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Cofermentation of Glucose, Xylose, and Arabinose by Mixed Cultures of Two Genetically Engineered *Zymomonas mobilis* Strains

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ABSTRACT

Cofermentation of xylose and arabinose, in addition to glucose, is critical for complete bioconversion of lignocellulosic biomass, such as agricultural residues and herbaceous energy crops, to ethanol. A factorial design experiment was used to evaluate the cofermentation of glucose, xylose, and arabinose with mixed cultures of two genetically engineered *Zymomonas mobilis* strains (one ferments xylose and the other arabinose). The pH range studied was 5.0–6.0, and the temperature range was 30–37°C. The individual sugar concentrations used were 30 g/L glucose, 30 g/L xylose, and 20 g/L arabinose. The optimal cofermentation conditions obtained by data analysis, using Design Expert software, were pH 5.85 and temperature 31.5°C. The cofermentation process yield at optimal conditions was 72.5% of theoritical maximum. The results showed that neither the arabinose strain nor arabinose affected the performance of the xylose strain; however, both xylose strain and xylose had a significant effect on the performance of the arabinose strain. Although cofermentation of all three sugars is achieved by the mixed cultures, there is a preferential order of sugar utilization. Glucose is used rapidly, then xylose, followed by arabinose.

Index Entries: Recombinant *Zymomonas;* ethanol; cofermentation; xylose; arabinose; mixed culture fermentation

INTRODUCTION

Lignocellulosic feedstocks are composed predominantly of cellulose, hemicellulose, and lignin, and are naturally resistant to chemical and bio-

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logical conversion. Because the feedstock can represent more than 40% of all process costs *(1)*, an economical biomass-to-ethanol process critically depends on the rapid and efficient conversion of all sugars (e.g., glucose, xylose, and arabinose) present in their cellulosic and hemicellulosic fractions *(2)*. Many organisms can ferment the glucose component of cellulose to ethanol, but efficient conversion of the pentose sugars, particularly xylose and arabinose, in the hemicellulose fraction has been hindered by the lack of a suitable biocatalyst *(3)*. Xylose is the predominant pentose sugar derived from the hemicellulose of most hardwood feedstocks, but arabinose can constitute a significant amount of the pentose sugars derived from various agricultural residues and other herbaceous crops, e.g. switchgrass, that are being considered for use as dedicated energy crops. Although arabinose makes up only 2–4% of the total pentoses in hardwoods, it represents 10–20% of the total pentoses in many herbaceous crops *(4)*. Arabinose contents can be as high as 30–40% of the total pentoses in corn fiber, a byproduct of corn processing *(5)*.

Zymomonas mobilis is a unique organism with the ability to anaerobically ferment sucrose, glucose, and fructose via the Entner-Doudoroff pathway, which is used primarily by aerobic organisms *(6)*. Highly expressed pyruvate decarboxylase and ethanol dehydrogenase genes rapidly convert pyruvate to ethanol *(7)*. Comparisons of high glucose (25% w/v) fermentations between *Z. mobilis* and *Saccharomyces carlsbergenesis* show that *Z. mobilis* is 2.5× faster in specific glucose uptake rate and 3× faster in specific ethanol productivity (8). Higher theoretical ethanol yields are also achieved because of lower cell biomass formation. Although *Z. mobilis* demonstrates many characteristics needed for an ideal ethanol-producing strain, its substrate utilization range is restricted to the fermentation of glucose, fructose, and sucrose *(9)*. Wild-type strains are not naturally suited for fermenting the xylose found in lignocellulosic feedstocks, because they lack the essential xylose assimilation and pentose metabolism pathways *(10)*.

Enzymatic analyses have confirmed that *Z. mobilis* completely lacks xylose isomerase, xylulokinase, and transaldolase activities, and contains insufficient levels of transketolase activity *(10)*. Consequently, introducing and expressing xylose isomerase, xylulokinase, transaldolase, and transketolase into *Z. mobilis* allowed the authors to complete a functional metabolic pathway that converts xylose to central intermediates of the Entner-Doudoroff pathway, and enables *Z. mobilis* to ferment xylose to ethanol *(10)*. Introducing and expressing L-arabinose isomerase, L-ribulokinase, L-ribulose-5-phosphate-4-epimerase, transaldolase, and transketolase from *Esherichia coli* led to the creation of an arabinose-fermenting strain of *Z. mobilis* (11).

In this study, two full factorial experiments are designed to study cofermentation of glucose, xylose, and arabinose by mixed culture. Experiment 1 examines the effects of each strain (individual and mixed) and each

sugar (glucose, xylose, and/or arabinose) on mixed sugar cofermentation performance. The parameters evaluated were process yield (Yp), % xylose, and arabinose utilization. The strains used are xylose-fermenting *Z. mobilis* ATCC 39676 (pZB4L) *(10)* and arabinose-fermenting *Z. mobilis* ATCC 39676 (pZB206) *(11)*. The ratios of sugars are similar to those found in agricultural residues such as corn fiber. The full factorial experiment examines the effect of each strain, individually and combined, by analyzing each fermentation for sugar consumption, ethanol yield, and byproduct formation. Glucose, xylose, and arabinose combinations are varied to determine any effects the sugar combination would have on the performance of the organisms. Experiment 2 examines the effects of pH and temperature on the cofermentation process. The authors hope to demonstrate that a mixed-culture fermentation, capable of fermenting glucose, xylose, and arabinose to ethanol, has potential applications to industries with feedstocks that contain hexose and mixed pentose sugars.

