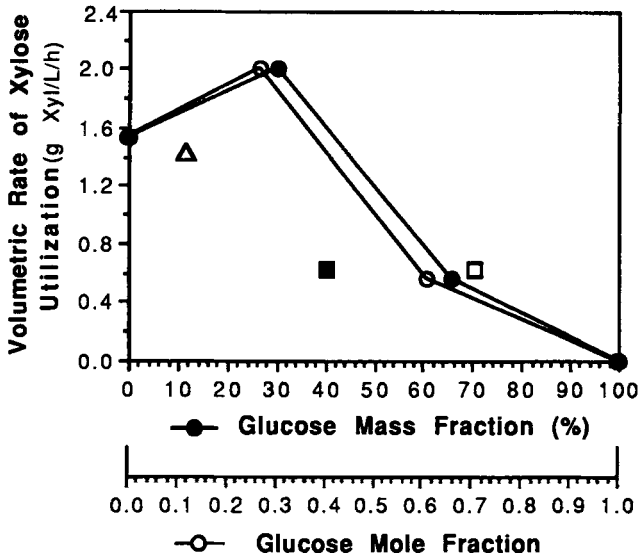

Fig. 3. The volumetric rate of xylose utilization as a function of the glucose mass or mole fraction. The glucose mass fraction (%) (solid circles) was calculated as the concentration of glucose divided by the total concentration of sugars in the medium (multiplied by 100). The open circle symbols represent the Q_s^{max} for xylose as a function of the glucose mole fraction. The solid square symbol represents the glucose mass fraction for the synthetic SW prehydrolysate medium based solely on the glucose and xylose content, whereas the open square symbol represents the hexose mass fraction. The open triangle represents the glucose mass fraction for the synthetic CR prehydrolysate medium.

Recombinant *E. coli* was constructed in response to the need for an ethanologen capable of producing ethanol from hemicellulose hydrolysates (*17–21*), and until recently the focus of attention has been directed primarily to efficient xylose utilization (*21–25*) because xylose is the major component of hemicellulose from the lignocellulosic feedstocks originally targeted for fuel ethanol production (*3,5*). From an operational engineering perspective, what is of practical importance in terms of productivity is a model that predicts the degree of inhibition of xylose utilization by another sugar or sugars. Furthermore, to be of practical utility, the relationship should be described in terms of the mass concentrations of the fermentable sugars. Figure 3 illustrates graphically the effect of glucose on the rate of xylose utilization in terms of the mass of glucose relative to the total mass of glucose and xylose in the medium (i.e., glucose mass fraction as % glucose). Inhibition of xylose utilization did not occur until the glucose component exceeded about 30% of the sugar mass in the medium (Fig. 3). Xylose utilization was 65% inhibited when glucose represented two-thirds (66%) of the sugar mass in the medium (Fig. 3).

Both glucose and xylose enter *E. coli* by means of energy-requiring specific carrier-mediated membrane transport systems (*33,36*). Whereas glucose enters *E. coli* by group translocation (*36*), xylose is transported across the membrane by proton symport (*38*). From a biochemical perspective, membrane transport is viewed in terms of the molar concentration of the substance being transported as well as the potential interfering substance. For this reason, the effect of glucose on xylose utilization is also represented in terms of the glucose mole fraction (Fig. 3). Assuming that membrane transport is rate limiting for xylose utilization, xylose uptake is inhibited when the molar ratio of glucose:xylose exceeds 1:4 (Fig. 3). Xylose utilization is 50% inhibited at equimolar concentrations of glucose and xylose (i.e., glucose mole fraction = 0.5) (Fig. 3). The nature of these data is not instructive regarding the possible mechanism of the inhibition of xylose utilization by glucose, but since both sugars are transported in an energy-dependent manner, it is conceivable that there is competition for a common source of energy.

In contrast to the effect of glucose on xylose utilization, mannose does not inhibit the rate of xylose utilization even at a mannose:xylose mass ratio of 2:1 (Fig. 1B, star symbol; Fig. 2).

Effect of Glucose and Xylose on Mannose Utilization

The effect of either glucose or xylose on mannose utilization was examined using media in which the mass concentration of the additional sugar was half that of mannose (i.e., mass ratio of mannose:glucose or xylose was 2:1). The fermentation time courses are shown in Fig. 1C. The values for Q_s^{max} are given in Table 1 and compared graphically in Fig. 2. Comparison of the Q_s^{max} values reveals that, at these specified concentration ratios, neither glucose nor xylose caused the rate of mannose utilization to be decreased, but to the contrary, both sugars produced a slightly enhanced rate of mannose utilization (Table 1 and Fig. 2).

