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Effect of Oxygen on Ethanol Production by a Recombinant Ethanologenic *E. coli*

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ABSTRACT

Escherichia coli strain B, bearing the *pet* plasmid pLOI297, and the wild-type culture, lacking the plasmid, responded to aeration of the complex medium by an approximate three- and fourfold increase in both growth rate and growth yield with glucose and xylose, respectively. At a relatively low oxygen transfer rate (8 mmol O_2/L/h), the sugar-to-ethanol conversion efficiency exhibited by the recombinant strain decreased 40% and 30% for glucose and xylose, respectively. At a high aeration efficiency (100 mmol O_2/L/h), the ethanol yield with respect to xylose was 0.15 g/g for the recombinant and 0.25 g/g for the culture lacking the plasmid. These observations suggest that oxygen reduces the ethanologenic efficiency of recombinant *E. coli* by diverting carbon to growth and end products other than ethanol. Previous observations, by others, on the effect of oxygen on ethanogenic recombinant *E. coli* were made with different strains bearing different plasmids. In addition to the possibility of strain and plasmid specificity, the results of this study suggest that previous conclusions were influenced by the particular environmental conditons imposed on the culture, including poor aeration efficiency and lack of pH control.

Index Entries: Fuel ethanol; aerobic; *Escherichia coli;* recombinant *E. coli;* xylose; growth yield; oxidative metabolism; *Zymomonas* genes.

Abbreviations: DO, dissolved oxygen; OTR, oxygen transfer rate (mmol O_2/L/h); Q_p, volumetric productivity (g ethanol/L/h); q_p, specific productivity (g ethanol/g cell/h); $Y_{p/s}$, product yield (g ethanol/g sugar); $Y_{x/s}$, growth yield coefficient (g dry wt cells/g sugar).

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INTRODUCTION

Environmental concerns relating primarily to air quality and global warming are creating a new opportunity for fermentation ethanol as an alternative transportation fuel (*1*). However, the present practice of using sources of carbohydrate that are highly valued as either food or feed means that the fermentation feedstocks account for about two-thirds of the cost of producing ethanol (*2,3*). The use of such expensive raw materials prohibits bioethanol from being competitive with gasoline in today's market. The existing fuel ethanol industry in the United States survives only by virtue of the economic assistance provided through various government incentives and tax credits (*3–5*).

Lignocellulosic biomass is considered an appropriate alternative fermentation feedstock for fuel ethanol production because it is inexpensive, plentiful, and renewable (*5*). However, lignocellulose remains recalcitrant to bioconversion because the yeast cultures presently employed in starch-based fermentations are unable to utilize the C_5-sugars (pentoses) that comprise the hemicellulose component of biomass (*5*). Research relating to the fermentation aspect of bioconversion technology has focused recently on various bacterial ethanologens that have demonstrated promise for the large-scale production of ethanol from lignocellulosic biomass (*5–7*).

Escherichia coli is able to metabolize all the sugars present in lignocellulose efficiently; however, ethanol is produced only as a minor end product of sugar metabolism (*8,9*). The bacterium *Zymomonas* is an ethanologen with demonstrated high-performance fermentation characteristics with respect to both yield and productivity (for reviews, *see 10–13*), but like *Saccharomyces* yeast, it is unable to utilize all the sugars that comprise lignocellulosic biomass (*14*). Recently, the "ethanol production pathway" of *Zymomonas mobilis*, consisting of the enzymes pyruvate decarboxylase and alcohol dehydrogenase II, has been cloned and plasmid vectors containing the so-called *pet* operon have been used to transform *Escherichia coli* (*15*). The genetically engineered *E. coli* recombinants combine the positive traits of both organisms (*16,17*). In *pet*-containing recombinants, ethanol is the predominant end product of fermentation (*15–18*). Although the pioneering work was done with *E. coli* K12(*15,19,20*), a subsequent physiological screening of several different recombinant *E. coli* cultures (*16,18,21*) led to the identification of *E. coli* B (ATCC 11303) as a "hardy strain and a suitable host for the *pet* plasmid pLOI297" (*21*). The resulting recombinant has been shown to produce ethanol efficiently from nutrient-rich lab media containing either glucose or xylose as the principal carbon sources (*16,18,21*). For the past three years, we have been assessing the fermentation performance characteristics of this patented recombinant *E. coli* (*17*) using both synthetic lab media (*22–26*) and biomass prehydrolysates prepared by different thermochemical processers from a variety of

biomass/waste feedstocks, including both hardwood (aspen) (*27*) and softwood (pine) (*7*) newsprint (*28*), spent sulfite liquors (*29*), and corn crop residues (*30*).