MATERIALS AND METHODS

Microorganisms and Media

Z. mobilis ATCC 39676 (pZB4L) (xylose-fermenting strain) *(10)* and ATCC 39676 (pZB206) (arabinose-fermenting strain) *(11)* were used for mixed-culture cofermentation. Both strains were stored in concentrated working stocks in rich media (RM) + 10% glycerol, and stored at −70°C until use. RM medium (1% yeast extract + 2 g/L KH_2PO_4) was prepared as a 10X concentrated stock solution. Glucose and xylose were prepared as 50% w/v stock solutions, and arabinose as a 25% w/v stock solution. All the solutions were filter-sterilized using a 0.2-μm filter (Nalgene, Rochester, NY).

Cultivation of Inoculum

A 500-mL Erlenmeyer flask that contained 400 mL of RM medium + 30 g/L glucose + 5 g/L xylose (or arabinose) was inoculated with 1.0 mL of thawed culture stock (one flask for each strain). Both flasks were placed in a rotary shaker (New Brunswick, Edison, NJ) at 150 rpm, at 30°C for 15 h, or until the optical density of the culture measured 2.0–3.0 at 600 nm. Cultures were transferred to sterile 150-mL centrifuge bottles and centrifuged at 2800 *g* for 10 min. The cell pellets were resuspended in 10 mL of RM medium, and used to inoculate the fermenters.

Fermentations

Fermentations were conducted in Biostat-Q fermenters from B. Braun (Allentown, PA) that contained 500 mL working volume of RM medium + 10 mg/L tetracycline + sugars. In the first factorial experiment, which was a 2-level, 5-factor full factorial, the sugar concentrations and type

Table 1
List of Fermentations and Their Results for Design 1, Performed to Study Interaction of Two Strains 39676(pZB4L) and 39676(pZB206) and Three Sugars (Glucose, Xylose, and Arabinose) at Constant pH 5.5 and T 35°C

Fermentation no.	Strains		Glucose (g/L)	C-Source xylose (g/L)	Arabinose (g/L)	Yp (%)	Xylose consumed (%)	Arabinose consumed (%)
	39676 (pZB4L)	39676 (pZB206)						
1	Y	Y	0.0	29.0	0.0	88.8	97.9	0.0
2	Y	N	30.7	30.5	0.0	85.0	96.4	0.0
3	N	Y	31.1	30.1	23.1	73.8	22.9	78.4
4	N	Y	33.8	0.0	0.0	82.7	0.0	0.0
5	Y	N	32.4	0.0	22.3	89.0	0.0	2.6
6	Y	Y	0.0	0.0	22.0	57.4	0.0	72.5
7	N	Y	0.0	28.8	23.4	14.7	0.9	16.0
8	Y	Y	30.9	31.4	0.0	83.8	94.2	0.0
9	Y	N	0.0	30.1	0.0	84.6	97.8	0.0
10	Y	N	0.0	0.0	22.5	0.0	0.0	0.0
11	Y	Y	32.5	0.0	22.5	74.7	0.0	74.7
12	Y	N	30.9	29.8	23.1	84.9	94.6	0.0
13	Y	N	33.1	0.0	0.0	86.4	0.0	0.0
14	Y	Y	0.0	27.2	23.2	53.5	96.6	13.8
15	N	Y	31.0	30.8	0.0	94.3	9.4	0.0
16	N	Y	33.6	0.0	22.1	79.4	0.0	98.6
17	N	Y	0.0	0.0	22.0	82.7	0.0	97.1
18	Y	Y	31.3	30.2	23.2	70.4	91.0	70.7
19	N	Y	0.0	28.6	0.0	1.7	0.3	0.0
20	Y	Y	33.8	0.0	0.0	83.2	0.0	0.0
21	Y	N	0.0	27.5	23.1	49.9	97.5	0.4

Table 2
List of Fermentations and Their Results for Design 2, Performed to Study Effect of pH and Temperature on Mixed Culture Cofermentation of Glucose, Xylose, and Arabinose by Two Strains 39676(pZB4L) and 39676(pZB206)

Fermentation no.	T (C°)	pH	Yp (%)	Xylose consumed (%)	Arabinose consumed (%)
22	30.0	5.0	68.50	84.88	51.50
23	33.5	5.0	65.90	82.42	52.83
24	37.0	5.0	57.20	60.57	47.32
25	30.0	5.5	67.80	89.41	56.23
26	33.5	5.5	69.80	87.67	66.12
27	33.5	5.5	71.60	89.45	68.13
28	37.0	5.5	62.80	71.48	57.72
29	30.0	6.0	73.20	93.57	73.70
30	33.5	6.0	70.20	90.80	70.92
31	37.0	6.0	61.50	66.12	70.07

varied with the conditions. Fermentations were controlled at 300 rpm agitation, 35°C, and pH 5.5, controlled using 2 *M* KOH. For the second factorial experiment, which was a 3-level, 2-factor full-factorial design with duplicate centerpoints, pH and temperature varied, depending on the condition being tested. The medium used was RM + 10 mg/L tetracycline + 30 g/L glucose + 30 g/L xylose + 20 g/L arabinose at a agitation rate of 300 rpm. All fermenters were inoculated with the concentrated cells to achieve an initial OD_{600} of 0.2. Data from 72 h fermentation were used for data analysis.

Analyses

Samples were taken periodically throughout the course of the fermentations and analyzed for sugars, ethanol, and byproducts by HPLC. HPLC analysis was conducted using a Hewlett-Packard (Wilmington, DE) series 1090 HPLC with a Bio-Rad (Hercules, CA) HPX-87H column. Process yield was calculated as ethanol concentration × 100 ÷ initial sugar concentration × 0.51.

Experimental Design and Data Analysis

The statistical design programs Design Ease and Design Expert (Stat-Ease, Minneapolis, MN) were used to design and analyze the results of the experiments. The designed experiments are shown in Tables 1 and 2.

