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EXPERIMENTAL ARTICLES

Optimization of Culture Medium and Growth Conditions for Production of L-Arabinose Isomerase and D-Xylose Isomerase by *Lactobacillus bifermentans*[1]

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Abstract—*Lactobacillus bifermentans* was used to produce the intracellular enzymes L-arabinose isomerase and D-xylose isomerase. Various factors of cultivation (temperature, pH, and incubation period) and culture medium composition (mineral salts, carbon source, and nitrogen source) were studied to select the conditions that maximize production of these enzymes. Arabinose isomerase and xylose isomerase activities were 9.4 and 7.24 U/ml, respectively. They were highest at 9 h of cultivation in the optimized medium, 1.6 times higher than that in the basic MRS broth. The optimal medium composition and cultivation conditions were determined. For optimal growth, the strain required Tween 80 (1 g/l) and a source of inorganic nitrogen (e.g., ammonium citrate). The bacterium had no requirement for sodium acetate for either growth or production of isomerases. The production rate of enzymes was increased when metal ions were added, primarily manganese (2.5 mM).

Keywords: L-arabinose isomerase, *Lactobacillus bifermentans*, MRS medium, optimization, production, D-xylose isomerase.

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Hemicelluloses account for approximately 20–35% of lignocellulose biomass. Unlike cellulose, hemicelluloses are not chemically homogeneous. They are heterogeneous polymers made up of pentoses (xylose, arabinose), hexoses (mannose, glucose, galactose), and sugar acids [1–3].

Many microorganisms have the capacity to use pentoses such as xylose or arabinose as the sole carbon source. In the case of bacteria, the two enzymes which play an important part in this conversion of pentose are L-arabinose isomerase (L-arabinose ketol isomerase EC 5.3.1.4) and D-xylose isomerase (D-xylose ketol isomerase EC 5.3.1.5), which catalyse in vivo the reversible isomerization of L-arabinose in L-ribulose and D-xylose in D-xylulose, respectively [4, 5]. This isomerization represents the first step in the way of conversion of pentoses into D-xylulose-5-phosphate, which is an intermediary of the microbial pentose phosphate pathway [4, 6–7].

Since interest in the production of biofuel from the cereal biomass is presently growing, these two enzymes attract the attention of researches both in the genetic construction [8] as described in the *Zymomonas mobilis* strain for D-xylose [9] and L-arabinose [10] or *Saccharomyces cerevisiae* yeast [10, 11] and in the realization of isomerization and simultaneous fermentation with *Saccharomyces cerevisiae* [12]. Development of biofuel from wheat increases the need to use pentoses, since wheat contains higher quantities of pentoses than corn, which is currently used in U.S. biofuel plants.

But at the industrial level the most important application for these enzymes is by immobilization; xylose isomerase (also referred to as glucose isomerase, GI) converts D-glucose to D-fructose and is one of the largest-volume commercial enzymes used today [13]. This catalytic activity is commonly used in industry for the production of high-fructose corn syrup (HFCS) [14–16].

With regard to arabinose isomerase, there is interest in the conversion of D-galactose into D-tagatose at the industrial level. Indeed, D-tagatose has a potential for use as a sugar-substitute in food, since it has a taste and sweetness similar to sucrose. D-tagatose is also a non-caloric sweetener with no laxative effect. It is also useful as an intermediate for synthesis of other optically active compounds and as an additive in detergent and cosmetic and pharmaceutical formulations [4].

In this manuscript we report work on the growth medium and the cultivation conditions needed in order to optimize the production of L-arabinose isomerase

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Table 1. Experimental range and levels of the independent variables

Variable name	Levels		
	–1	0	+1
Peptone (g/l)	2.5	10	17.5
Meat extract (g/l)	2.5	10	17.5
Yeast extract (g/l)	1	5	9
Potassium phosphate dibasic (g/l)	0.5	2	3.5
Sodium acetate (g/l)	1	5	9
Ammonium phosphate (g/l)	0.5	2	3.5

and D-xylose isomerase from *Lactobacillus bifermentans* DSM 20003^T.

