0273-2289/95/5152—0179$07.40

Comparative Energetics of Glucose and Xylose Metabolism in Ethanologenic Recombinant *Escherichia coli* B

HUGH G. LAWFORD* AND JOYCE D. ROUSSEAU

*Department of Biochemistry, University of Toronto,
Toronto, Ontario, Canada M5S 1A8*

ABSTRACT

This study compared the anaerobic catabolism of glucose and xylose by a patented, recombinant ethanologenic *Escherichia coli* B 11303:pLOI297 in terms of overall yields of cell mass (growth), energy (ATP), and end product (ethanol). Batch cultivations were conducted with pH-controlled stirred-tank bioreactors using both a nutritionally rich, complex medium (Luria broth) and a defined salts minimal medium and growth-limiting concentrations of glucose or xylose. The value of Y_{ATP} was determined to be 9.28 and 8.19 g dry wt cells/mol ATP in complex and minimal media, respectively. Assuming that the nongrowth-associated energy demand is similar for glucose and xylose, the mass-based growth yield ($Y_{x/s}$, g dry wt cells/g sugar) should be proportional to the net energy yield from sugar metabolism. The value of $Y_{x/s}$ was reduced, on average, by about 50% (from 0.096 g/g glu to 0.051 g/g xyl) when xylose replaced glucose as the growth-limiting carbon and energy source. It was concluded that this observation is consistent with the theoretical difference in net energy (ATP) yield associated with anaerobic catabolism of glucose and xylose when differences in the mechanisms of energy-coupled transport of each sugar are taken into account. In a defined salts medium, the net ATP yield was determined to be 2.0 and 0.92 for glucose and xylose, respectively.

Index Entries: ATP yield; xylose; recombinant *E. coli*; ethanol; bioenergetics; defined medium.

---

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

INTRODUCTION

Xylose is a major constituent of hemicellulose (*1,2*), and its efficient utilization represents a major obstacle to economic large-scale fermentation fuel ethanol production from lignocellulosic biomass and wastes (*3,4*). The facultative gram-negative bacterium *Escherichia coli* is capable of utilizing a broad spectrum of carbon substrates, but it is heterofermentative (*5,6*). Under anaerobic conditions, catabolism of sugars, such as glucose and xylose, can lead to the generation of several different end products. Wild-type *E. coli* contains low levels of native alcohol dehydrogenase, but pyruvate decarboxylase is absent (*7*), and hence, ethanol is not a major end product. Under anaerobic conditions, lactic acid is the predominant end product (*5,6*). Recombinant DNA technology has been employed recently to engineer *E. coli* metabolically into a homofermentative ethanologen (*8–10*). Recombinant *E. coli*, carrying a multicopy plasmid containing cloned genes for both pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) from *Zymomonas mobilis* (the *pet* operon), converts hemicellulose-derived sugars to ethanol at near maximum theoretical efficiency (*8–12*). Although much of the pioneering metabolic engineering with *E. coli* was done with derivatives of strain K12 (*8–12*), most of the subsequent research and development have focused on *E. coli* B (ATCC 11303) as the host strain (*13–22*). The high affinity (low K_m) of the *Zymomonas* PDC for pyruvate (23), coupled to its abundance owing to high expression from the multicopy vector, results in the effective direction of pyruvate metabolism to ethanol as the near exclusive end product (*13–22*). Figure 1 illustrates diagrammatically the metabolism of glucose and xylose by ethanologenic recombinant *E. coli*.

Bioenergetic Parameters— Energy Conservation and Consumption

An understanding of the physiological characteristics of microorganisms, including factors that regulate carbon and energy metabolism during growth, can furnish useful information when engineering a bioconversion processes involving different substrates (*24,25*). Growth yield measurements can provide useful information concerning the relationship between substrate (carbon source) utilization, energy conservation (production of high-energy phospho-metabolites, such as ATP, phosphenolpyruvate [PEP], and acetylphosphate), and the synthesis of cell mass (growth) (*26–28*). During growth, a portion of the carbon source is diverted to cell mass, whereas the remainder is metabolized for the purpose of generating energy and conserving it in a biologically useful form (Fig. 2 adapted from ref. 26). Substrate-level phosphorylations (SLP) catalyzed by 3-phosphoglycerate kinase, pyruvate kinase, acetate kinase, and PEP carboxykinase are responsible for energy conservation (ATP production) during anaerobic metabolism by *E. coli* (Fig. 2). The energy-con-