RESULTS AND DISCUSSION

Two factorial experiments were carried out to evaluate the performance of the mixed-culture, mixed-sugar cofermentation system. First, a

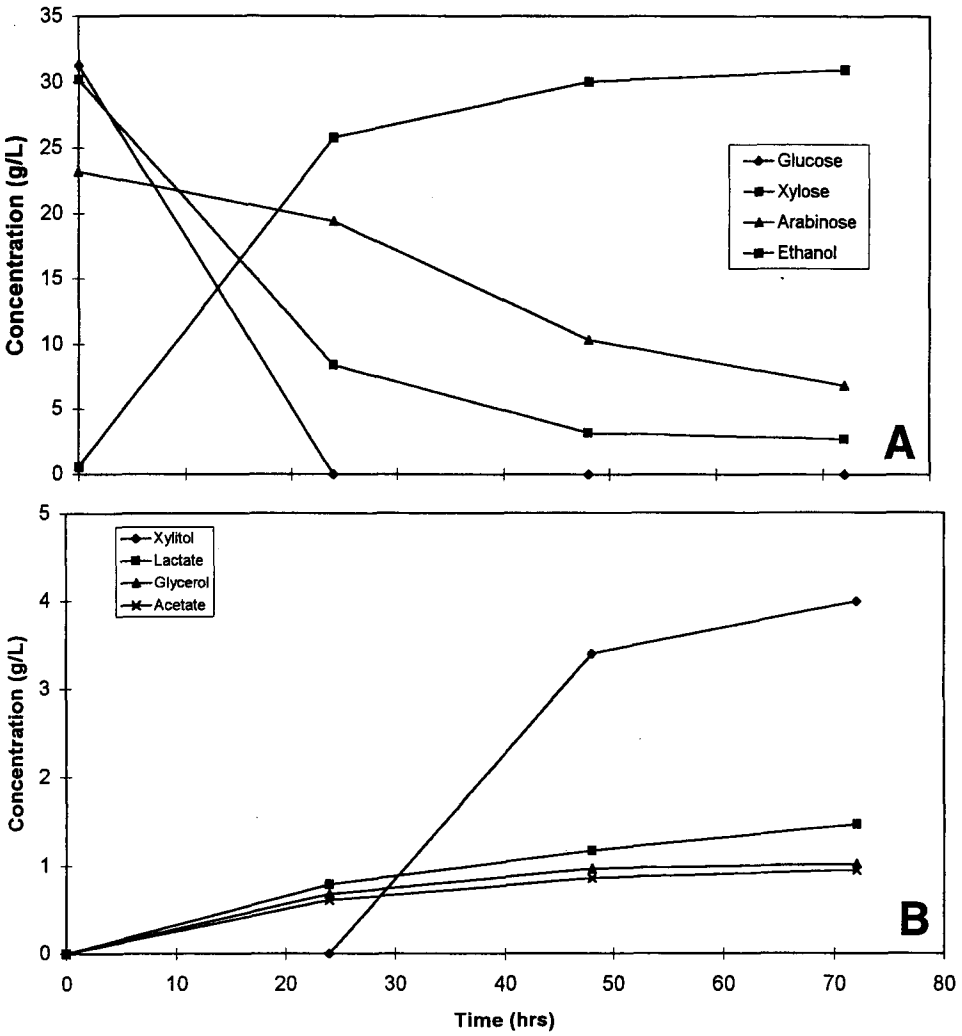

Fig. 1. Coculture fermentation profile of strains 39676(pZBAL) and 39676(pZB206) on mixed sugar of 30 g/L glucose, 30 g/L xylose, and 20 g/L arabinose at pH 5.5 and *T* 35°C. **(A)** Growth profile. **(B)** Byproduct profile.

two-level, five-factor full factorial (2^5) design was used to evaluate the main and two-way interaction effects of each strain (39676 (pZB4L) [xylose strain] and/or 39676 (pZB206) [arabinose strain]) and each sugar (glucose, xylose, and/or arabinose) on mixed-sugar cofermentation performance (fermentations 1–21). This first factorial experiment was run at 35°C and pH 5.5. Following this experiment, a 3-level 2-factor full-factorial (3^2) design with duplicate centerpoints was run at a fixed sugar concentration of 80 g/L (30 g/L glucose; 30 g/L xylose; and 20 g/L arabinose), to characterize the effect of pH and temperature on the cofermentation process (fermentations 22–31). In this second factorial experiment, the pH was varied

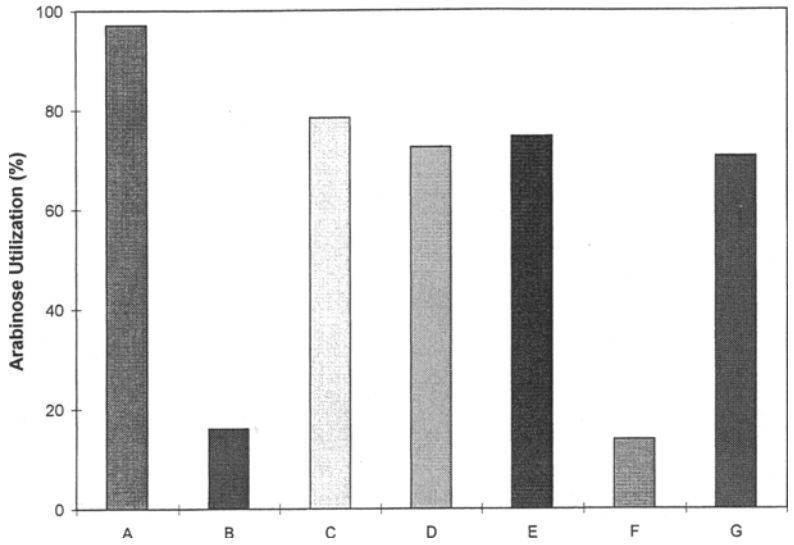

Fig. 2. Effect of glucose, xylose, and xylose strain (39676[pZB4L]) on arabinose utilization by arabinose strain (39676[pZB206]) at pH 5.5 and *T* 35°C. **(A)** Control (arabinose strain grown on arabinose only). **(B)** Arabinose strain grown on arabinose and xylose (effect of xylose). **(C)** Arabinose strain grown on arabinose plus xylose and glucose (effect of xylose and glucose). **(D)** Arabinose and xylose strain grown on arabinose (effect of xylose strain). **(E)** Arabinose and xylose strain grown on arabinose and glucose (effect of xylose strain and glucose). **(F)** Arabinose and xylose strain grown on arabinose and xylose (effect of xylose strain and xylose). **(G)** Arabinose and xylose strain grown on arabinose, xylose, and glucose (effect of xylose strain, xylose, and glucose).