Fermentation of Synthetic Prehydrolysate Media

Figure 4 shows the time-course of a batch fermentation by recombinant *E. coli* using a synthetic prehydrolysate medium (SW) formulated to mimic a softwood (pine) hemicellulose hydrolysate in which the total reducing sugar concentration was 45.7 g/L (20 wt% glucose, 30 wt% xylose, and 50 wt% mannose). The ethanol yield together with the volumetric rates of utilization for the individual sugars are summarized in Table 1. For comparison purposes, the Q_s^{max} value for each of the sugars is presented in Figure 2. The Q_s^{max} associated with both glucose and xylose is decreased relative to the Q_s^{max} exhibited in single substrate media, but the Q_s^{max} associated with mannose in the SW medium is somewhat higher than the corresponding Q_s^{max} value exhibited with mannose as the sole substrate (Fig.

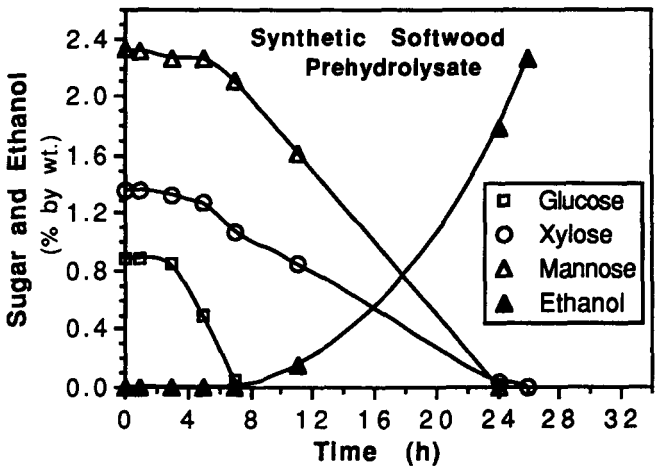

Fig. 4. Fermentation of synthetic softwood prehydrolysate by recombinant *E. coli.*

2). These observations with the more complex synthetic prehydrolysate medium containing all three sugars are consistent with the observed interactive effects using the various two sugar media. However, concerning the effect of glucose on xylose utilization, the relationship represented in Fig. 3 in terms of the Q_s^{max} for xylose and the glucose mass fraction proved not to be useful in predicting the rate of xylose utilization. When expressed as percentage of the combined concentrations of glucose and xylose, at 40% glucose the rate of xylose utilization is considerably less than would be predicted from the model relationship described in Fig. 3 (filled square symbol). However, when glucose and mannose together are expressed as percentage of the total reducing sugar, at 70% hexose (instead of glucose), the observed Q_s^{max} for xylose is close to the value predicted by the model relationship described in Fig. 3 (open square symbol). Although this interpretation implies a role for mannose in decreasing the rate of xylose utilization in the SW medium, this is not consistent with the observations in connection with the two substrate fermentations in which mannose did not appear to affect xylose utilization even at a mass ratio of 2:1 (Fig. 2 and Table 1).

Figure 5 shows the fermentation time-course using a synthetic crop residue prehydrolysate medium (CR) formulated to mimic a corn cob hemicellulose hydrolysate in which the total reducing sugar concentration was 46.9 g/L (10 wt% glucose, 73 wt% xylose, and 17 wt% arabinose). The ethanol yield together with the volumetric rates of utilization for the individual sugars are summarized in Table 1. The arabinose is consumed at a rate that is similar to xylose (Fig. 5). Since the ethanol yield (based on the total reducing sugar content of the medium) is close to the theoretical maximum, it is concluded that the arabinose is efficiently converted to ethanol by the recombinant *E. coli.* The rate of xylose utilization in the CR

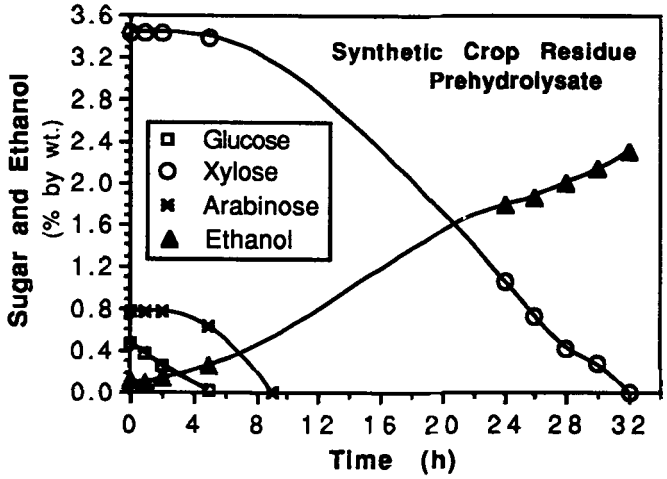

Fig. 5. Fermentation of synthetic crop residue prehydrolysate by recombinant *E. coli*.

medium is similar to the rate observed when xylose was the sole substrate and the inhibition of glucose utilization in the CR medium is consistent with the effect of xylose on glucose utilization that was observed with the two substrate media (Fig. 2).