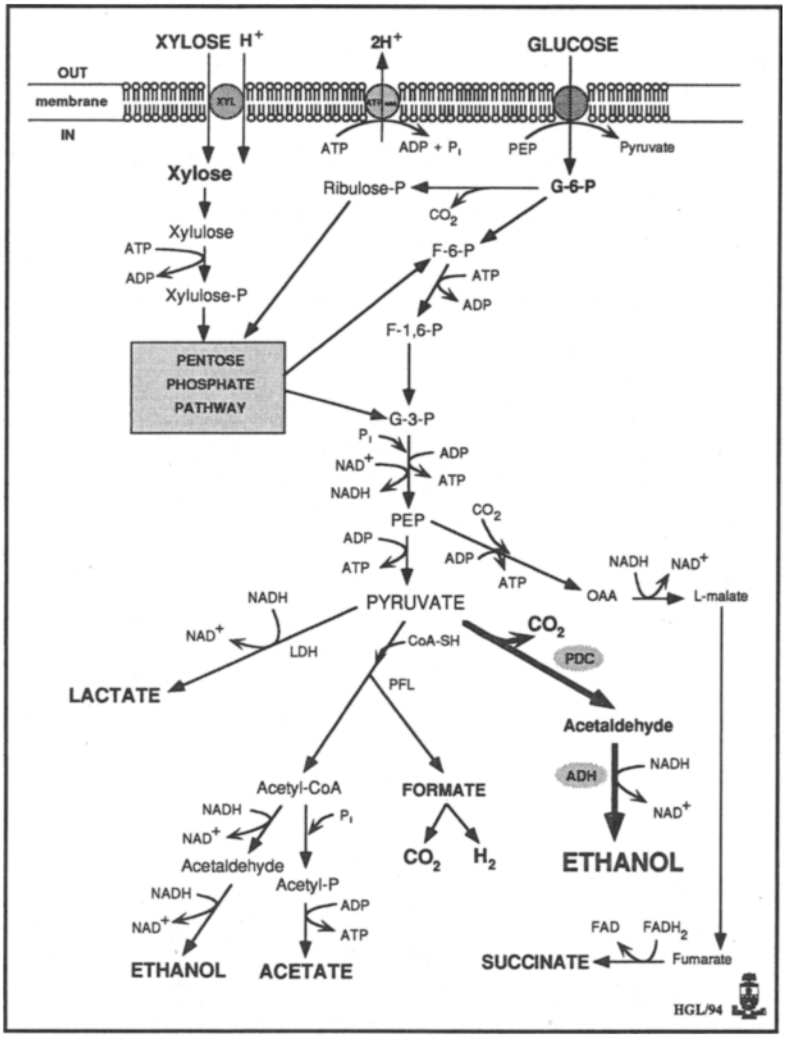

Fig. 1. Anaerobic metabolism of glucose and xylose by recombinant *E. coli*. The plasmid-borne *Zymomonas* genes for pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) catalyze the ethanol production pathway shown in bold arrows. Membrane-transporting elements responsible for (1) xylose-proton symport, (2) proton export by the ATPase, and (3) glucose uptake by the glucose phosphotransferase system are shown as filled circles embedded in the membrane phospholipids. Abbreviations used are: LDH, lactate dehydrogenase; PFL, pyruvate-formate lyase; PEP, phosphoenolpyruvate; G-3-P, glyceraldehyde-3-phosphate.

suming processes can be collectively referred to as the "work" performed by the living cell (*26*). These processes can be classified as chemical (e.g., biosynthesis), osmotic (e.g., active transport), and mechanical (e.g., movement) (*26*), but for the purposes of this investigation, they are classified as either growth-associated or nongrowth-associated (i.e., all others) (Fig.

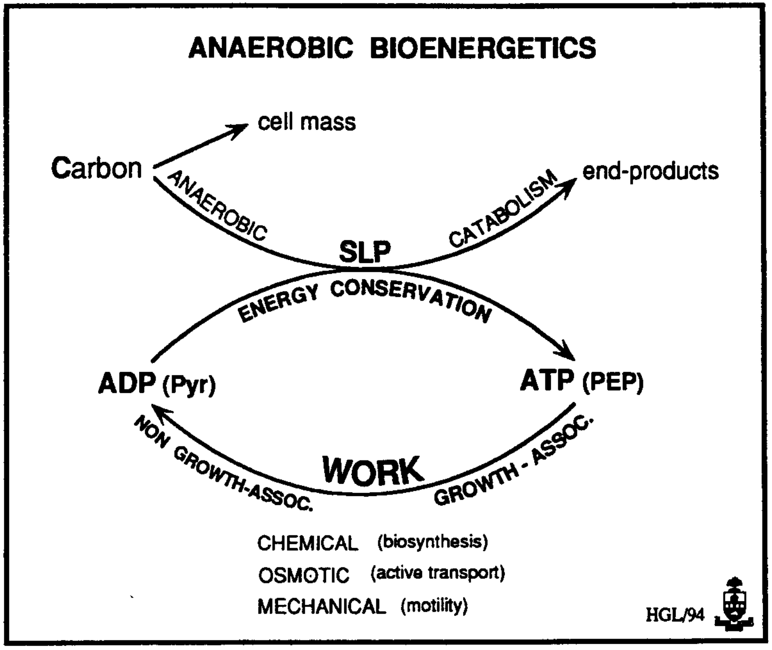

Fig. 2. Anaerobic bioenergetics. Abbreviations used are: SLP, substrate-level phosphorylations; Pyr, pyruvate; PEP, phosphoenolpyruvate. The arrows representing sugar catabolism and energy conservation are described fully in Fig. 1.

2). One of the axioms of modern microbial bioenergetics is that during growth, the consumption of energy is partly growth dependent and partly growth-independent (*26–29*). Energy that is consumed independent of growth is often referred to as "maintenance energy" (*26–29*), and Pirt (*29*) has described a method for estimating the "maintenance energy" requirement on the basis of the relationship between the specific rate of sugar utilization and the specific growth rate in carbon-limited, steady-state, continuous cultures.

Thauer et al. (*26*) point out that "maintenance" is a poor descriptive for this portion of the energy that is consumed independent of growth (chemical work or biosynthesis), because it involves much more than maintenance functions and includes such functions as osmotic and mechanical work. Hill et al. (*25*) have included a separate term called "wastage" energy to denote that portion of the energy that is recognized by others (*30,31*) to be clearly neither growth-related nor maintenance energy.

The amount of cell mass produced from a given amount of growth-limiting carbon and energy source (substrate) will depend on the amount of energy generated during catabolism and the amount of energy required for the synthesis of cell mass. The amount of ATP required to synthesize 1 g (dry wt) of bacterial cells is equal to $1/Y_{ATP}$. The concept of Y_{ATP} was introduced in 1960 by Bauchop and Elsden (*32*), and has since been the subject of several reviews (*27,33–35*).Although, at first, Y_{ATP} was believed to be a biological constant with an average value of 10.5 g dry wt cells/mol ATP (*32,36*), more extensive testing under diverse environmental conditions and involving more microorganisms has suggested that Y_{ATP} is not a constant (*26–28*). It has been postulated (*27–29*) that a possible explanation for the wide variance in values for Y_{ATP} and the discrepancy between experimental and theoretical Y_{ATP} values (*27*) lies in the fact that no account is made for the energy required to perform functions that are independent of growth. If, for example, under certain environmental conditions, more energy were to be diverted to performing such "work," less energy would be available for growth and the value for Y_{ATP} would be diminished proportionately. It has been estimated that about 50% of the energy consumed is not related to biosynthetic reactions or growth (*26–28*).