from 5.0 to 6.0, and the temperature from 30 to 37°C (centerpoint at pH 5.5 and 33.5°C).

Evaluation of Cofermentation Performance as a Function of Sugar Mixture and Strain-Combination Fixed pH and Temperature

Figure 1 shows an example of the growth and byproduct formation profiles observed during mixed-culture co-fermentation carried out at pH 5.5 and temperature 35°C (fermentation 18). As this figure illustrates, in the presence of all three sugars, first, glucose is used most rapidly, then xylose and arabinose appear to coferment simultaneously. Another characteristic of this system is that the rate of xylose utilization is somewhat faster than that of arabinose. Finally, except for xylitol and/or arabitol, very little byproduct formation is observed, although low levels of acetate, lactate, and glycerol are produced.

The results of this 2^5 factorial experiment are summarized in bar graph form in Figs. 2,3 (fermentations 3,6,7,11,14,17,18),4, and 5 (fermentations 1,8,9,12,14,18,21), and in three-dimensional (3-D) form in Figs. 6–8 (fermen-

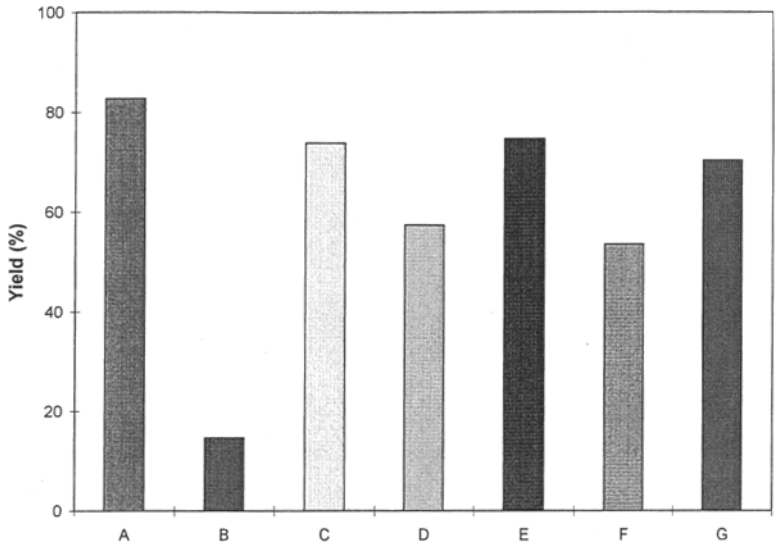

Fig. 3. Effect of glucose, xylose, and xylose strain (39676[pZB4L]) on ethanol process yield (Yp) for arabinose utilization by arabinose strain (39676[pZB206]) at pH 5.5 and *T* 35°C. **(A)** Control (arabinose strain grown on arabinose only). **(B)** Arabinose strain grown on arabinose and xylose (effect of xylose). **(C)** Arabinose strain grown on arabinose plus xylose and glucose (effect of xylose and glucose). **(D)** Arabinose and xylose strain grown on arabinose (effect of xylose strain). **(E)** Arabinose and xylose strain grown on arabinose and glucose (effect of xylose strain and glucose). **(F)** Arabinose and xylose strain grown on arabinose and xylose (effect of xylose strain and xylose). **(G)** Arabinose and xylose strain grown on arbinose, xylose, and glucose (effect of xylose strain, xylose, and glucose).

tation 1–21). A complete summary of the key performance results (process yields, xylose utilization [%], and arabinose utilization [%] is also provided in Table 1.

The key findings from this experiment are that xylose, and, to a lesser extent, the xylose strain inhibit the cofermentation performance of the arabinose strain. Figures 2 and 3 show the effects of glucose, xylose, and the xylose strain on arabinose utilization and ethanol Yp, respectively, for the arabinose strain. These figures show that the presence of both xylose and the xylose strain negatively affect the arabinose strain. Although virtually all the arabinose (97%) was utilized by the arabinose strain in medium that contained only arabinose, arabinose utilization decreased substantially when xylose or the xylose strain, or both, were present (to only 16, 72, and 14%, respectively). Similarly, the Yp was reduced in the presence of xylose and the xylose strain, decreasing from 83% in medium that contained arabinose alone to 15 and 57% in medium that contained arabinose and xylose or the xylose strain, respectively. Based on these results, xylose greatly inhibits arabinose utilization by the arabinose strain. However, experimental results show that the addition of glucose diminishes the in-