MATERIALS AND METHODS

Microorganisms and culture method. The strain *Lactobacillus bifermentans* (DSM 20003^T) was obtained from Deutsche Sammlung von Mikroorganismen (DSMZ), Braunschweig, Germany. The strain was cultivated at 37°C on a medium MRS at pH 7.5: peptone (10 g/l), meat extract (10 g/l), yeast extract (5 g/l), Tween 80 (1 g/l), K_2HPO_4 (2 g/l), sodium acetate (5 g/l), ammonium acetate (2 g/l), $MgSO_4 \cdot 7H_2O$ (0.05 g/l). Enzyme production was investigated on media supplemented with arabinose or xylose as substrates. Growth was monitored by measuring absorbance at 600 nm. Cells were harvested in the late exponential growth phase by centrifugation at 10000 *g* for 30 min.

Determination of cell density. Cell numbers were determined by direct cell counting using a Thoma chamber (0.02 mm depth) under phase contrast in an Olympus model BH-2 microscope (Olympus America Inc., United States). When necessary, the samples were diluted with sterile 0.5 M NaCl solution.

Enzyme and protein assays. Cells were isolated by centrifugation of culture broths (1 ml). After centrifugation, they were suspended in a 50 mM MES solution pH 7.5 (45 µl. The arabinose isomerase reaction was carried out with 45 µl of the cell suspension incubated together with 30 µl of the test solution (50 mM MES pH 7.5; 0.1 M L-arabinose; 5 mM $CoCl_2$) at 60°C for 10 min. For D-xylose isomerase activity, L-arabinose was replaced with D-xylose at the same concentration. The formation of L-ribulose or D-xylulose from L-arabinose and D-xylose respectively was measured using the modified colorimetric assay of Kulka at 535 nm and 630 nm, respectively. The absorption was measured with a UVIKON spectrophotometer (Kontron Instruments, Switzerland).

One unit of L-arabinose isomerase or D-xylose isomerase catalyzes the formation of 1 µmol ketose (ribulose or xylulose) min^{-1} at 60°C in this assay system.

Effect of initial pH. While optimizing the initial pH of the medium, the pH was adjusted within the range from 5.0 to 9.0 with 10 N NaOH or 10 N hydrochloric acid. The fermentation was carried out at 35°C until the beginning of the stationary phase.

Effect of incubation temperature. To study the effect of incubation temperature on the enzymes, the flasks were incubated at various temperatures (30, 35, 37, and 40°C). The other parameters, like media composition and pH, were kept at their optimum level and the fermentation was run until the beginning of the stationary phase.

Effect of inoculum volume. The effects of the inoculum volume on isomerase production were studied by adding different concentrations of cell mass to the medium; the fermentation was carried out until the beginning of the stationary phase. The medium used was medium MRS; pH and incubation temperature were kept at their optimum levels.

Experimental optimization. A series of studies was performed to investigate the effect of various media components on AI and XI activity to optimize the media. To study the effect on enzyme production, the basal medium MRS was used as model and modified (Table 1). Varying quantities of different organic and inorganic nitrogen sources or other components were added and tested to determine their effect on arabinose isomerase and xylose isomerase production. Different carbon sources were added to the medium and tested at various concentrations. Salts of various metal ions, including Ca^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, Mg^{2+}, Mn^{2+}, and Zn^{2+}, were applied to the culture medium.

Materials. Meat extract (Sigma Chemical Co.), yeast extract, peptone, and casamino acids (Difco Laboratories) were used. All other chemicals were of analytical grade from Sigma Chemical Co and were used without further purification.

RESULTS AND DISCUSSION

Effect of temperature on enzyme production. The cultivation was carried out to the entry stationary phase. The results are presented in Fig. 1. The maximum biomass and enzymatic production (AI: 3.5 U/ml and XI: 2.6 U/ml) were attained at 37°C (Fig. 1). A decrease in the yield of AI or XI was observed when the temperature was higher or lower than the optimum incubation temperature. Both suboptimal and superoptimal temperatures were found to have an adverse effect on the activity of *L. bifermentans enzymes* (Fig. 1).

Consequently, the incubation temperature was crucial because its changes were likely to result in a longer generation time and the lag phase was considerably longer at non-optimal temperatures, thereby decreasing the yield of enzymes.