Disregarding the involvement of the portion of the conserved energy that is consumed independent of growth, the value of Y_{ATP} can be calculated from the following relationship (*27,28*):

$$Y_{ATP}\ (\text{g dry wt cells/mol ATP}) = (\text{molar } Y_{x/s}/G_{ATP}) \qquad (1)$$

where $Y_{x/s}$ represents the molar growth yield coefficient (g dry wt cells/mol carbon substrate) and G_{ATP} represents the net ATP gain (mol ATP produced/mol of substrate fermented).

Calculating the Net Yield of Energy from Metabolism

Determination of the value of the net yield of energy, or ATP gain (G_{ATP}), associated with carbon substrate catabolism depends on a complete understanding of the metabolic fate of the growth-limiting carbon (energy) source (Fig. 1). According to Stouthamer, "only under anaerobic conditions can the ATP yield for substrate breakdown be calculated exactly, because the catabolic pathways for anaerobic breakdown of substrates are known" (p. 13 in ref. 27). Metabolic engineering by recombinant DNA technology provides an opportunity to simplify a complex metabolic pathway through the introduction of well-defined perturbations (*24*). In the case of *E. coli*, the transformation of the organism from heterofermentative to homofermentative through the expression of cloned *Zymomonas* genes for PDC and ADH (*11*) greatly facilitates the determination of the ATP gain by means of end-product analysis (Fig. 1). This is especially the case where glucose is used as carbon source, because the transport and metabolism of glucose under anaerobic conditions are well documented in the literature (*36,37*). In marked contrast to the vast literature concerning growth with glucose, very little has been done using xylose.

In this study, the growth yields of recombinant ethanologenic *E. coli* growing anaerobically in pH-controlled batch cultivations with growth-limiting concentrations of either glucose or xylose were compared using both a nutrient-rich complex medium and a defined mineral salts medium.

Assuming that the Y_{ATP} was similar for each type of fermentation medium, the growth yield for the different sugars should be a direct reflection of the net yield of energy associated with the catabolism of the sugar used to support growth (*see* Eq. [1]).

MATERIALS AND METHODS

Organism

Recombinant *E. coli* B (ATCC 11303 carrying the *pet* plasmid pLOI297) (*13*) was a gift from L. O. Ingram (University of Florida, Gainesville, FL) and was maintained in an antibiotic-supplemented medium as described previously (*18*).

Preparation of Inocula

Single colony isolates were transferred to about 100 mL of complex medium (LB) containing added sugar (about 2% glucose or xylose) in 125-mL screw-cap flasks and grown overnight at 30°C and low agitation in a shaker water bath. In the case of the plasmid-bearing culture, the medium was supplemented with antibiotics (40 mg/L ampicillin and 10 mg/L tetracycline). Batch fermentations were inoculated by transferring approx 100 mL of the overnight LB culture directly to 1400 mL of medium in the stirred-tank bioreactor. The same sugar was used for preculture and fermentation. The initial cell density was monitored spectrophotometrically to give an OD_{550} in the range 0.1–0.2 corresponding to 30–50 mg dry wt cells/L.

Fermentation Media

The nutrient-rich, complex culture medium was Luria broth (LB) (*38*), and the defined salts medium (DS) was as described previously (*18*). Sugar (glucose or xylose) was added at the concentraton specified. Antibiotics were not added to the fermentation media. All media were sterilized by autoclaving. The sugar supplements were autoclaved separately.

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). The pH was controlled at 6.3 by the addition of 2*N* KOH.

Analytical Procedures

Growth 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 using an HPX-H column (Bio-Rad Labs, Richmond, CA) as described previously (*18*).

Determination of Fermentation and Bioenergetic Parameters

The ethanol (product) yield ($Y_{p/s}$) was calculated as the mass of ethanol produced (final concentration) per mass of sugar added to the medium, and was not corrected for the dilution caused by the addition of alkali. In calculating $Y_{p/s}$, no account was made for the assimilable/fermentable carbon present in the complex LB medium. The mass-based cell yield (obs. $Y_{x/s}$, where s = glucose or xylose) was calculated by dividing the maximum cell density (dry wt cells/L) by the concentration of sugar consumed. When LB was used as the fermentation medium, the cell yield was corrected for the contribution of the LB nutrients to the total cell mass (cor. $Y_{x/s}$). This value was experimentally determined to be 0.41 g dry wt cells/L.

Fermentations with Glucose

The net ATP gain (G_{ATP}) was calculated as mol ATP/mol of sugar metabolized to ethanol and mixed-acid end products. For the purpose of calculating G_{ATP}, the amount of sugar utilized was adjusted for the amount of sugar diverted to cell mass. The value of G_{ATP} was based on the assumption that 1 mol of ATP was produced for each mol of ethanol, lactic acid, and succinic acid, and that 2 mol of ATP were produced/mol of acetic acid. The value for Y_{ATP} (g dry wt cells/mol ATP) was determined by dividing the molar cell yield for glucose by the ATP gain associated with glucose catabolism, as shown below:

$$Y_{ATP}\ (\text{g dry wt cells/mol ATP}) = (\text{obs. } Y_{x/\text{glu}} \cdot 180/(\text{glu})\, G_{ATP}) \qquad (2)$$