Oxygen is an essential element for the growth of aerobic organisms and exerts its effect on cellular functions primarily by its effect on the respiratory generation of metabolic energy. Oxygen affects not only the growth rate and growth yield, but also the replication of plasmid DNA and the expression of plasmid genes (*31*). However, *E. coli* is capable of energy generation by either fementative (anaerobic) or respiratory (aerobic) processes (*32*). The transition from anaerobiosis to aerobiosis is accompanied by the biosynthesis of certain citric acid cycle enzymes and elements of the electron transport chain (*33*). Oxygen replaces certain organic compounds (pyruvate or acetyl CoA) as electron acceptor in the oxidation of reduced pyridine nucleotide, and the rate of ATP generation, as well as the yield of ATP from sugar metabolism, is significantly increased. The decrease in the rate of sugar consumption caused by oxygen is known as the "Pasteur effect" (*33*). However, these effects of oxygen can be significantly repressed by elevated sugar concentrations ("Crabtree effect") and by acid metabolites produced during fermentative metabolism (*34*).

Because of the relatively low solubility of oxygen in aqueous media, it is important that the rate of supply of oxygen to the medium (oxygen transfer rate, OTR) be sufficient to meet the respiratory demand of the microbial culture (*35,36*). The dissolved oxygen (DO) concentration is the differential between oxygen supply (OTR) and demand (respiration), and can be monitored with a polarographic or galvinometric electrode (DO probe) (*36*). The respiratory oxygen demand of microbial cultures, of even moderate density, can exceed the OTR associated with shake flasks, which is low compared to stirred-tank bioreactors (*37*). When the OTR is insufficient to meet the respiratory demand of the culture, the DO is zero and the culture is oxygen-limited.

Previous reports on the effect of oxygen on genetically engineered ethanologenic *E. coli* containing *pdc* and *adh* genes from *Zymomonas* used two different host cultures, namely strains TC4 (*15*) and JM101 (*38*). Furthermore, these studies employed shake-flask cultures and nonbuffered media (*15,38*). Under such conditions, the effect of oxygen may have been masked both by poor aeration efficiency (i.e., low OTR) and by suboptimal pH, which is a direct consequence of inadequate buffering and the absence of pH control. Observations made under these conditions could have influenced the conclusions of the study with respect to the effect of oxygen on recombinant *E. coli* (*9,39*). The effect of oxygen on recombinant *E. coli* strain B (carrying plasmid pLOI297) has not been reported.

The objective of the present study was to assess quantitatively the effect of varying degrees of culture aeration on growth and metabolism of

recombinant *E. coli* B (pLOI297) with a specific focus on the techno-economically important parameters of ethanol yield and productivity. By including in this study the host culture *E. coli* B (ATCC 11303), which lacked the plasmid, we were able to examine the specific effect of the *Zymomonas* enzymes being expressed in the recombinant under aerobic conditions. This investigation employed pH-controlled batch cultures with either glucose or xylose as the source of carbon and energy.

MATERIALS AND METHODS

Organisms

Escherichia coli B (ATCC 11303) was obtained from the American Type Culture Collection (Rockville, MD), and this culture was designated as "11303." Recombinant *Escherichia coli* B (ATCC 11303 carrying the *pet* plasmid pLOI297) (*16*) was a gift from L. O. Ingram (University of Florida, Gainsville, FL) and was designated as "+p297." These cultures were maintained in an antibiotic-supplemented medium as described previously (*22*). Fermentation media were inoculated at an initial cell density of 30–50 mg dry wt cells/L (OD_{550} approx 0.1–0.2).

Culture Media

The principal culture medium was Luria broth (*40*), which was supplemented with 5 m*M* $MgSO_4$ and 17 m*M* phosphate, and designated as "sLB" (*25*). Either glucose or xylose was added at the concentrations specified. Antibiotics were not added to the sLB medium. All media were sterilized by autoclaving. The magnesium, phosphate, and sugar supplements were autoclaved separately. Unless specified otherwise, the pH was controlled at 6.3.

Fermentation Equipment

pH-stat batch fermentations of 1500-mL volume were conducted in MultiGen™ (model F2000) stirred-tank bioreactors with variable-speed agitation (3 Rushton turbine impellers), flow-controlled gas sparging, pH (adjusted using 2*N* KOH), and temperature control (30°C) (New Brunswick Scientific Co., Edison, NJ).

Oxygen Transfer Rate (OTR)

The OTR (mmol O_2/L/h) associated with this specific bioreactor design was determined by the sulfite oxidation method (*41*) as previously described (*36*). The concentration of DO was monitored using a polarographic DO electrode and recording analyzer (model 50—New Brunswick Scientific Co., Edison, NJ). The OTR was fixed by adjusting the agitation rate and air sparging velocity as illustrated in Table 1.

Table 1
Effect of Agitation and Aeration on the Oxygen Transfer Rate

Agitation rate, RPM	Air sparging rate, cc/min	OTR, mmol O_2/L/h
500	500	8
600	500	24
850	750	100

Analytical Procedures

Growth was measured turbidometrically at 550 nm (1-cm lightpath), and culture dry weight was measured by microfiltration followed by washing and drying the filter to constant weight under an infrared heatlamp. Linear growth rates (mg dry wt cells/L/h) were determined from plots of optical density (OD) vs time using the relationship 1.0 OD_{550} = 0.34 g dry wt cells/L (*16*). Compositional analyses of fermentation media and cell-free spent media were determined using an HPLC equipped with an RI monitor and computer-interfaced controller/integrator (Bio-Rad Labs, Richmond, CA) (*22*). Separations were performed at 65°C using an HPX-87H column (300 × 7.8 mm) (Bio-Rad Labs, Richmond, CA). The mobile phase was 0.005*N* sulfuric acid (flow rate = 0.6 mL/min), and the injection vol was 0.02 mL.