Effect of pH on enzyme production. Enzyme production and growth of *L. bifermentans* were markedly influenced by initial pH of the culture medium. *L. bifer-*

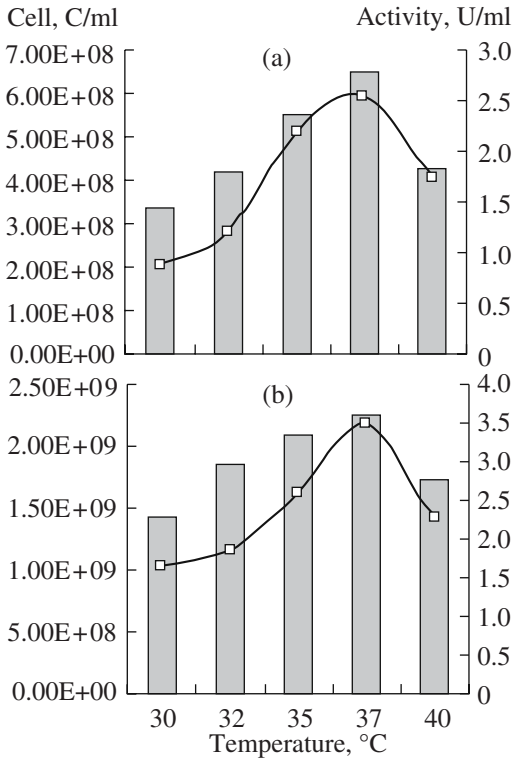

Fig. 1. Effect of initial culture temperature on growth (lines) and AI (a) and XI (b) production (columns) of *L. bifermentans.* The bacterium was cultivated at pH 6.8 in the MRS medium, supplemented with 10 g/l arabinose (a) or xylose (b). The results shown are the means of duplicate assays of duplicate shake cultures.

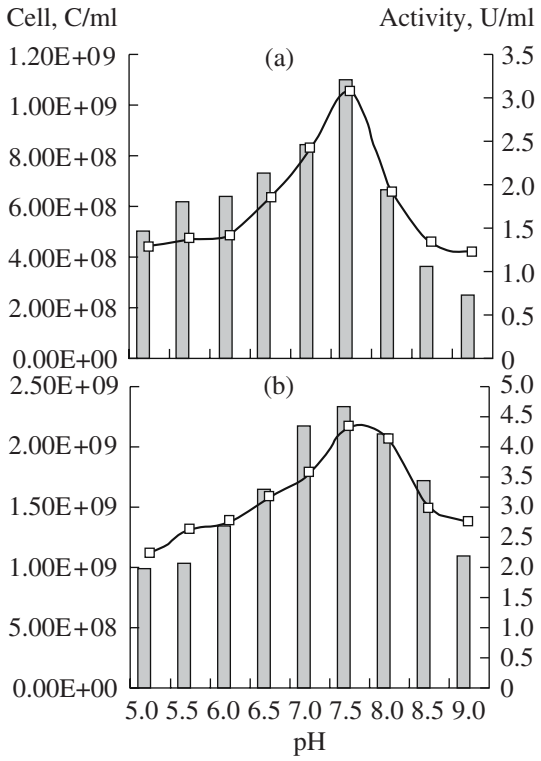

Fig. 2. Effect of initial medium pH on growth (lines) and XI (a) and AI (b) production (columns) of *L. bifermentans.* The bacterium was cultivated at 37°C in the MRS medium supplemented with 10 g/l xylose (a) or arabinose (b). The results shown are the means of duplicate assays of duplicate shake cultures.

mentans could grow and produce desired enzymes within a rather broad pH range of 5.0–9.0; however, both growth and enzyme yield were higher between 7.0 and 8.0 (Fig. 2). In pH < 7.0 or pH > 8.0 the growth decreased and the lag phase was longer. The highest activity of both enzymes was obtained when the initial pH of the medium was adjusted to 7.5.

Effect of inoculum age and size. An inoculum level of 1×10^6 to 1×10^8 cells/ml was used to establish the effect of inoculum size on enzyme production by *L. bifermentans*. A 1×10^7 cells/ml inoculum was optimal for growth as well as AI and XI production; the lag phase was also minimal. For a higher inoculum no notable increase in the activity was observed. With 1×10^6 cells/ml inoculum size, lag phase was increased significantly and maximal activity was obtained at a longer incubation time. Moreover, old inoculum (more than 48 h) resulted in a lag phase increase, although the final AI and XI activities were not affected.