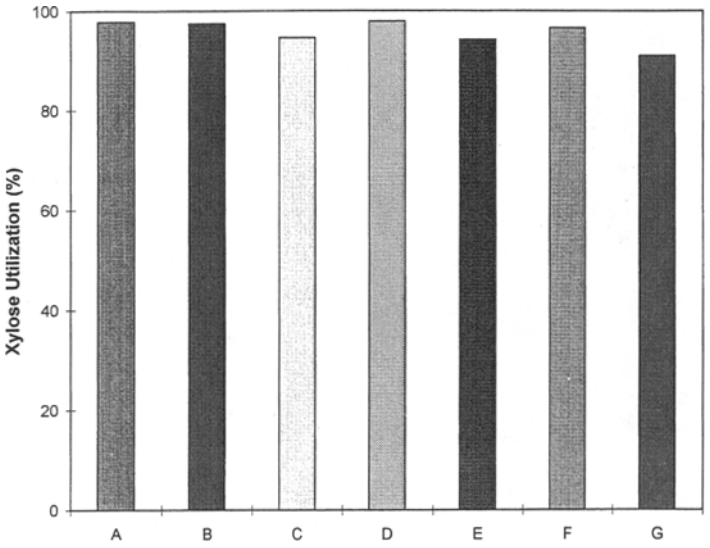

Fig. 4. Effect of glucose, arabinose, and arabinose strain (39676[pZB206]) on xylose utilization by xylose strain (39676[pZB4L]) at pH 5.5 and *T* 35°C. **(A)** Control (xylose strain grown on xylose only). **(B)** Xylose strain grown on xylose and arabinose (effect of arabinose). **(C)** Xylose strain grown on xylose plus arabinose and glucose (effect of arabinose and glucose). **(D)** Xylose strain and arabinose strain grown on xylose (effect of arabinose strain). **(E)** Xylose and arabinose strain grown on xylose and glucose (effect of arabinose strain and glucose). **(F)** Xylose and arabinose strain grown on arabinose and xylose (effect of arabinose strain and arabinose). **(G)** Xylose and arabinose strain grown on arabinose, xylose, and glucose (effect of Arabinose strain, arabinose, and glucose).

hibitory effect of xylose and the xylose strain on arabinose utilization. Figures 2 and 3 show that arabinose utilization and Yp increased to 78 and 74%, respectively, when the arabinose strain was grown in the presence of all three sugars.

Figures 4 and 5 show that the neither arabinose nor the arabinose strain had a major effect on xylose utilization by the xylose strain. As Fig. 4 demonstrates, xylose utilization was above 95% on medium that contained only xylose, regardless of whether glucose or arabinose were present. Similarly, Fig. 5 shows that the Yp achieved by the xylose strain was around 85% in all cases, except when arabinose was present (in which case, the Yp yield decreased to about 50%, because arabinose was not utilized.

The main factor effects and the two-way factor–factor interaction effects identified in this experiment can be visualized more easily when the results are represented in 3-D form as cubic plots. Figures 6–8 show cubic representations of the results generated using the Design-Ease software. In the cubic plots shown in these figures, the three orthogonal axes (A, B, and C) represent the three factors being varied in the experiment. The

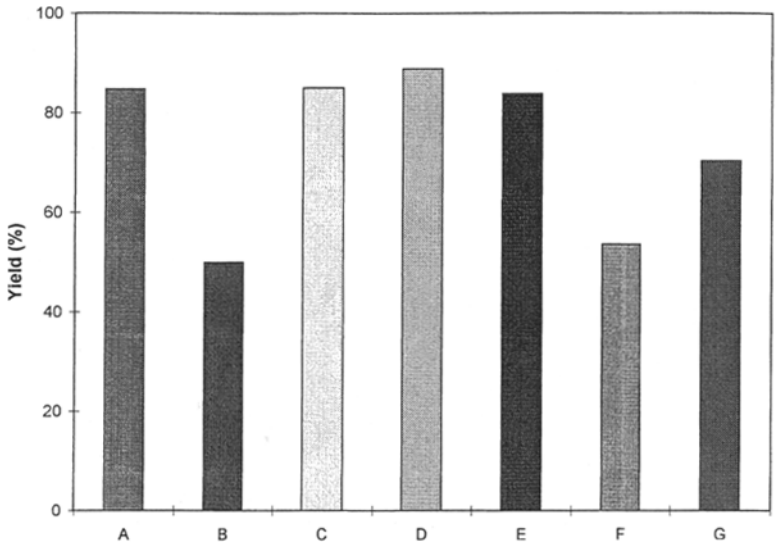

Fig. 5. Effect of glucose, arabinose, and arabinose strain (39676[pZB206]) on ethanol process yield (Yp) for xylose utilization by xylose strain (39676[pZB4L]) at pH 5.5 and *T* 35°C. **(A)** Control (xylose strain grown on xylose only). **(B)** Xylose strain grown on xylose and arabinose (effect of arabinose). **(C)** Xylose strain grown on xylose plus arabinose and glucose (effect of arabinose and glucose). **(D)** Xylose strain and arabinose strain grown on xylose (effect of arabinose strain). **(E)** Xylose and arabinose strain grown on xylose and glucose (effect of arabinose strain and glucose). **(F)** Xylose and arabinose strain grown on arabinose and xylose (effect of arabinose strain and arabinose). **(G)** Xylose and arabinose strain grown on arabinose, xylose, and glucose (effect of arabinose strain, arabinose, and glucose).

eight corners of the cubes correspond to the eight independent cases investigated for each possible subset of the cofermentation system (negative sign is indication of absence and positive sign indicates the presence of the factor), and the numerical values are the performance results achieved at each condition. For example, Fig. 6 summarizes the effect of the xylose strain (axis A), glucose (axis B), and xylose (axis C) on the performance of the arabinose strain in arabinose-containing media. Figure 6A graphically illustrates how the Yp varied under the conditions studied, i.e., at each corner of the cube. As Fig. 6A shows, a Yp of 83% was achieved when the fermentation was carried out using only arabinose, i.e., in the absence of glucose, xylose, and the "4L" xylose strain (A−, B−, C− lower left-hand front corner of the cube). Figure 6A readily shows that the Yp falls to 57% when the xylose strain is added (A+, B−, C− lower right-hand front corner of the cube), and to only 15% when xylose is added (A−, B−, C+ lower left hand back corner of the cube); a Yp of 54% is achieved when both xylose and the xylose strain are present (A+, B−, C+ lower right hand back corner of the cube). The cubical representation of the results makes the beneficial effect of glucose on alleviating the inhibitory effect