Determination of Fermentation Parameters

In batch fermentations, the average volumetric productivity (av. Q_p) was determined by dividing the final ethanol concentration by the time required to achieve complete sugar (either glucose or xylose) utilization. The maximum volumetric productivity (Q_p^{max}) was estimated as the maximum slope in plots of ethanol concentration vs elapsed fermentation time. The specific productivity (q_p) was estimated by dividing the value for Q_p^{max} by the maximum biomass (dry wt) concentration. 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. The growth yield coefficient ($Y_{x/s}$) was calculated as the dry biomass per mass of sugar added to the medium.

RESULTS

A quantitative physiological assessment of the effect of oxygen on growth and ethanol production by recombinant *E. coli* B, and its host

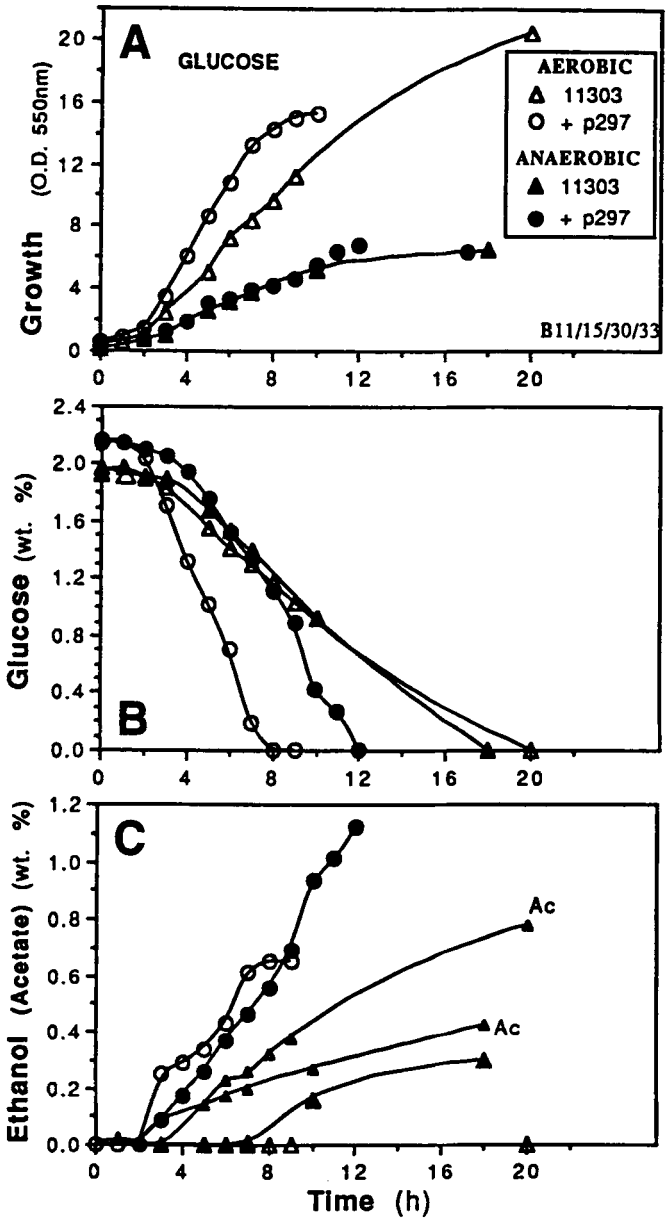

Fig. 1. Time-course of aerobic and anaerobic growth and metabolism by wild-type and recombinant *E. coli* B with glucose. (**A**) Growth, (**B**) glucose utilization, and (**C**) production of ethanol or acetate (Ac). Symbols: *see* insert.

strain (ATCC 11303) lacking the *pet* plasmid (pLOI297), was conducted in pH-stat batch cultures under anaerobic and aerobic conditions. Figures 1 and 2 are typical of the type of data collected. Figures 1 and 2 illustrate typical time-courses of pH-stat batch cultures of *E. coli* B (ATCC 11303) and recombinant *E. coli* B (pLOI297), under both anaerobic and aerobic