Effect of nitrogen source. *L. bifermentans* was not able to develop without an organic nitrogen source such as yeast extract, meat extract, or peptone [17]. Yeast extract had the greatest effect on activity; increasing its concentration resulted in higher enzymatic activity. Increase in peptone or meat extract concentration did not enhance the enzymatic activity; however, decreased concentration of peptone caused a loss of activity, whereas in the case of decreased meat extract concentration the enzymatic activity was the same as under control conditions, in spite of a decrease in population density. The presence of inorganic nitrogen sources such as ammonium citrate or ammonium phosphate in the culture medium did not have a significant effect on enzyme production (Table 2). However, addition of ammonium sulphate resulted in a slight decrease in AI activity. Moreover, growth of the strain decreased in the absence of inorganic nitrogen.

Lactic acid bacteria are known to be fastidious microorganisms with complex nutrient requirements due to their limited ability to biosynthesize B-vitamins and amino acids [18].

Effect of metal ions. Many microorganisms that produce XI require divalent metal ions as cofactors (Mg^{2+}, Mn^{2+}, and Co^{2+}) [16, 18]; AI was also known to be a metalloprotein [19]. The growth of *L. bifermentans* was stimulated greatly in the presence of manganese. The stimulatory effect of Mn^{2+} could be observed already at a concentration of 1 mM; at higher concentrations (5 mM) the population decreased (Table 3).

Table 2. Effect of different nitrogen sources on *L. bifermentans* growth and AI and XI production

Nitrogen source	Carbor source			
	Xylose		Arabinose	
	XI (U/ml)	Cell (C/ml)	AI (U/ml)	Cell (C/ml)
Control (MRS medium)	3.47	8.20E+08	5.07	1.12E+09
Organic				
Peptone –1	2.72	6.72E+08	3.87	1.01E+09
Peptone +1	3.65	9.09E+08	5.10	1.28E+09
Tryptone –1	2.69	6.13E+08	3.78	1.00E+09
Tryptone +1	3.35	8.91E+08	4.99	1.13E+09
Meat extract –1	3.43	4.46E+08	4.96	9.25E+08
Meat extract +1	3.54	9.59E+08	5.23	1.40E+09
Yeast extract –1	3.92	7.28E+08	5.45	1.04E+09
Yeast extract +1	4.05	9.01E+08	6.42	1.19E+09
Inorganic				
Ammonium citrate –1	3.76	8.26E+08	5.43	1.19E+09
Ammonium citrate +1	3.81	7.98E+08	5.04	1.22E+09
Ammonium phosphate –1	3.71	8.33E+08	5.30	1.14E+09
Ammonium phosphate +1	3.79	8.04E+08	5.11	1.16E+09
Ammonium sulfate –1	3.13	8.16E+08	5.01	1.05E+09
Ammonium sulfate +1	3.55	7.74E+08	4.78	1.08E+09

Notes: The bacterium was cultivated at 37°C and pH 7.5 in the MRS medium. Results shown are the means of duplicate assays of duplicate shake cultures.

Table 3. Effect of different metal ions on *L. bifermentans* growth and AI and XI production

Ions mM		Carbon source			
		Xylose		Arabinose	
		AI (U/ml)	Cell (C/ml)	AI (U/ml)	Cell (C/ml)
Control (without metal ions)		3.87	1.17E+09	4.36	1.38E+09
Magnesium					
	1	4.29	1.62E+09	5.30	1.71E+09
	2.5	4.18	1.80E+09	5.29	2.04E+09
Copper					
	2.5	4.47	1.44E+09	4.01	1.28E+09
Iron					
	2.5	4.00	2.20E+09	4.07	2.21E+09
Zinc					
	2.5	4.24	1.41E+09	5.14	1.51E+09
Calcium					
	2.5	4.58	1.73E+09	5.25	1.80E+09
Cobalt					
	2.5	5.02	1.97E+09	5.35	1.50E+09
Manganese					
	0.5	5.35	2.03E+09	5.94	2.46E+09
	1	5.75	2.24E+09	6.30	2.47E+09
	2.5	5.78	2.38E+09	6.57	2.51E+09
	5	5.46	2.30E+09	5.91	2.43E+09

Notes: The bacterium was cultivated at 37°C and pH 7.5 in the MRS medium supplemented with 10 g/1 arabinose or xylose. The results shown are the means of duplicate assays of duplicate shake cultures.