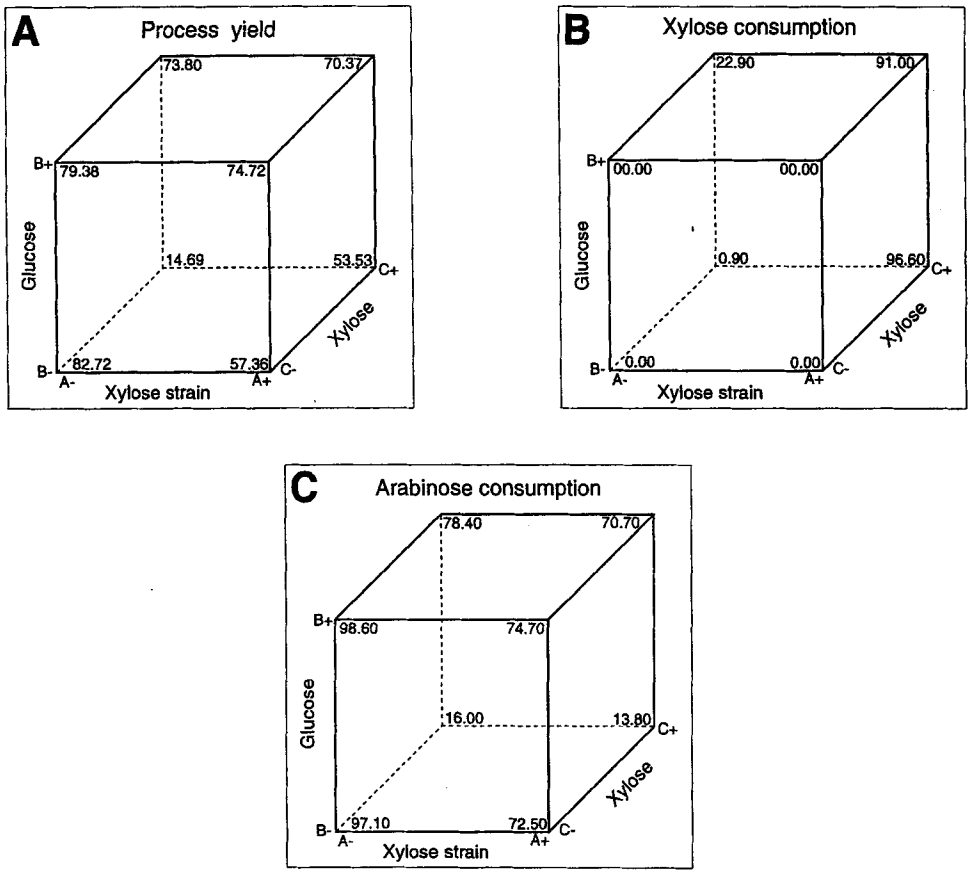

Fig. 6. Cubical presentation of the effect of xylose strain 3967PZB4L), xylose, and glucose on the performance of arabinose strain 39676(pZB206). The effects are predicted by the software program Design Expert. The effects of three factors (glucose, xylose, and xylose strain) on the Yp, xylose utilization, and arabinose utilization are shown in Figs. **6A–C**, respectively. Note: sign (−) means absence of the factor, and sign (+) means presence of the factor.

of xylose and the xylose strain on Yp readily apparent. All conditions under which glucose is present (i.e., the B+ corner conditions depicted in the upper plane of the cube) achieve higher Yps than when glucose is absent (i.e., the B− corners depicted in the lower plan of the cube), except for the arabinose-only control. Figure 6B,C shows similar cubic representations of the xylose and arabinose utilization performance results achieved for the arabinose–arabinose strain co-fermentation system.

Figure 7 shows similar plots that summarize the performance of the xylose–xylose strain system. Figure 8 shows a summary of cofermentation system performance as a function of the tertiary sugar mixture, when both strains are present. As Fig. 8 shows, when all three sugars were present, the mixed-culture cofermentation system achieved an ethanol Yp of 70%,