Fermentations with Xylose

It was assumed that the value of Y_{ATP} was constant, and therefore, the net ATP gain associated with xylose catabolism was calculated as follows:

$$(\text{xyl})\, G_{ATP}\ (\text{mol ATP/mol Xyl}) = (\text{obs. } Y_{x/\text{xyl}} \cdot 150/Y_{ATP}) \qquad (3)$$

Carbon Balancing and Determination of %C Recovery

Carbon balances were calculated as described previously (*39,40*). The amount of carbon (C) in the sugar added (as carbon and energy source) and end products is presented as meq C (mol wt divided by number of carbons). For cell mass, meq C was determined as the dry wt cells divided by 0.0246 (*40*). For growth in LB media, the max biomass (g dry wt cells/L) was adjusted for the contribution from LB nutrients by subtracting 0.41 g dry wt cells/L. It was assumed that 1 mol CO_2 was produced/mol of ethanol or acetic acid. No account was made for the possible conversion of formic acid to CO_2 and H_2 or the consumption of CO_2 in the production of succinic acid.

RESULTS AND DISCUSSION

Almost all the previous research with recombinant ethanologenic *E. coli* strains has employed complex media (usually LB) (*10–17*). Nutrient-rich complex media are used in applied microbiology and industrial processes, because they promote rapid and robust growth, but this advantage is overshadowed by the compositional uncertainties in investigations of a fundamental metabolic nature. In this study, a complex medium (LB) was employed to provide a point of comparison with reference to the work of others (*13–17*); however, because carbon balancing is made easier through the use of defined salts media, this type of medium, in which the added sugar was the sole source of carbon and energy (*18*), was also employed in this investigation into the energy yield associated with xylose metabolism in *E. coli*.

Fermentations with a Nutritionally Rich Complex Medium

Figure 3 illustrates typical time-courses of an anaerobic batch fermentations with recombinant *E. coli* B (11303:pLOI297) conducted in the controlled environment of a stirred bioreactor with the temperature at 30°C and the pH maintained at 6.3. The nutrient-rich complex medium was LB (*38*) to which either glucose or xylose was added. In determinations of carbon (sugar)-based growth yield ($Y_{x/s}$), it is important to ensure that the sugar is the growth-limiting nutrient. Figure 4 shows that the final cell density in LB medium is directly proportional to the concentration of added sugar (glucose or xylose) up to a maximum sugar concentration of about 30 g/L. Without any added sugar, the LB medium supports a cell mass concentration of 0.41 g dry wt/L (Fig. 4). Figure 4 also shows the magnesium deficiency of the complex LB medium. By supplementing LB with 0.5 m*M* $MgSO_4$, it was possible to extend the linear relationship between cell yield and sugar concentration to about 50 g/L. These observations are in confirmation of earlier studies using LB and this same recombinant culture (*13,18,20*). The slope of the lines in Fig. 4 represent the average mass-based growth yield coefficients (corrected for the contribution from endogenous carbon sources) (av. cor. $Y_{x/s}$) with respect to glucose and xylose, and are 0.096 and 0.051 g dry wt cells/g sugar, respectively (*see also* bracketed values in Table 1).

Another advantage of employing relatively low sugar concentrations in these experiments is that it helps in avoiding possible ethanol toxicity problems that are inherently of concern with this type of yield analyses owing to the fact that overall yield determinations reflect the average of integral effects over the entire growth (fermentation) interval, which potentially could include end-product-inhibitory effects (*24*).

The various yield parameters with respect to growth ($Y_{x/s}$), energy (Y_{ATP}), and ethanol ($Y_{p/s}$) for the batch fermentations represented in Fig. 3 are summarized in Table 1. Details of the methods by which these various yield

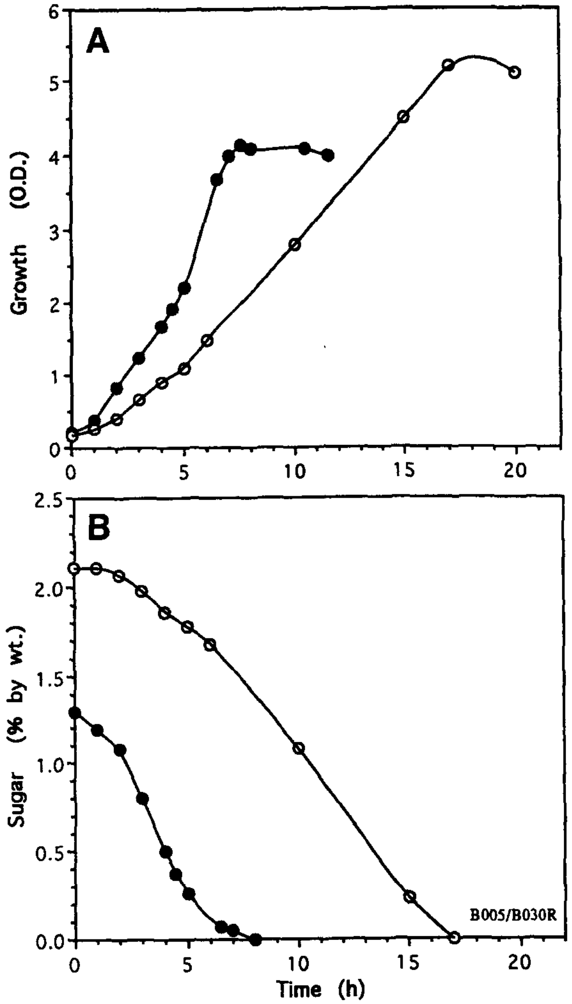

Fig. 3. A typical pH-stat anaerobic batch fermentation time-course with recombinant ethanologenic *E. coli* (pLOI297) using a complex medium. The medium was LB and the pH was kept constant at 6.3. (**A**) growth; (**B**) sugar utilization. (●) Glucose; (○) xylose.