The maximum enzymatic activity was at 2.5 mM of Mn^{2+}.

Addition of other ions (Mg^{2+}, Co^{2+}, Ca^{2+}, and Zn^{2+}) also resulted in increased activity, although their effect was less pronounced than that of manganese. Moreover, in the presence of Co^{2+}, Ca^{2+}, and Zn^{2+} ions, the yield was less than in the presence of manganese. Other ions (Cu^{2+} and Fe^{2+}) inhibited the isomerase production with arabinose as carbon source, although it was still higher than in the control with xylose.

Effect of carbon source. Carbohydrates are important carbon and energy sources for cultures of *L. bifermentans* Effects of various carbon sources on XI production have been reported, but only D-xylose was an obligate requirement to induce enzyme production. However, it was possible to substitute starch, glucose, sorbitol, or glycerol for 75% of xylose [16, 18]. In order to identify a suitable carbon source for AI and XI production by *L. bifermentans*, different carbohydrates, i.e., xylose, arabinose, glucose, and galactose, were studied in the media containing peptone (10 g/l), meat extract (2.5 g/l), and yeast extract (10 g/l). Other carbon sources, including fructose, sorbitol, xylan, and starch, were also tested in preliminary experiments and did not cause AI/XI production (data not shown).

The results are shown in Table 4. It appears that arabinose was a better carbon source than xylose both for AI and XI production. Moreover, the biomass was highest when arabinose was used as a carbon source. With glucose as a carbon source, the biomass was higher; both AI and XI activity were detected, but the yield was lower compared to pentoses. Galactose was not suitable for cell growth. In order to determine the

Table 4. Effect of initial carbon source on the bacterial growth and AI and XI production of *L. bifermentans*

Carbon source %	AI (U/ml)	XI (U/ml)	Cell (C/ml)
Arabinose			
0.5	5.05	3.38	1.62E+09
1	5.30	3.76	2.52E+09
2	5.30	3.53	2.55E+09
Xylose			
0.5	3.73	3.61	1.37E+09
1	4.29	3.93	1.68E+09
2	4.15	3.89	1.90E+09
Arabinose + Xylose			
0.5 + 0.5	4.72	3.80	1.80E+09
1 + 1	4.88	3.93	2.34E+09
Glucose + Arabinose			
0.2 + 0.5	4.91	3.67	2.90E+09
0.2 + 1	5.43	4.24	2.92E+09
Glucose + Xylose			
0.2 + 0.5	4.73	3.93	2.49E+09
0.2 + 1	4.88	4.79	2.47E+09
Galactose + Arabinose			
0.2 + 0.5	4.98	4.24	2.47E+09
0.2 + 1	5.57	4.47	2.53E+09
Galactose + Xylose			
0.2 + 0.5	4.46	3.82	1.29E+09
0.2 + 1	4.57	4.15	1.77E+09

Notes: The bacterium was cultivated at 37°C and pH 7.5 with the MRS medium. Results shown are the means of duplicate assays of duplicate shake cultures.

optimum concentration of pentose for enzyme production, different concentrations (5–20 g/l) of carbon sources were used in the media. With increasing concentrations of arabinose or xylose, AI/XI activity increased up to 10 g/l pentoses in medium and decreased thereafter.

On the contrary, addition of glucose or galactose to arabinose or xylose makes it possible to increase AI/XI production very slightly; the mixture of galactose (2 g/l) and arabinose (10 g/l) exhibited maximum activity. However, since this increase is not really significant compared to the use of pentoses alone, the subsequent experiments were carried out with arabinose (10 g/l).

Effect of the other components of the medium. We tested the effect of other components of the medium such as sodium acetate, dibasic potassium phosphate, and Tween 80 (Table 5).