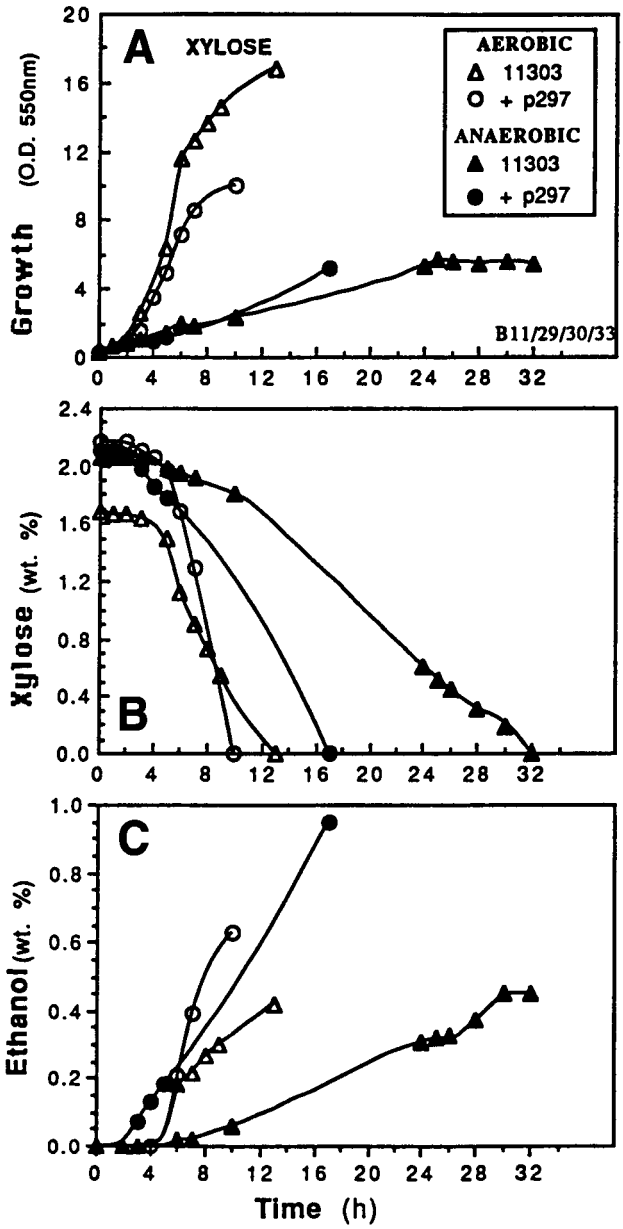

Fig. 2. Time-course of aerobic and anaerobic growth, and metabolism by wild-type and recombinant *E. coli* B with xylose. (**A**) Growth, (**B**) xylose utilization, and (**C**) production of ethanol. Symbols: *see* insert.

conditions, using a nutrient-rich glucose (Fig. 1) and xylose medium (Fig. 2), respectively. The results of these experiments and others are summarized quantitatively with respect to final cell mass and ethanol concentrations, product (ethanol) yield, sugar-to-ethanol conversion efficiency, and productivity (volumetric and specific) in Tables 2 and 3.

Table 2
Effect of Oxygen on Glucose Fermentation
by *Escherichia coli* B (ATCC 11303) and a Recombinant (pLOI297)

Conditions Medium Comp.	Expt.	Products [Glu], g/L	Products [Biomass], g dry wt	Products [EtOH], g/L	Productivity av Q_p, g P/L/h	Productivity Q_p^{max} g P/L/h	Productivity q_p g P/g cell/h	Yield $Y_{p/s}$ g P/g S	Yield Conversion, Effic., %
E. coli B (pLOI297)									
Anaerobic	B30	21.5	2.18	11.2	0.93	1.40	0.64	0.52	102
	B05	23.2	2.43	13.0	1.18	1.53	0.63	0.56	110
	B15	47.6	4.97	24.5	1.36	2.05	0.41	0.51	100
Aerobic									
OTR = 8 mmol O_2/L/h									
	B15	22.6	5.21	6.49	0.81	0.75	0.14	0.30	59
E. coli B (ATCC 11303)									
Anaerobic	B11	19.3	2.09	3.00	0.18	0.40	0.19	0.16	31
	B17	10.8	1.43	2.10				0.18	35
Aerobic									
OTR = 8 mmol O_2/L/h									
	B15	22.6	6.80	0	0	0	0	0	0
OTR = 100 mmol O_2/L/h									
	B33*	19.7	6.62	0	0	0	0	0	0

P = ethanol.
S = glucose.
*In these expts., the pH was controlled at 7.0.

Table 3
Effect of Oxygen on Xylose Fermentation
by *Escherichia coli* B (ATCC 11303) and a Recombinant (pLOI297)

Conditions Medium Comp.	Expt.	Products [Xyl], g/L	Products [Biomass], g dry wt	Products [EtOH], g/L	Productivity av Q_p, g P/L/h	Productivity Q_p^{max} g P/L/h	Productivity q_p g P/g cell/h	Yield $Y_{p/s}$ g P/g S	Yield Conversion, Effic., %
E. coli B (pLOI297)									
Anaerobic	B30	21.1	1.54	9.5	0.56	0.64	0.42	0.45	88
	B21	42.2	2.56	18.8	0.75	1.39	0.54	0.45	88
Aerobic									
OTR = 8 mmol O_2/L/h									
	B29	21.6	4.26	6.3	0.63	1.05	0.25	0.29	57
OTR = 24 mmol O_2/L/h									
	B32*	38.5	4.25	7.5	0.38	0.57	0.13	0.19	37
OTR = 100 mmol O_2/L/h									
	B31	34.2	8.06	5.2	0.21	0.85	0.11	0.15	29
	B32*	47.7	8.80	9.1	0.26	0.31	0.04	0.19	37
E. coli B (ATCC 11303)									
Anaerobic	B17	11.5	1.08	2.9	0.21			0.27	53
	B11	20.5	1.53	4.5	0.14	0.17	0.11	0.25	49
Aerobic									
OTR = 24 mmol O_2/L/h									
	B31*	19.1	3.59	5.20	0.27	0.34	0.09	0.27	53
OTR = 100 mmol O_2/L/h									
	B33*	16.9	4.07	4.23	0.30	0.35	0.09	0.25	49