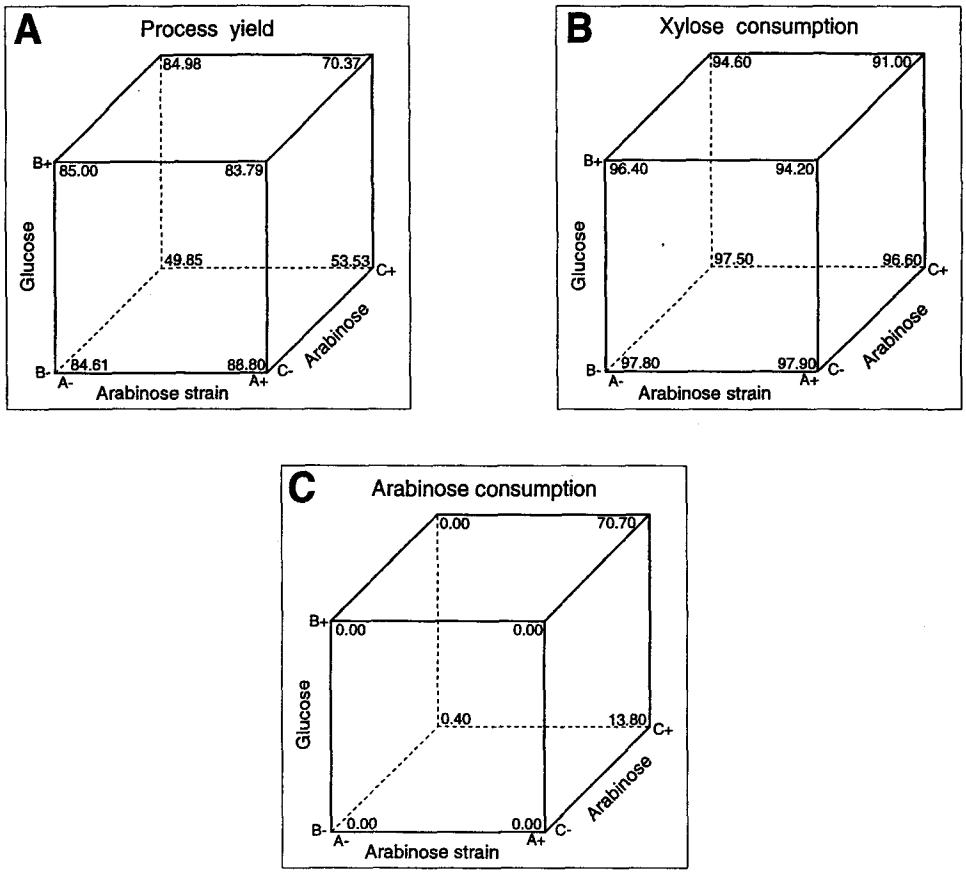

Fig. 7. Cubical presentation of the effect of arabinose strain 39676(pZB206), arabinose, and gluose on the performance of xylose strain 39676(pZB4L). The effects are predicted by the software program Design Expert. The effects of three factors (glucose, arabinose, and arabinose strain) on the Yp, xylose utilization, and arabinose utilization are shown in Fig. **7A–C**, respectively. Note: sign (−) means absence of the factor, and sign (+) means presence of the factor.

with 91% xylose utilization and 71% arabinose utilization. The marginal Yp of 70% is mostly a result of incomplete arabinose utilization.

Evaluation of Mixed-Culture Cofermentation Performance as a Function of pH and Temperature

The effect of pH and temperature on the mixed culture cofermentation process was studied using a 3-level 2-factor full-factorial (3^2) experiment carried out at a fixed sugar concentration (30 g/L glucose; 30 g/L xylose; and 20 g/L arabinose). The pH ranged from 5.0 to 6.0 and temperature from 30 to 37°C. Duplicate centerpoints were run at pH 5.5 and 33.5°C. Results of this experiment are summarized in Table 2 and Fig. 9 (fermentations 22–31).

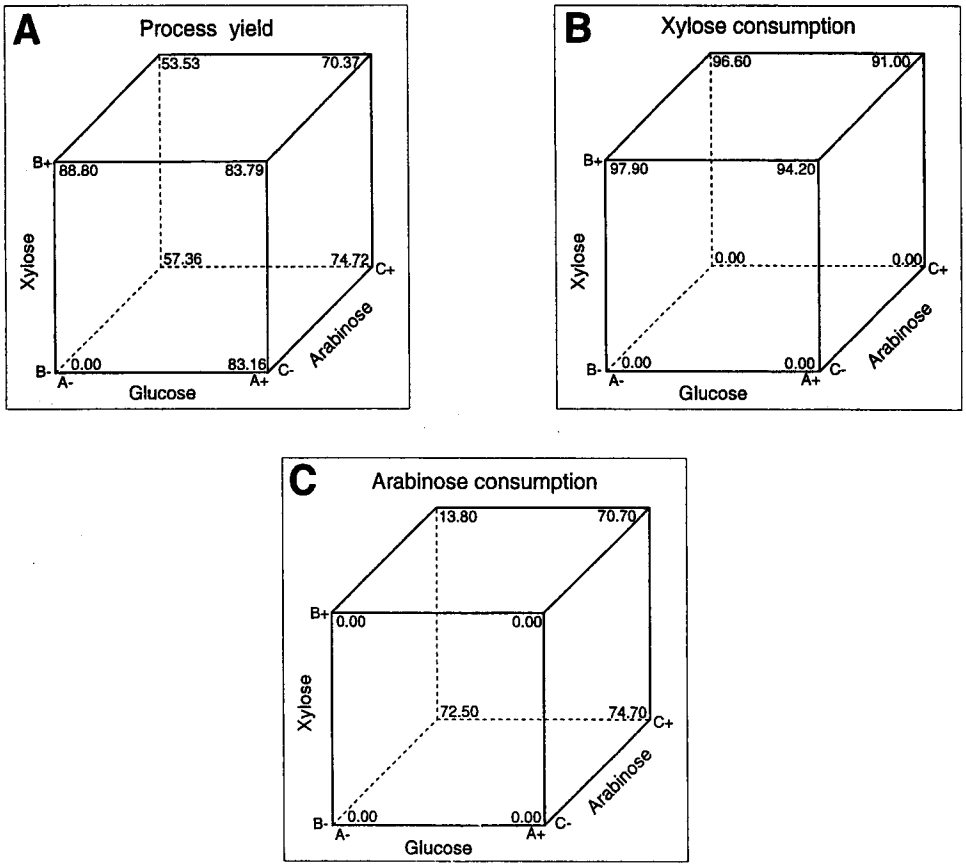

Fig. 8. Cubical presentation of the effect of glucose, xylose, and arabinose on the cofermentation by mixed cultures of xylose strain 39676(pZB4L) and arabinose strain 39676ZP206). The effects are predicted by the software program Design Expert. The effects of three factors (glucose, xylose, and arabinose) on the Yp, xylose utilization, and arabinose utilization are shown in Figs. **8A–C**, respectively. Note: sign (−) means absence of the factor, and sign (+) means presence of the factor.

As shown in both Table 2 and Fig. 9, the highest Yp and xylose and arabinose utilization were 73, 94, and 74%, respectively, and were achieved at pH 6.0 and 30°C (fermentation 29). The lowest values for these same performance metrics were 57, 61, and 47% at pH 5.0 and 37°C (fermentation 24). In general, increasing pH and decreasing temperature had positive effects on the cofermentation process.