Table 5. Effect of different components of medium MRS on the bacterial growth and AI and XI production of *L. bifermentans*

Source	Carbon source			
	Xylose		Arabinose	
	XI (U/ml)	Cell (C/ml)	AI (U/ml)	Cell (C/ml)
Control (MRS medium)	3.47	8.20E+08	5.07	1.12E+09
Potassium phosphate dibasic –1	3.91	8.19E+08	5.23	1.06E+09
Potassium phosphate dibasic +1	3.12	8.20E+08	5.06	1.12E+09
Sodium acetate –1	3.40	6.03E+08	3.93	8.38E+08
Sodium acetate +1	3.65	8.97E+08	5.33	1.45E+09
Sodium citrate –1	3.49	6.77E+08	4.95	8.91E+08
Sodium citrate +1	3.77	79.43E+08	5.27	1.51E+09

Notes: The bacterium was cultivated at 37°C and pH 7.5 with the MRS medium supplemented with 10 g/1 arabinose or xylose. Results shown are the means of duplicate assays of duplicate shake cultures.

Tween 80 was added as a surfactant to study its effects on the permeability of bacterial cell wall and accelerate bacterial growth and production rate by facilitating mass transfer through the cell wall [20]. The effect of increased Tween 80 concentration on AI and XI activity was not significant apart from its decrease, which had an effect on cell multiplication.

Sodium acetate or citrate enhanced cell growth and increased weakly the AI and XI production. These compounds were used as components of the buffer and could be removed from the medium if the culture pH during batch fermentations was maintained at 7.5 by automatic addition of 10 N NaOH.

Increased concentration of the phosphate source had no effect or resulted in a very slight reduction in activity. On the other hand, the decrease in phosphate concentration appreciably improved AI and XI production. However, no effect on cell growth was observed.

Profile of growth and enzyme production. The time course of growth and enzyme production by *L. bifermentans* was determined in batch culture under

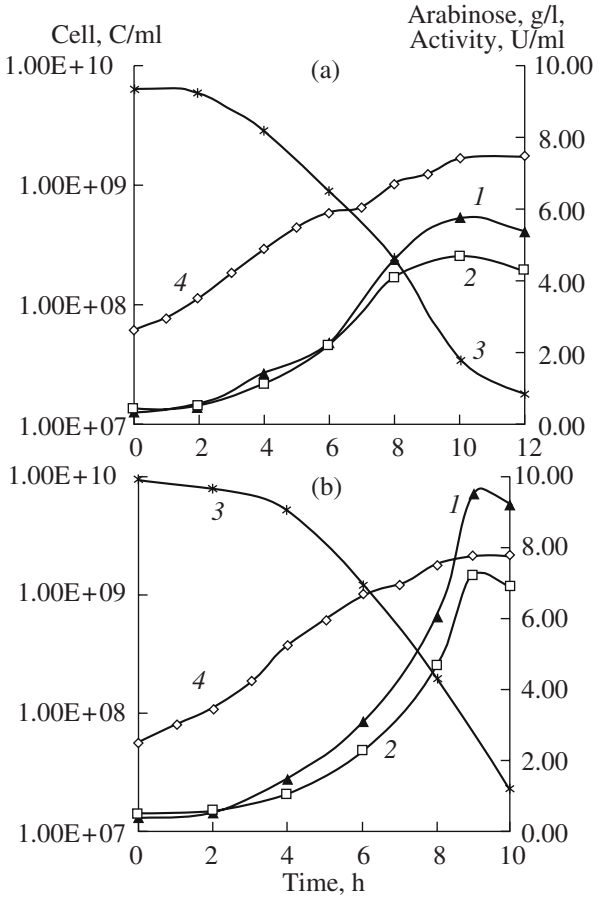

Fig. 3. Profile of growth and AI and XI production by *Lactobacillus bifermentans* in batch culture: AI activity (*1*), XI activity (*2*), arabinose (*3*), biomass (*4*). The bacterium was cultivated at 37°C and pH 7.5 with Mn^{2+}, 10 g/l arabinose, Tween 80 in (a) MRS medium or (b) optimized medium. Results shown are the means of duplicate assays of duplicate shake cultures.

agitation on unmodified MRS medium (a) and optimized medium (b). Moreover, we tested the effect of strictly anaerobic growth conditions (incubation under nitrogen) as compared to an oxygen-limited culture. In anaerobic conditions, *L. bifermentans* had a generation time of approximately 115 min and a lag phase of approximately 1 h, compared to a generation time of 91 min and no lag phase in oxygen-limited conditions. Consequently, the enzymatic activity of the strain was weak (data not shown).