Note: In expt. B32* (OTR=24), the DOT fell to zero at about 9 h and remained here for the duration of the run (next 11 h). In expts. where OTR = 100, the DOT was never < 20% saturation
P = ethanol.
S = xylose.
*In these expts., the pH was controlled at 7.0.

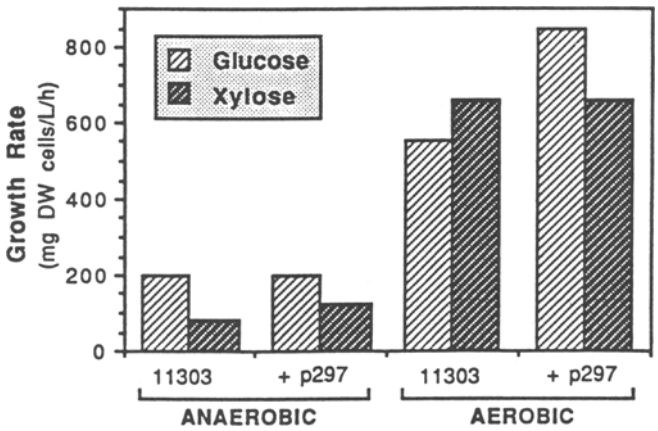

Fig. 3. Comparative growth rates for wild-type and recombinant *E. coli* B under anaerobic and aerobic conditions using either glucose or xylose as carbon source. Linear growth rates (mg dry wt cells/L/h) were determined from data shown in Figs. 1A and 2A for glucose and xylose, respectively.

Effect of Oxygen on Growth Rate

Figures 1A and 2A are plots of culture turbidity vs time, which illustrate the aerobic and anaerobic growth on the recombinant *E. coli* B, and its host strain (ATCC 11303) lacking the *pet* plasmid (pLOI297), in magnesium and phosphate-supplemented Luria broth (sLB) containing either glucose or xylose as the major source of carbon. Anaerobic growth in sLB medium without added sugar was logarithmic, with a mean generation time of 70 min (results not shown). Under anaerobic conditions, growth of the recombinant *E. coli* B in Luria broth, supplemented with either glucose or xylose, has previously been shown to be biphasic, with a short period of exponential growth (lasting for only about three generations) followed by a more sustained period of "linear" growth (*22–24,26*). The nonexponential nature of the anaerobic growth of ethanologenic recombinant *E. coli* cultures has also been reported by others (*16,21,38*). The host strain, *E. coli* B (ATCC 11303), exhibits similar linear growth characteristics in this medium (*26*).

From turbidometric plots of growth, similiar to those shown in Figs. 1A and 2A, the linear growth rate (dry wt cells/L/h) was determined as a function of the presence or absence of oxygen, and the results are presented, for comparative purposes, in Fig. 3. The anaerobic growth rates of the recombinant culture and the culture lacking the plasmid were identical in the glucose medium and were double the rates that were achieved using the xylose medium (Fig. 3). With xylose, air sparging at an oxygen transfer rate (100 mmol O_2/L/h) that was not growth-limiting produced about a sevenfold increase in the growth rate of both cultures (Fig. 3).

With glucose, the aerobic growth rate of the recombinant was faster than that of the strain lacking the plasmid, which was a threefold increase and a fourfold increase for the recombinant and host strains of *E. coli*, respectively, over their anaerobic growth rates (Fig. 3).

Effect of Oxygen on Growth Yield

Since the growth yield coefficient ($Y_{x/s}$) for a particular sugar substrate is directly proportional to the amount of energy (ATP) derived from the catabolism of that sugar, and since the energy yield from respiratory oxidative metabolism is more than from anaerobic fermentative metabolism, the value for $Y_{x/s}$ would be expected to increase, but only if the culture is able to benefit energetically when oxgyen is introduced into the medium. Because of the "Crabtree effect," the magnitude of the enhancing effect of oxygen on growth yield is comprised at elevated sugar concentrations (*33*). Ingram and Conway (*15*) noted that "recombinant (*E. coli*) strains grew under aerobic and anaerobic conditions to cell densities equivalent to, or higher than, those of strain TC4 lacking the plasmid." However, the medium used by Ingram and Conway (*15*) was not buffered, and because of the different nature of the end products produced, and pH decreased considerably less with the recombinant cultures. Nevertheless, the strain TC4 lacking the plasmid, the aerobic growth for glucose was 2.3 times the anaerobic growth yield (*15*).