parameters were determined are described in the Materials and Methods section. Determinations of metabolic end products that were used to construct carbon balances for the experiments shown in Fig. 3 are summarized in Table 2. For the purpose of carbon balancing, the elemental composition of the cell mass was considered to be constant ($CH_{1.8}O_{0.5}N_{0.2}$) with a mol wt of 24.6 g/mol, and it was assumed that cell composition is independent of the sugar used for growth (*39,40*). At 48.7% carbon, this composition of *E. coli* cell mass compares favorably to ones used by others at 47.1% (*24,41*) and 47.4% (*27*). Apart from cell mass, the major end products for both glucose and xylose fermentations were ethanol and CO_2 (Table 2). Succinic acid was the only other detectable metabolic end product (Table

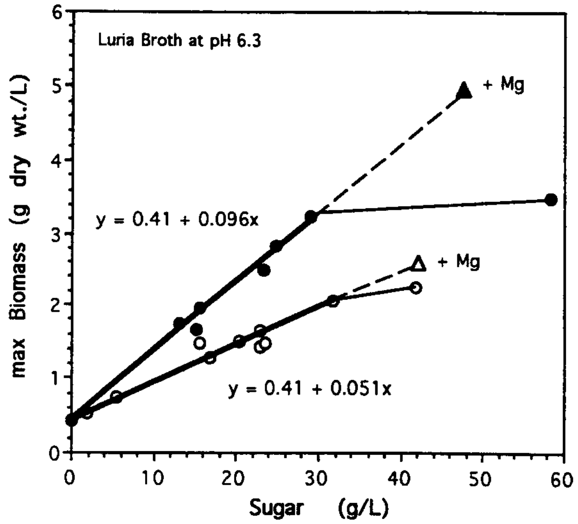

Fig. 4. Plot of final cell mass concentration as a function of sugar concentration. The medium was LB and the pH was kept constant at 6.3. (●) Glucose; (○) xylose. Two experiments are shown where the LB medium was supplemented with 0.5 m*M* $MgSO_4$: (△) xylose with LB + Mg^{2+}; (△) xylose with LB + Mg^{2+}. Equations are shown for solid lines that were computer-generated using linear regression. The slope of the lines represent the averages for $Y_{x/s}$ (corrected for the contribution from endogenous carbon sources) for glucose and xylose.

Table 1
Summary of Yield Parameters with Respect to Growth, Energy, and Ethanol for Recombinant *E. coli* B (11303:pLOI297)[a]

Medium type	Sugar, g/L	Biomass, g dry wt cells/L	$Y_{p/s}$, g/g	obs $Y_{x/s}$	cor $Y_{x/s}$	Y_{ATP}	G_{ATP}
				g dry wt cells/g sugar			
Complex (LB)							
Glucose	13.0	1.74	0.54	0.133	0.102 (0.096)	9.28	2.58
Xylose	21.1	1.54	0.45	0.073	0.054 (0.051)		0.87
Defined salts							
Glucose	17.1	1.55	0.46	0.091	0.091	8.19	2.00
Xylose	13.9	0.70	0.47	0.050	0.050		0.92

[a] Methods for calculating $Y_{p/s}$ (g EtOH/g sugar), obs. $Y_{x/s}$ and cor. $Y_{x/s}$ (g dry wt cells/g sugar utilized), Y_{ATP} (g dry wt cells/mol ATP) and G_{ATP} (mol ATP/mol sugar) are described in the Materials and Methods section. Bracketed values represent averages (*see* Fig. 1). Abbrev.: LB, Luria broth; DS, defined salts medium; dry wt, dry weight; EtOH, ethanol.

Table 2
End-Product Analyses and Carbon Balances
for Glucose and Xylose Fermentations by Recombinant *E. coli* B (11303:pLOI297)[a]

	Carbon IN	Carbon OUT								
Medium	Sugar	Biomass	EtOH	Succ.	Ac	Lac	Form	CO_2	% C for Biomass	% C Recovery
LB + G	433.2	54.0	303.8	32.0	0	0	0	151.9	12.0	125
LB + X	703.5	45.9	412.4	47.6	0	0	0	206.2	6.5	101
DS + G	570.0	63.0	343.0	0	0	0	0	171.5	11.1	101
DS + X	463.5	28.5	286.6	0	0	0	0	143.3	6.2	99

[a] The elemental composition of the cell mass was assumed to be constant and represented by $CH_{1.8}O_{0.5}N_{0.2}$ with a mol wt of 24.6 g/mol. The amount of carbon (C) is represented as meq C. Abbrev: LB, Luria broth; DS, defined salts medium; G, glucose; X, xylose; Succ, succinate; Ac, acetate; Form, formate.

2). These observations confirm the very effective manner in which the expression of the plasmid-borne *Zymomonas* genes for PDC and ADH create an ethanol production pathway in this organism (*11,12*).