Fermentation of Biomass Prehydrolysates

Beall et al. (*30*) observed that xylose utilization was incomplete (50% xylose remaining after 3 d) in the fermentation of an acid hydrolysate of corn hull hemicellulose (73 wt% glucose and 17 wt% xylose) by recombinant *E. coli* (strain KO11). From studies with model fermentation media (data not shown), it was concluded that "xylose sparing is a problem for strain KO11 when the glucose content exceeds 50% of the fermentable sugar" (*30*). Whereas xylose is the major component of hardwood hemicellulose hydrolysates, mannose predominates in softwood prehydrolysates (*8*). In contrast to the fermentation of hardwood prehydrolysates (aspen) by recombinant *E. coli* (*25*), fermentation of softwood prehydrolysates is protracted owing to the much slower utilization of xylose (*4,23, 27,31*). This observation led Barbosa et al. (*31*) to suggest that glucose and hexose sugars are used *preferentially* by ethanologenic *E. coli*. Unfortunately, the nature of the kinetic data reported by Barbosa et al. (*31*) does not permit one to distinguish between complete or partial inhibition of xylose utilization by the hexose sugars. The term "used preferentially" (*30,31*) is ambiguous because, in the context of aerobic sugar metabolism by *E. coli*, "preferred substrate" (*31*) is interpreted as meaning the utilization of one sugar to the exclusion of all others until that "preferred" sugar has been completely exhausted from the medium. Under aerobic conditions, this type of sequential sugar utilization is reflected in diauxie (biphasic

growth), and the biochemical mechanism responsible for this phenomenon involves the repression and/or induction of specific enzymes and membrane transporters. However, diauxic growth has not been observed for recombinant *E. coli* (pLOI297) fermenting a mixture of glucose and xylose (*24*). This work with synthetic media, together with previous studies employing mixtures of glucose and xylose (*23,24*), clearly establishes that there is not a sequential order in the anaerobic utilization of hexose and pentose sugars whereby the consumption of pentose sugars does not commence until the complete exhaustion of hexose sugars from the medium. The complete inhibition of either hexose or pentose sugar utilization was never observed during this investigation, and we conclude that, under anaerobic conditions, hexose and pentose sugars are consumed simultaneously by recombinant *E. coli*. In the absence of any evidence of either induction or repression, we suggest that the apparent *preference* for fermentation of hexose sugars by recombinant *E. coli* may be owing to the decreased rate of xylose transport caused by hexose sugars. Glucose has been shown to be a more potent modulator of xylose utilization than mannose; however, it is not known whether the biochemical mechanism involves a direct or indirect interaction with the membrane carrier responsible for xylose transport. Since it has also been shown that xylose can affect the rate of glucose utilization, the results of this study also point to the importance of the concentration of the different sugars in terms of the relative rates of utilization by recombinant *E. coli*.

ACKNOWLEDGMENTS

This research was supported by an Operating Grant from the Natural Sciences and Engineering Research Council of Canada and, in part, by funding from Energy, Mines and Resources Canada. We are grateful to Lonnie Ingram for the recombinant *E. coli* B(pLOI297).

REFERENCES

1. Wright, J. D. (1988), *Chem. Eng. Progress* **84,** 62–68.
2. Wyman, C. E. and Hinman, N. D. (1990), *Appl. Biochem. Biotechnol.* **24/25,** 735–753.
3. Bull, S. R. (1991), in *Energy from Biomass & Wastes XIV,* Klass, D. L., ed., Institute of Gas Technology, Chicago, IL, pp. 1–14.
4. Lawford, H. G. and Rousseau, J. D. (1993), in *Energy from Biomass & Wastes XVI,* Klass, D. L., ed., Institute of Gas Technology, Chicago, IL, pp. 559–597.
5. Lynd, L. R. (1990), *Appl. Biochem. Biotechnol.* **24/25,** 695–719.
6. Lynd, L. R., Cushman, J. H., Nichols, R. J., and Wyman, C. E. (1991), *Science* **251,** 1318–1323.
7. Grethlein, H. E. (1985), *Bio/Technology* **3,** 155–160.
8. Grohmann, K., Himmel, M., Rivard, C., Tucker, M., Baker, J., Torget, R., and Graboski, M. (1984), *Biotech. Bioeng. Symp.* **14,** 139–157.