The growth yield coefficient ($Y_{x/s}$) can be determined from the mass ratio of cells (max cell density) to sugar added to the medium. The final cell density was proportional to the amount of sugar added (over the range of sugar used in these experiments). Without any added sugar, the growth yield in the sLB medium was 0.42 g dry wt cells/L (equivalent to OD = 1.24) (results not shown). The maximum cell densities (g dry wt cells/L) for glucose and xylose media are given in Tables 2 and 3, respectively.

The effect of varying degrees of aeration on $Y_{x/s}$ for both the recombinant and the host culture grown in sLB with either glucose or xylose as carbon source is shown in Fig. 4. Beall et al. (*21*) reported that the growth yield for the recombinant culture, under anaerobic conditions, was 0.064 g/g for both glucose and xylose; however, it has been our experience that the anaerobic growth yield coefficient is greater for glucose than for xylose (*22,24*). The present study confirms these observations and further reveals that this relationship also holds for the strain lacking the plasmid (Fig. 4). With aeration such that the OTR (100 mmol O_2/L/h) was not growth-limiting, the value of $Y_{x/s}$ was about 3.4 times higher for both cultures independent of the sugar used (Fig. 4). When the cultures were oxygen-limited (OTR values of 8 or 24), the response in terms of $Y_{x/s}$ was intermediate (Fig. 4).

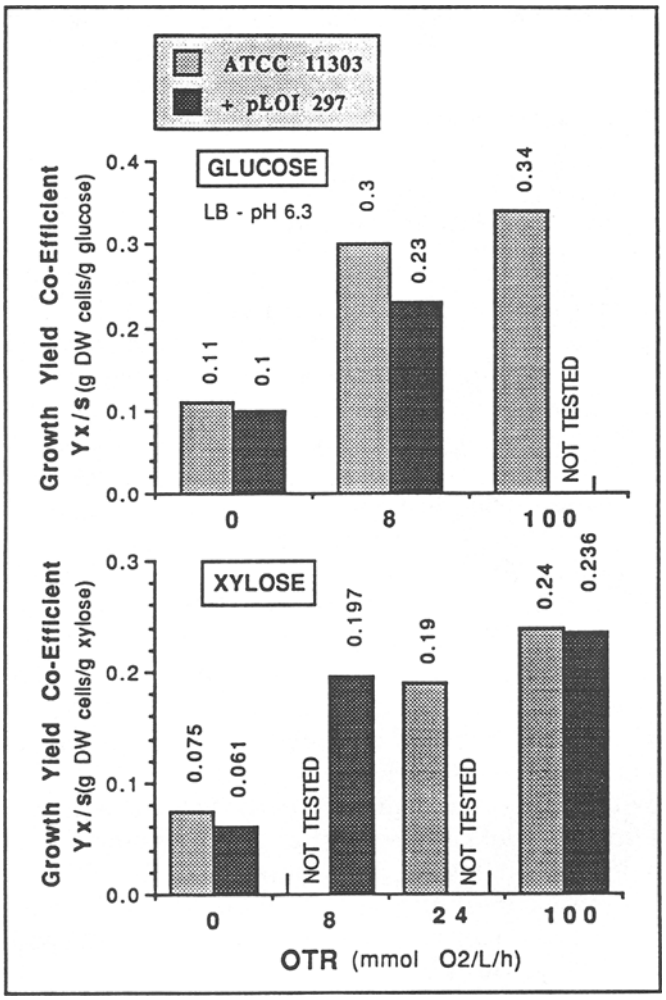

Fig. 4. Comparative growth yield coefficients for wild-type and recombinant *E. coli* B under anaerobic and aerobic conditions using either glucose or xylose as carbon source. *See* Materials and Methods for method of calculating $Y_{x/s}$.

Effect of Oxygen on Ethanol Yield

Ingram and Conway (*15*) reported that, for *E. coli* strain TC4 lacking the *pet* plasmid, acetate was the main product under aerobic conditions and no ethanol was produced. Under anaerobic conditions, lactic acid was the major end product (*15*). However, under aerobic conditions, the major end product produced by recombinant *E. coli* strain TC4 (with plasmid pLOI308) was ethanol ($Y_{p/s}$ = 0.44 g/g) (*15*).