The net energy yield associated with anaerobic glucose catabolism (G_{ATP}) can be derived from a combined knowledge of the mechanism of energy-coupled glucose transport and the enzymes involved in the metabolism of glucose, with particular focus on those reactions in which ATP (or its equivalent) is either consumed or generated. It is known that glucose enters *E. coli* by the PEP-energized phosphotransferase system and that during the transport process, glucose is phosphorylated (*36,37*). Hence, the glucose transport process in *E. coli* substitutes in effect for the reaction normally catalyzed by hexokinase (glucokinase) in the Embden-Myerhof-Parnas pathway, and consequently, the theoretical value of G_{ATP} for anaerobic glucose catabolism in recombinant ethanologenic *E. coli* is 2.0 mol ATP/mol glucose consumed (*see* Fig. 1). The value for G_{ATP} is based solely on the metabolism of the sugar added to the medium with account being made for the portion directed to cell mass and therefore unavailable for generating ATP. However, endogenous carbon sources contained in a complex medium complicate the experimental determination of G_{ATP} that is based on end-product analysis. This explains why the value determined for G_{ATP} for glucose with the LB medium is >2.0 (Table 1) and why the carbon recovery was >100% (Table 2). Nevertheless, in the case of glucose metabolism in the complex medium, it is possible to calculate the Y_{ATP} by dividing the value of the observed molar growth yield by the experimentally determined G_{ATP} (glu) (*see* Materials and Methods). Using this method, the value for Y_{ATP} is 9.28 g dry wt cells/mol ATP (Table 1). This means that, under these conditions, it takes 108 mmol ATP to synthesize 1 g (dry wt) of cell mass. This value for Y_{ATP} is close both to the value of 10.5 given by Bauchop and Elsden (*32*) in their pioneering study of growth yield and also to the value of 10.6 (±1.0) quoted by Forrest and Walker (*42*) that was based on an average from 47 determinations with a variety of different microorganisms.

In this investigation, it is assumed that a knowledge of the value for Y_{ATP} that is obtained under specified environmental conditions provides a means to estimate a value of G_{ATP} associated with xylose utilization by recombinant *E. coli*. The value for G_{ATP} (xyl) was obtained by dividing the observed molar growth yield coefficient by 9.28 (Table 1). By this method, the net ATP gain associated with anaerobic xylose utilization by recombinant *E. coli* was 0.87 mol ATP/mol xylose (Table 1). Although the net energy yield from anaerobic xylose catabolism can be estimated in this manner, certain assumptions are implicit. Since Y_{ATP} does not take into account that portion of the energy that is consumed independent of growth (sometimes referred to as the "maintenance energy"), it is assumed that this is unaffected by the substitution of xylose for glucose under otherwise identical environmental conditions. Since the determination of growth yield depends on measurement of cell mass, it is assumed that cell composition is relatively invariant. Although determinations of dry cell mass involve washing the cells subsequent to harvesting by ultrafiltration, it is not known if exopolymers (e.g., colanic acid) are produced or to what extent they might be removed by this procedure.

Reports in the literature involving determinations of microbial growth yields involving xylose (or other pentose sugars) are rare. However, the assumption has been made that the net ATP gain associated with anaerobic pentose catabolism is 1.67 (*25,32,43*). This is based on the following metabolic pathway for xylose catabolism involving xylose isomerase, xylulose kinase, and the conversion of pentose monophosphates into fructose-6-phosphate and glyceraldehyde-3-phosphate by the transaldolases and transketolases of the pentose phosphate pathway (Fig. 1).

$$\begin{array}{llll} & \text{3 Xylose} & \rightarrow & \text{3 Xylulose} \\ & \text{3 Xylulose} + \text{3 ATP} & \rightarrow & \text{3 Xylulose-P} + \text{3 ADP} \\ & \text{3 Xylulose-P} & \rightarrow & \text{2 Fructose-6-P} + \text{Glyceraldehyde-3-P} \\ & \text{2 F-6-P} + \text{6 ADP} + 4\ P_i & \rightarrow & \text{4 EtOH} + 4\ CO_2 + \text{6ADP} + \text{6 ATP} \\ & \text{G-3-P} + \text{2 ADP} + P_i & \rightarrow & \text{EtOH} + CO_2 + \text{2ADP} + \text{2 ATP} \\ \text{Sum:} & \text{3 Xylose} + \text{5 ADP} + 5\ P_i & \rightarrow & \text{5 EtOH} + 5\ CO_2 + \text{5 ATP} \end{array} \qquad (4)$$

According to this summary of the metabolic fate of intracellular xylose, the net yield of energy (ATP) from xylose is 1.67 mol ATP/mol xylose. If the net ATP gain (G_{ATP}) for xylose were 1.67, it would mean that, on a mass basis, the cell was capable of deriving the same amount of energy from glucose and xylose. Assuming that Y_{ATP} is constant, this would mean that the mass-based growth yields for these sugars would be identical. Clearly, this is not the case, since the average corrected values for $Y_{x/s}$ are 0.096 and 0.051 g dry wt/cells/mol of glucose and xylose, respectively (Fig. 4 and Table 1). These observations are at variance with those of Beall et al. (*16*), who reported that, for this same recombinant *E. coli* growing in LB with pH control, the observed $Y_{x/s}$ was 0.064 g/g for both glucose and xylose.

However, the assumption that G_{ATP} (xyl) has a value of 1.67 does not take into account the energy required for active transport of xylose. It is reported (*44*) that xylose enters *E. coli* by proton symport with a stoichiometry of 1 proton/xylose (Fig. 1). If the stoichiometry associated with membrane proton-translocating ATPase is 2 protons/mol ATP hydrolyzed (*45–47*), then the active transport of xylose would require the equivalent of 0.5 mol ATP/mol of xylose transported into the cell. When the energy required for xylose transport is taken into consideration, the net ATP gain associated with anaerobic xylose catabolism is reduced from 1.67 to 1.17. This lower value for G_{ATP} (xyl) is still considerably less than experimentally determined from the observed growth yield and Y_{ATP}.