Statistical analysis of the results showed that both Yp and xylose utilization were more sensitive to temperature than to pH. By increasing the pH from 5.0 to 6.0, with temperature ranging from 30 to 37°C, the ethanol Yp yield and xylose utilization increased only by 5 and 9%, respectively; increasing temperature from 30 to 37°C caused the ethanol Yp and xylose utilization to drop 10 and 20%, respectively. On the other hand, the relative sensitivity of arabinose utilization to pH and temperature was the

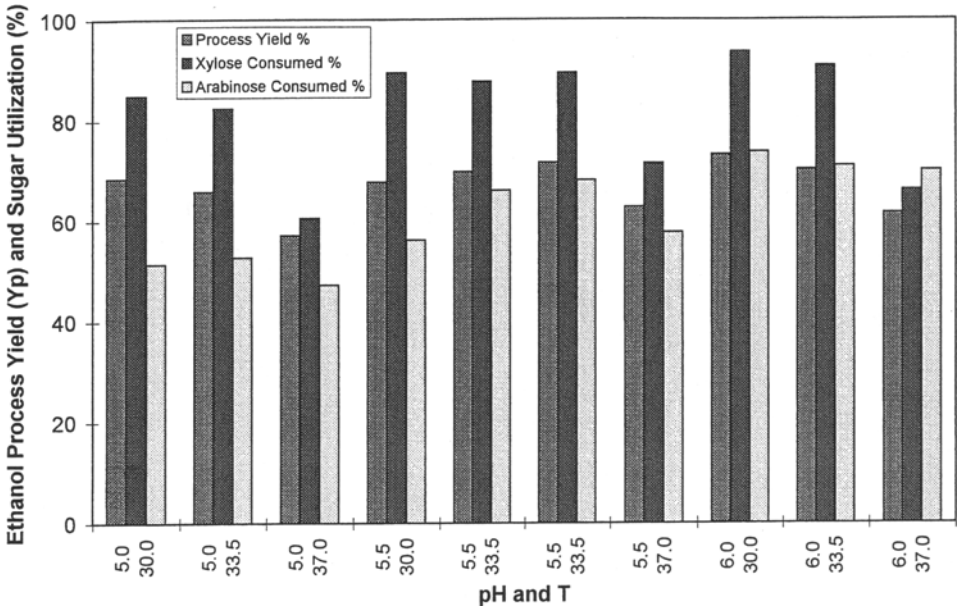

Fig. 9. Effect of pH and temperature on the ethanol process yield (Yp), xylose, and arabinose utilization by cofermentation of glucose, xylose, and arabinose, using mixed cultures of xylose strain 39676(pZB4L) and arabinose strain 39676(pZB206).

opposite, with pH exerting a more significant effect than temperature. Arabinose utilization increased by about 20% when pH was increased from 5.0 to 6.0, at temperature range of 30 to 37°C, but decreased only by 5% when temperature was raised from 30 to 37°C. The optimum conditions for mixed-culture cofermentation with sugar mixture (glucose:xylose:arabinose at 30:30:20 g/L) was estimated to be approx pH 5.85 and 31.5°C, using the Design-Expert program. Under these optimal conditions, the Yp and xylose and arabinose utilization were predicted to be 72.5, 94, and 71%, respectively.

As in previous experiments, low levels of byproducts were produced by the mixed culture system over the range of conditions tested. Major byproducts were xylitol and lactate, which were produced at concentrations as high as 4 g/L and 2 g/L, respectively. The concentrations varied, depending on the experimental conditions. Other minor byproducts formed were glycerol and acetate; both accumulated to less than 1 g/L under all conditions. *Zymomonas* generally produces few by-products *(12)*. Byproducts found in this work are similar to those reported earlier *(12)*.

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REFERENCES

1. Hinman, N. D., Wright, J. D., Hoagland, and W., Wyman, C. E. (1989), *Appl. Biochem. Biotechnol.* **20/21,** 391–401.
2. Wright, J. D. (1987), AIChE National Meeting, Minneapolis, MN.
3. Zhang, M., Franden, M. A., Newman, M., McMillan, J., Finkelstein, M., and Picataggio, S. (1995), *Appl. Biochem. Biotechnol.* **51/52,** 527–536.
4. McMillan, J. D. and Boynton B. L. (1994), *Appl. Biochem. Biotechnol.* 45/46, 569–584.
5. Carlson, T. (1994), *Fifth Corn Utilization Conference,* June 8–10, St. Louis, MO, National Corn Growers Association.
6. Doelle, H. W., Kirk, L., Crittenden, R., Toh, H., and Doelle M. B. (1993), *Crit Rev in Biotechnol.* **13,** 57–98.
7. Ingram, L. O., Eddy, C. K., Mackenzie, K. F., Conway, T., and Alterthum F. (1989), *Dev. Indust. Microbiol.* **30,** 53–69.
8. Rogers, P. L., Lee, K. J., and Tribe, D. E. (1979), *Biotechnol. Lett.* **1,** 165–170.
9. Eveleigh, D. E., Stokes, H. W., and Dally, E. L., (1983), *Organic Chemicals from Biomass,* Wise, D., ed., Benjamin/Cummings, Menlo Park, CA.
10. Zhang, M., Eddy, C., Deanda, K. A., Finkelstein, M., and Picataggio, S. (1995), *Science* **267,** 240–243.
11. Deanda, K. A., Eddy, C., Zhang, M., and Picataggio, S. (1996), *Appl. Environ. Microbiol.* **62,** 4465–4470.
12. Swings, J. and DeLay, J. (1977), Bacteriol. Rev. **41,** 1–46.