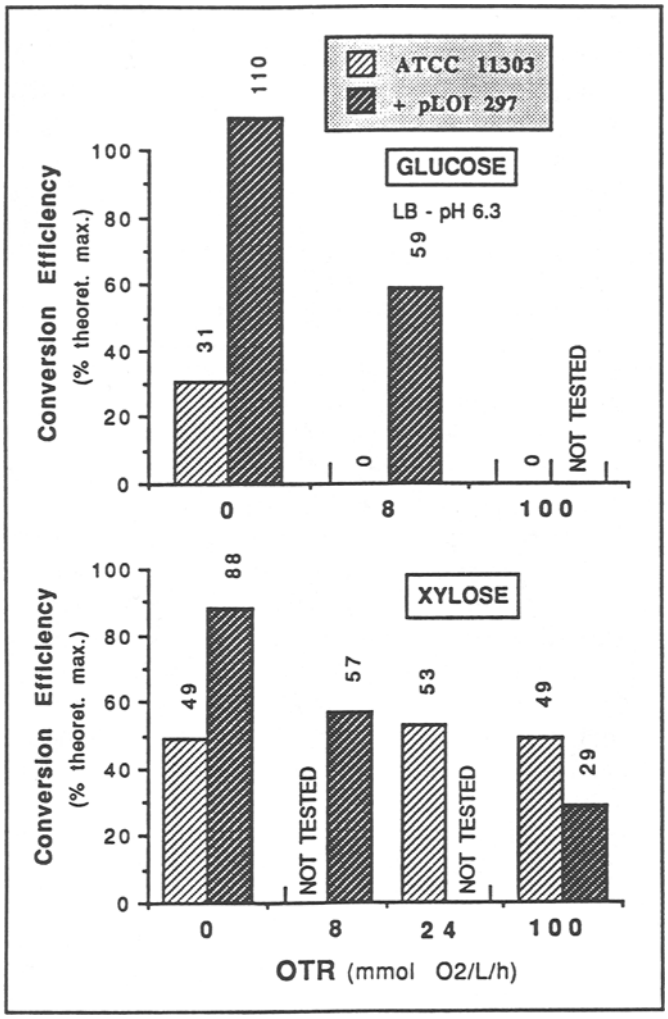

Fig. 5. Comparative sugar-to-ethanol conversion efficiencies for wild-type and recombinant *E. coli* B under anaerobic and aerobic conditions using either glucose or xylose as carbon source. Percentage of conversion efficiency was determined as the $Y_{p/s}$ divided by 0.51 × 100.

Figure 1C shows that, with glucose as carbon source, *E. coli* B (lacking the plasmid) produced acetate as the major end product of both aerobic and anaerobic metabolism. Even under anaerobic conditions, *E. coli* B produced ethanol at a conversion efficiency of 31% of max theoretical (Fig. 5). The conversion efficiency of the recombinant *E. coli* was decreased 50% when the OTR was raised from 0 mmol O_2/L/h to 8 mmol O_2/L/h (Fig. 5).

With xylose as carbon source, *E. coli* B produced ethanol as the major end product of both aerobic and anaerobic metabolism (Fig. 2C) at a relatively constant conversion efficiency of about 50% (Fig. 5). By contrast, xylose-to-ethanol conversion efficiency displayed by the recombinant, reflected the degree of aeration, ranging from 88% with no air to 57% with limiting oxygen and to 29% for a condition of oxygen sufficiency (Fig. 5).

Effect of Oxygen on Ethanol Productivity

Even at constant sugar concentrations, because the growth yield coefficients for glucose and xylose are not the same, and because oxygen affects the growth yield coefficient, the biomass (cell) concentration in these batch cultures is quite variable (Tables 2 and 3). For this reason, across-the-board comparisons of the effect of aeration on the two cultures (in glucose and xylose media), based on volumetric productivities (Q_p) (Tables 2 and 3) or the apparent rate of ethanol production (*see* Figs. 1C and 2C), are not appropriate. Rather, comparisons should be made in terms of the specific productivity (q_p), which takes into account the differences in biomass concentration under the different environmental conditions. As expected, under anaerobic conditions, the recombinant produces ethanol from both glucose and xylose much faster than the strain lacking the *pet* plasmid (Figs. 1C and 2C). Using q_p as the basis of comparison, it appears that the recombinant produces ethanol from glucose at a rate that is about 50% faster than from xylose when the sugar concentration is about 20–25 g/L, but when the sugar concentration is increased to 40–50 g/L, the specific rate of ethanol production is the same for the two sugars (Tables 2 and 3). Figure 2C appears to suggest that aeration stimulates ethanol production from xylose by the strain lacking the plasmid, but the faster rate is owing to the threefold increase in cell concentration, and in reality, the specific ethanol productivity is relatively unaffected by oxygen (Table 3).

DISCUSSION

It has been claimed that "the presence of the *Z. mobilis* ethanol pathway enzymes in *E. coli* under oxidative conditions diverts over 90% of the carbon and prevents the additional energy generation which normally occurs during oxidative growth" (*39*). This conclusion appears to be supported by Neale et al. (*38*), who observed that aeration resulted in only a 10% reduction in the efficiency of ethanol production from glucose (92 to 82%) or xylose (82 to 72%) by *E. coli* strain JM101 carrying the same *Z. mobilis* genes on plasmid pZAN4. However, the recombinant cultures used in these studies were not *E. coli* B, which was the strain that was subsequently selected as the most suitable host for the *pet* plasmid, presumably

because of its resilience to environmental variances (*18*). Prior to our present investigation, it was not known specifically how recombinant *E. coli* B reacted to oxygen. Furthermore, previous studies had been made under conditions of poor aeration efficiency (shake flasks) and in the absence of pH control (*15,38*).