Fermentations with a Defined Salts Minimal Medium

In order to eliminate or at least to minimize complications of interpretation caused by endogenous carbon sources contained in the complex

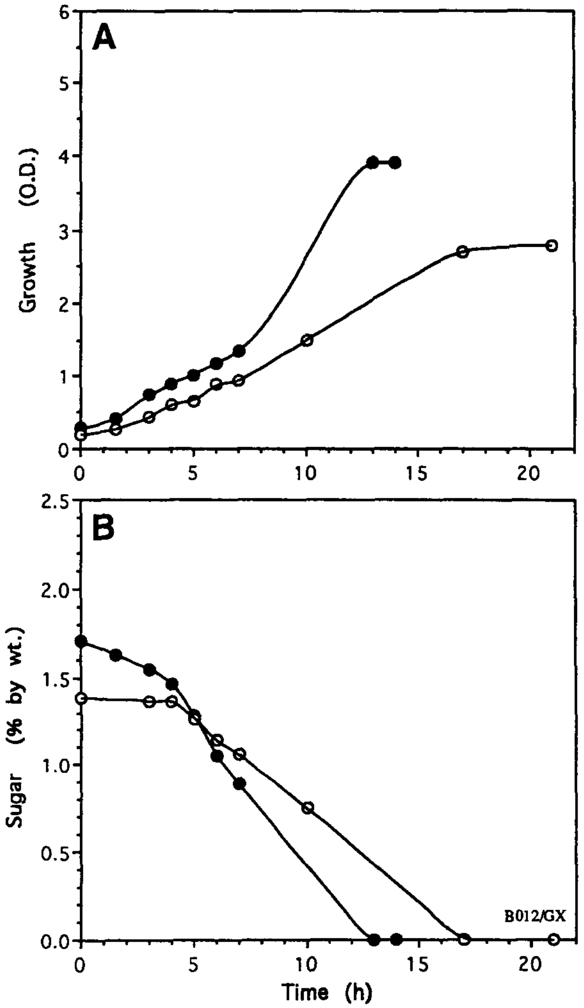

Fig. 5. A typical pH-stat anaerobic batch fermentation time-course with recombinant ethanologenic *E. coli* (pLOI297) using a defined salts medium. pH was kept constant at 6.3. (**A**) Growth; (**B**) sugar utilization. (●) Glucose; (○) xylose.

LB medium, experiments of similar design were performed with the same recombinant *E. coli* using a defined salts minimal medium (Fig. 5). The growth rate in chemically lean, mineral salts medium (Fig. 5A) was only 20–25% of the rate observed with the nutritionally rich, complex medium (Fig. 3A) as reported previously (*18,21*). The yield parameters and carbon balances are summarized in Tables 1 and 2, respectively. With both sugars, there was very good closure of the carbon balance with near 100% carbon recovery as cell mass, ethanol, and carbon dioxide (Table 2). The decrease in ethanol yield with the glucose minimal medium (Table 1) confirms the involvement of the LB carbon sources in contributing to ethanol production as has been postulated previously by others (*13,16,18*). It is not known why there was not a proportionately similar decrease in ethanol yield when xylose was used as the sole carbon source (Table 1).

Using glucose as sole carbon and energy source, the experimentally determined value for G_{ATP} was 2.0, which is slightly in excess of the theoretically predicted value since a small portion of the glucose is used anabolically (Table 1). The calculated value of Y_{ATP} was 8.19 g dry wt cells/mol ATP, which means that 122 mmol of ATP are required under these condtions to synthesize 1 g of cell mass. Surprisingly, this is only 14 mmol of ATP in excess of the amount required to produce the same amount of cell mass from a nutritionally rich medium. Because of the absence of the vitamins and amino acids in the minimal medium, it was expected that the energetic demand for growth would be considerably greater with this medium compared to the complex medium. Literature values of Y_{ATP} for different microorganisms are generally much higher when complex media are employed (for reviews, *see 27,33–35*). It is postulated that this is because of the greater proportion of energy that is consumed in "work" that is not growth-related in the nutritionally lean growth environment (*26,27*).

Using the same approach as described previously with the complex medium, the experimentally determined value for the net yield of ATP associated with anaerobic xylose catabolism (G_{ATP}) is 0.92 (Table 1), which is close to the value of 0.87 that was obtained with the complex medium.

From this investigation on growth yields, it is concluded that, on a mass basis, *E. coli* derives only about half as much energy from xylose compared to glucose. With experimental error, this difference can be rationalized on the basis of theoretical net energy yields when proper consideration is given to the differences with respect to the active transport of glucose and xylose. This difference in energy yield may explain, in part, the slower growth rate that occurs with xylose (Figs. 3A and 5A) (*18,21*).

Future experiments will involve determining the specific rate of sugar utilization as a function of the specific growth rate in carbon-limited steady-state chemostat cultures as a means of assessing the role of so-called maintenance energy with respect to the observed differences in growth yield associated with different sugars. The extent to which the observations of the present investigation might be applicable to other organisms is not known.

ACKNOWLEDGMENTS

This research was supported by the Natural Sciences and Engineering Research Council of Canada (Research Grant OPG0046623). We are grateful to Lonnie Ingram for the gift of the recombinant *E. coli* culture.