Figure 3 showed the time profiles of cell growth, sugar consumption and AI/XI production in the medium with arabinose (10 g/l). *L. bifermentans* developed quickly and reached the stationary phase after 8 h of cultivation on optimized medium, whereas on basic MRS medium it was reached only after 10 to 11 h. On the other hand, the maximal cell density obtained (2.5×10^9 cells/ml) was identical for the two media and the residual arabinose was almost exhausted at that time. The maximal AI and XI production and productivity was reached at the early stationary phase (9.52 and 7.24 U/ml for AI and XI, respectively); these values were much higher than those of control. The decrease of the enzyme activity during the stationary phase may be explained by the detrimental effects of acidic pH or some by-products such as proteases [21].

In this work, a process for production of AI and XI by *L. bifermentans* was demonstrated. The effects of major nutrients, i.e., ions, carbon sources, and carbon/nitrogen ratios, on AI and XI production were studied in order to obtain a suitable fermentation medium. Arabinose was an optimal carbon source for the enzyme production, although cell growth was the best in glucose medium. The incubation temperature and pH were 37°C and 7.5, respectively, the concentration of peptone, meat extract, yeast extract, dibasic potassium phosphate, and ammonium citrate were at 10 g/l, 2.5 g/l, 10 g/l, 0.5 g/l, and 0.5 g/l, respectively, in addition to mineral salts including $MnSO_4 \cdot H_2O$ (2.5 mM).

According to the response analysis, an optimal carbon source (10 g/l) was identified and a maximal AI and XI production (9.52 U/ml and 7.24 U/ml) and productivity (1.4 and 1.04 U/h) were successfully obtained.

REFERENCES

1. Sun, X.F., Xu, F., Sun, R.C., Geng, Z.C., Fowler, P., and Baird, M.S., Characteristics of Degraded Hemicellulosic Polymers Obtained from Steam Exploded Wheat Straw, *Carbohydr. Polym.*, 2005, vol. 60, pp. 15–26.
2. Sun, R.C. and Tomkinson, J., Characterization of Hemicelluloses Obtained by Classical and Ultrasonically Assisted Extractions from Wheat Straw, *Carbohydr. Polym.*, 2002, vol. 50, pp. 263–271.
3. Sun, R.C. and Hughes, S., Fractional Extraction and Physico-Chemical Characterization of Hemicelluloses and Cellulose from Sugar Beet Pulp, *Carbohydr. Polym.*, 1998, vol. 36, pp. 293–299.
4. Kim, B.C., Lee, Y.H., Lee, H.S., Lee, D.W., Choe, E.A., and Pyun Y.R., Cloning, Expression and Characterization of L-Arabinose Isomerase from *Thermotoga neapolitana*: Bioconversion of D-Galactose to D-Tagatose Using the Enzyme, *FEMS Microbiol. Lett.*, 2002, vol. 212, pp. 121–126.
5. Wovcha, M.G., Steuerwald, D.L., and Brooks, K.E. Amplification of D-Xylose and D-Glucose Isomerase Activities in *Escherichia coli* by Gene Cloning, *Appl. Environ. Microbiol.*, 1983, vol. 45, no. 4, pp. 1402–1404.
6. Richard, P., Verho, R., Putkonen, M, Londesborough, J., and Penttila, M., Production of Ethanol From L-arabinose by *Saccharomyces cerevisiae* Containing a Fungal L-Arabinose Pathway, *FEMS Yeast Res.*, 2003, pp. 85–189.
7. Patrick, J.W., and Lee, N., Purification and Properties of an L-Arabinose Isomerase from *Escherichia coli, J. Biol. Chem.*, 1968, vol. 243, pp. 4312–4318.
8. Bothast, R.J., Nichols, N.N. and Dien, B.S. Fermentation with New Recombinant Organisms, *Biotechnol. Prog.*, 1999, vol. 15, pp. 867–875.