Over the course of a pH-controlled batch fermentation with *E. coli* B, with excess glucose and oxygen (DO > 20% saturation), Landwall and Holme (*34*) have observed that the growth yield coefficient progressively decreased from about 0.4 to < 0.1 g cells/g glucose. The decrease in $Y_{x/s}$ was interpreted as reflecting changes in the degree to which energy was being produced by oxidative vs fermentative metabolism, together with a decreasing efficiency of oxidative phosphorylation (*42*). Unfortunately, the literature is correspondingly silent with respect to growth and metabolism with xylose as carbon and energy source. Our observations on the effect of oxygen on the *E. coli* culture lacking the plasmid whereby the growth yield increased threefold (with both gluose and xylose) are consistent with the existing lierature (*43,44*). Under aerobic conditions, the growth yield coefficient of the recombinant culture would be expected similarly to increase proportionately, but only if it were possible for the culture to derive more ATP from sugar metabolism when oxygen was added to the medium at a rate that surpassed the oxygen demand of the culture. Under such a condition, NADH would be oxidized by the respiratory chain NADH oxidase rather than by the ethanol production pathway (comprised of the *Z. mobilis* enzyme pyruvate decarboxylase and alcohol dehydrogenase II). As a consequence of the increase in oxidative metabolism, the ethanol yield would be expected to decrease. Both the cloned *Zymomonas* alcohol dehydrogenase and the *E. coli* native respiratory chain NADH oxidase enzymes exhibit equally high affinities for NADH (approx 50 μM) (*16*). The switch between these two routes for NADH oxidation might well be the redox state of the medium such that the respiratory chain would only predominant if the OTR exceeded the oxygen demand of the cells and the DO was greater than DO_{crit} (i.e., a value for DO below which the respiration rate is limited by the dissolved oxygen concentration of the medium).

It is clear from the results that under anaerobic conditions the *pet* plasmid fulfills the function for which it was desgined, namely to channel the flow of carbon to ethanol effectively as the predominant end product of hexose and pentose catabolism. However, under aerobic conditions, the recombinant and the culture lacking the plasmid appear to function similarly with respect to the growth and metabolic parameters examined. The possible exception was the reaction of the two cultures to the condition of low-aeration efficiency (OTR = 8) with glucose as carbon source. One curious anomaly was the capacity of *E. coli* B to produce ethanol from xylose at a conversion efficiency (about 50% of theoretical maximum) that was seemingly unaffected by the presence of oxygen in the medium.

Ingram et al. have stated

> Recombinant *E. coli* strains containing the *pet* operon are at a selective disadvantage during growth in the absence of abundant, fermentable sugar. The *Z. mobilis* ethanol pathway serves as both a carbon and energy drain in these recombinants, minimizing the utility of oxidative metabolism with the more complex substrates. Cells are starved for pyruvate and ATP by diversion of carbon skeletons and NADH to ethanol. Thus, recombinant *E. coli* strains expressing the *pet* operon are debilitated in comparison to wild-type organisms with respect to survival in the natural environment (*9*).

In the context of these statements by Ingram, it is interesting to note that under a condition of oxygen limitation (owing to a low-aeration efficiency), we observed that the response of the recombinant was intermediate with respect to both growth and ethanol yield. Since the experiments on the effect of oxygen reported by others (*15,38*) were performed using shake flasks under a condition that is likely to produce an oxygen-limiting rate of oxygen transfer (Lawford and Rousseau, unpublished observations), we suggest that those observations are more comparable to our observations made under a condition of oxygen deficiency, and that the conclusions made about the fate of this type of genetic construct under aerobic conditions (*9*) "in the natural environment" (*39*) should be reassessed in the light of the information presented in this study.

Under aerobic conditions, *E. coli* metabolizes pyruvate primarily by the pyruvate dehydrogenase complex and by lactate dehydrogenase with excess acetyl-CoA being converted to acetate (*8*). Ingram and Conway (*15*) observed that "appreciable quantities of acetate were produced by the parent strain (*E. coli* TC4) even under aerobic conditions" and *E. coli* B, lacking the *pet* plasmid, produced significant amounts of acetate from glucose under both anaerobic and aerobic conditions (Fig. 1C), but acetate was not produced from xylose. Lactate was also an end product of glucose metabolism under aerobic conditions and was almost the sole end product of aerobic xylose metabolism (results not shown).

Based on experiments with glucose, Lorowitz and Clark (*42*) reported that oxygen represses alcohol dehydrogenase in *E. coli* (strain K12), and this observation was corroborated by Neale et al. (*38*) with strain JM101 using glucose and xylose as carbon sources. Whereas the nonethanologenic nature of the *E. coli* B, lacking the *pet* plasmid, metabolizing glucose under aerobic conditions, would seem predictable in view of these previous observations by others (*38,42*), our observations with xylose under similar conditions, in which the xylose-to-ethanol conversion efficiency was 30% of the maximum theoretical, suggest a need to reassess the validity of the conclusion regarding repression of alcohol dehydrogenase by oxygen. It seems possible that the effect of oxygen might be strain-specific.

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