9. Kong, F., Engler, C. R., and Soltes, E. (1992), *Appl. Biochem. Biotechnol.* **34/35,** 23–35.
10. Grethlein, H. E., Allen, D. C., and Converse, A. O. (1984), *Biotech. Bioeng.* **26,** 1498–1505.
11. Grohmann, K., Torget, R., and Himmel, M. (1986), *Biotechnol. Bioeng. Symp.* **17,** 135–151.
12. Torget, R., Werdene, P., Himmel, M., and Grohmann, K. (1990), *Appl. Biochem. Biotechnol.* **24/25,** 115–126.
13. Torget, R., Walter, P., Himmel, M., and Grohmann, K. (1991), *Appl. Biochem. Biotechnol.* **28/29,** 75–86.
14. Jefferies, T. W. (1981), *Biotechnol. Bioeng. Symp.* **11,** 315–324.
15. Skoog, K. and Hahn-Hägerdal, B. (1988), *Enzyme Microbiol. Technol.* **10,** 66–88.
16. Prior, B. A., Kilian, S. G., and du Preez, J. C. (1989), *Process Biochem.* **24,** 21–32.
17. Ingram, L. O., Alterthum, F., Ohta, K., and Beall, D. S. (1990), in *Developments in Industrial Microbiology,* Pierce, G. E., ed., vol. 31, Elsevier, New York, pp. 21–30.
18. Ingram, L. O., Conway, T., and Alterthum, F. (1991), United States Patent 5,000,000.
19. Ingram, L. O. and Conway, T. (1988), *Appl. Environ. Microbiol.* **54,** 397–404.
20. Alterthum, F. and Ingram, L. O. (1989), *Appl. Environ. Microbiol.* **55,** 1943–1948.
21. Ingram, L. O. (1991), in *Energy from Biomass & Wastes XIV,* Klass, D. L., ed., Institute of Gas Technology, Chicago, IL, pp. 1105–1126.
22. Ohta, K., Alterthum, F., and Ingram, L. O. (1990), *Appl. Environ. Microbiol.* **56,** 463–465.
23. Lawford, H. G. and Rousseau, J. D. (1991), *Appl. Biochem. Biotechnol.* **28/29,** 221–236.
24. Lawford, H. G. and Rousseau, J. D. (1992), in *Energy from Biomass & Wastes XV,* Klass, D. L., ed., Institute of Gas Technology, Chicago, IL, pp. 583–622.
25. Lawford, H. G. and Rousseau, J. D. (1991), *Biotechnol. Letts.* **13,** 191–196.
26. Lawford, H. G. and Rousseau, J. D. (1992), *Appl. Biochem. Biotechnol.* **34/35,** 185–204.
27. Lawford, H. G. and Rousseau, J. D. (1993), *Biotechnol. Letts.* **15,** 505–510.
28. Lawford, H. G. and Rousseau, J. D. (1993), *Appl. Biochem. Biotechnol.* **39/40,** 667–685.
29. Lawford, H. G. and Rousseau, J. D. (1992), *Biotechnol. Letts.* **14,** 421–426.
30. Beall, D. S., Ingram, L. O., Ben-Bassat, A., Doran, J. B., Fowler, D. E., Hall, R. G., and Wood, B. E. (1992), *Biotechnol. Letts.* **14,** 857–862.
31. Barbosa, M. de F. S., Beck, M. J., Fein, J. E., Potts, D., and Ingram, L. O. (1992), *Appl. Environ. Microbiol.* **58,** 1382–1384.
32. Lawford, H. G. and Rousseau, J. D. (1993), *Biotechnol. Letts.* **15,** 615–620.
33. Roseman, S. and Meadow, N. D. (1990), *J. Biol. Chem.* **265,** 2993–2996.
34. Beall, D. S., Ohta, K., and Ingram, L. O. (1991), *Biotechnol. Bioeng.* **38,** 296–303.
35. Luria, S. E. and Delbruck, M. (1943), *Genetics* **28,** 491–511.
36. Gottschalk, G. (1985), in *Bacterial Metabolism,* Springer-Verlag, New York.
37. Furlong, C. E. (1987), in *Escherichia coli and Salmonella typhimurium—Cellular and Molecular Biology,* Neidhart, F. C., ed., Am. Soc. Microbiol. Washington, DC, pp. 768–796.
38. Lam, V. M. S., Daruwalla, K., Henderson, P. D. F., and Jones-Mortimer, M. C. (1980), *J. Bacteriol.* **143,** 396–402.