9. Zhang, M., Eddy, C, Deanda, K., Finkestein, M, and Picataggio, S., Metabolic Engineering of a Pentose Metabolism Pathway in Ethanologenic *Zymomonas mobilis, Science*, 1995, vol. 267, pp. 240–243.
10. Deanda, K., Zhang, M., Eddy, C., and Picataggio, S., Development of an Arabinose-Fermenting *Zymomonas mobilis* Strain by Metabolic Pathway Engineering, *Appl. Environ. Microbiol.*, 1996, vol. 62, no. 12, pp. 4465–4470.
11. Dien, B.S., Cotta, M.A., and Jeffries, T.W., Bacteria Engineered for Fuel Ethanol Production: Current Status, *Appl. Microbiol. Biotechnol*, 2003, vol. 63, pp. 256–266.
12. Chandrakant, P. and Bisaria, V.S., Simultaneous Bioconversion of Glucose and Xylose to Ethanol by *Saccharomyces cerevisiae* in the Presence of Xylose Isomerase, *Appl. Microbiol. Biotechnol.,* 2000, vol. 53, pp. 301–309.
13. Lee, C.Y., Bhatnagar, K., Saha, B.C., Lee, Y.E., and Takagi, M., Imanaka, T., Bagdasarian, M., and Zeikus, J.G., Cloning and Expression of the *Clostridium thermosulfurogenes* Glucose Isomerase Gene in *Escherichia coli* and *Bacillus subtilis, Appl. Environ. Microbiol.,* 1990, vol. 56, no. 9, pp. 2638–2643.
14. Borgi, A., Srih-Belguith, K., Ben Ali, M., Mezghani, M., Tranier, S., Haser, R., and Bejar, S., Glucose Isomerase of the *Streptomyces* sp. SK Strain: Purification, Sequence Analysis and Implication of Alanine 103 Residue in the Enzyme Thermostability and Acidotolerance, *Biochimie*, 2004, vol. 86, pp. 561–568.
15. Pawar, S.A. and Deshpande, V.V., Characterization of Acid-Induced Unfolding Intermediates of Glucose/Xylose Isomerase, *Eur. J. Biochem.*, 2000, vol. 267, pp. 6331–6338.
16. Bhosale, S.H., Rao, M.B., and Deshpande, V.V., Molecular and Industrial Aspects of Glucose Isomerase, *Microbiol. Rev.*, 1996, vol. 60, no. 2, pp. 280–300.
17. Deshmukh, S.S., Deshpande, M.V., and Shankar, V., Medium Optimization for the Production of Glucose Isomerase from Thermophilic *Streptomyces thermonitrificans, World J. Microbiol. Biotechnol.*, 1994, vol. 10, pp. 264–267.
18. Chauhan, K., Trivedi, U., and Patel, K.C., Statistical Screening of Medium Components by Plackett-Burman Design for Lactic Acid Production by *Lactobacillus* sp. KCP01 Using Date Juice, *Bioressour. Technol.*, 2006 (in press).
19. Lee, D.W., Choe, E.A., Kim, S.B., Eom, S.H., Hong, Y.H., Lee, S.J., Lee, H.S., Lee, D.Y., and Pyun, Y.R., Distinct Metal Dependence for Catalytic and Structural Functions in the L-Arabinose Isomerases from the Mesophilic *Bacillus halodurans* and the Thermophilic *Geobacillus stearothermophilus, Arch. Biochem. Biophys.*, 2005, vol. 434, pp. 333–343.
20. Lee, M.T., Chen, W.C., and Chou, C.C., Medium Improvement by Orthogonal Array Designs for Cholesterol Oxidase Production by *Rhodococcus equi*, No. 23, *Process Biochem.*, 1997, vol. 32, pp. 697–703.
21. Bakhtiari, M.R., Faezi, M.G., Fallahpour, M., Noohi, A., Moazami, N., and Amidi, Z., Medium Optimization by Orthogonal Array Designs for Urease Production by *Aspergillus niger* PTCC5011, *Process Biochem.,* 2006, vol. 41, pp. 547–551.