REFERENCES

1. Grohmann, K., Himmel, M., Rivard, C., Tucker, M., Baker, J., Torget, R., and Graboski, M. (1984), *Biotech. Bioeng. Symp.* **14,** 139-157.
2. Timell, T. E. (1967), *Wood Sci. and Technol.* **1,** 45-70.
3. Bull, S. R. (1989), in *Energy from Biomass & Wastes XIV,* D. L. Klass, ed., Institute of Gas Technology, Chicago, IL, pp. 1-14.
4. Hinman, N. D., Wright, J. D., Hoagland, W., and Wyman, C. E. (1989), *Appl. Biochem. Biotechnol.* **20/21,** 391-401.
5. Tempest, D. W. and Neijssel, O. M. (1987), in *Escherichia coli and Salmonella typhimurium,* F. C. Neidhart, ed., Academic, New York, pp. 800-802.
6. Gottschalk, G. (1986), in *Bacterial Metabolism,* 2nd ed. Springer-Verlag, New York, pp. 208-282.
7. Clark, D. and Rod, M. L. (1987), *J. Mol. Evol.* **25,** 151-158.
8. Brau, B. and Sahm, H. (1986), *Arch. Microbiol.* **144,** 296-301.
9. Neale, A. D., Scopes, R. K., and Kelly, J. M. (1988), *Appl. Microbiol. Biotechnol.* **29,** 162-167.
10. Ingram, L. O., Conway, T., Clark, D. P., Sewell, G. W., and Preston, J. F. (1987), *Appl. Environ. Microbiol.* **53,** 2420-2425.
11. Ingram, L. O., Conway, T., and Alterthum, F. (1988), United States Patent 5,000,000.
12. Ingram, L. O., Alterthum, F., Ohta, K., and Beall, D. S. (1990), in *Developments in Industrial Microbiology,* vol. 31, Elsevier, New York, pp. 21-30.
13. Alterthum, F. and Ingram, L. O. (1989), *Appl. Environ. Microbiol.* **54,** 397-404.
14. Ingram, L. O. (1991), in *Energy from Biomass and Wastes XIV,* Klass, D. L., ed., Institute of Gas Technology, Chicago, IL, pp. 1105-1126.
15. Ohta, K., Alterhum, F., and Ingram, L. O. (1990), *Appl. Environ. Microbiol.* **56,** 463-465.
16. Beall, D. S., Ohta, K., and Ingram, L. O. (1991), *Biotechnol. Bioeng.* **38,** 296-303.
17. Ohta, K., Beall, D. S., Meija, J. P., Shanmugam, K. T., and Ingram, L. O. (1991), *Appl. Environ. Microbiol.* **57,** 893-900.
18. Lawford, H. G. and Rousseau, J. D. (1991), *Appl. Biochem. Biotechnol.* **28/29,** 221-236.
19. Lawford, H. G. and Rousseau, J. D. (1991), *Biotechnol. Lett.* **13,** 191-196.
20. Lawford, H. G. and Rousseau, J. D. (1992), in *Energy from Biomass and Wastes XV,* Klass, D. L. ed., Institute of Gas Technology, Chicago, IL, pp. 583-622.
21. Lawford, H. G. and Rousseau, J. D. (1993), in *Energy from Biomass and Wastes XVI,* Klass, D. L. ed., Institute of Gas Technology, Chicago, IL, pp. 559-597.
22. Lawford, H. G. and Rousseau, J. D. (1993), *Appl. Biochem. Biotechnol.* **39/40,** 301-322.
23. Bringer-Meyer, S., Schimz, K.-L., and Sahm, H. (1986), *Arch. Microbiol.* **146,** 105-110.
24. Diaz-Ricci, J. C., Tsu, M., and Bailey, J. E. (1992), *Biotechnol. Bioeng.* **39,** 59.
25. Hill, P. W., Klapatch, T. R., and Lynd, L. R. (1993), *Biotechnol. Bioeng.* **42,** 873-883.

26. Thauer, R. K., Jungermann, K., and Decker, K. (1977), *Bacteriol. Rev.* **41,** 100–180.
27. Stouthamer, A. H. (1979), in *International Reviews of Biochemistry—Microbial Biochemistry,* vol. 21, Quayle, J. R., ed., University Park Press, Baltimore, pp. 1-47.
28. Batley, E. H. (1987), in *Energetics of Microbial Growth,* John Wiley, New York.
29. Pirt, J. S. (1975), in *Principles of Microbe and Cell Cultivation,* John Wiley, New York.
30. Gunsalus, I. C. and Shuster, C. W. (1961), in *The Bacteria,* vol. 2, Academic, New York.
31. Neijssel, O. M. and Tempest, D. W. (1976), *Arch. Microbiol.* **110,** 305-311.
32. Bauchop, T. and Elsden, S. R. (1960), *J. Gen. Microbiol.* **23,** 457-469.
33. Stouthamer, A. H. (1969), in *Methods in Microbiology,* vol. 1, Norris, J. R. and Ribbons, D. W., eds., Academic, New York, p. 629.
34. Stouthamer, A. H. (1977), in *Microbial Energetics,* 27th Symp. Soc. Gen. Microbiol., Haddock, B. A., and Hamilton, W. A., eds., Cambridge University Press, London, pp. 285-315.
35. Stouthamer, A. H. (1976), in *Yield Studies in Microorganisms,* Meadowfield, Dewbury, UK.
36. Roseman, S. (1969), *J. Gen. Physiol.* **54,** 138–184.
37. Roseman, S. and Meadow, N. D. (1990), *J. Biol. Chem.* **265,** 2993-2996.
38. Luria, S. E. and Delbruck, M. (1943), *Genetics* **28,** 491-511.
39. Lawford, H. G. and Rousseau, J. D. (1993), *Biotechnol. Lett.* **14,** 421–426.
40. Lawford, H. G. and Rousseau, J. D. (1993), *Biotechnol. Lett.* **15,** 615-620.
41. Luria, S. E. (1960), in *The Bacteria,* vol. 1, Gunsalus, I. C. and Stanier, I. Y., eds., Academic, New York, (chap. 1).
42. Forrest, W. W. and Walker, D. J. (1971), *Adv. Microbiol. Physiol.* **5,** 213.
43. Elsden, S. R. and Peel, J. L. (1958), *Ann. Rev. Microbiol.* **12,** 145.
44. Lam, V. M. S., Daruwalla, K., Henderson, P. D. F., and Jones-Mortimer, M. C. (1980), *J. Bacteriol.* **143,** 396-402.
45. Abrams, A. and Smith, J. B. (1974), in *The Enzymes,* 3rd ed., vol. 10, Boyer, P. D., ed., Academic, New York, pp. 395-429.
46. Nicholls, D. G. (1982), in *Bioenergetics,* Academic, New York.
47. Mitchell, P. (1975), *FEBS Lett.* **43,** 189-194.