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Short Communication

Production of Lactic Acid from Corn Cobs Through Fermentation by *Lactobacillus delbruekii*

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Abstract. Corn cobs were used as the source of reducing sugars for conversion into lactic acid through fermentation by a local strain of *Lactobacillus delbruekii*, under varying parameters of time, temperature, pH and glucose concentration. The production of lactic acid significantly increased with increase in pH, fermentation time and glucose concentration (1-5%) and was significantly high (8.40 g/l) at pH 6, while significantly low (7.67 g/l) at pH 5.

Keywords: corn cobs, fermentation, lactic acid, *Lactobacillus delbruekii*

Lactic acid is a weak organic acid occurring widely in nature; it can be easily broken down in water and other solvents. Using hydrolysates of pretreated wood, newspaper or corn cobs, 10-30 g/l of lactic acid had been produced using *Lactobacillus* spp. (Vishnu *et al*., 2000).

Corn cobs account for 16-19% of the whole corn; they contain significant amount of cellulosic material, which may be the best source of fermentable sugars. Thus, it can be used for the production of organic acids e.g. lactic acid through simultaneous saccharification and fermentation (SSF), using species of genus *Lactobacillus* (Nakasaki and Adachi, 2003).

In the present study, corn cobs were used for producing lactic acid under varying parameters through fermentation by *Lactobacillus delbruekii*. The samples of corn cobs were analyzed for acid detergent fibre (ADF) and neutral detergent fiber (NDF) (AOAC, 1990) and for cellulose, hemicellulose and lignin content (Krishna and Ranjhan, 1980). pH was determined before and after the hydrolysis with the help of pH meter (INOLAB) (AOAC, 1990). The reducing sugars (as glucose) in the hydrolyzed samples were determined by spectrophotometer (Gadgil *et al.,* 1995). The samples were delignified with 1% (w/w) sodium hydroxide (Tsay, 1983), hydrolysed (Wang, 2003) and later neutralized by addition of small volume of concentrated NaOH solution.

The hydrolysate was evaporated at 100 °C for 1 h and different glucose concentrations (1% to 5%) were used for fermentation through adding starter culture of strains of *Lactobacillus delbruekii* IFST-1, (obtained from the Microbiology and Biotechnology Laboratory of the Institute of Food Science and Technology, University of Agriculture, Faisalabad), at different sets of variables i.e. temperature, pH, time and glucose concentration.

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During fermentation process, the medium was daily evaluated for reducing sugars and lactic acid. Lactic acid was determined using HPLC Perkin Elmer Series 200 (Bevilacqua and Califano, 1989).

Triplicate analyses were performed on all the samples. The data was subjected to statistical analysis using completely randomized design (CRD) and Duncan's new multiple range test (Steel *et al.,* 1997).

The results indicated that variables such as pH, time and concentration of glucose had highly significant effect on the reducing sugar content and lactic acid production, whereas variation in temperature (34 °C to 40 °C) was found to have non-significant effect.

As pH of the fermentation medium was increased from 5 to 6, there was a significant decrease in the reducing sugar content (from 29.56 g/l to 2.28 g/l) due to more utilization as raw material; with progress of time, utilization of glucose increased from 3.06 to 27.28 g/l. The findings are in line with those of Bustos *et al.* (2004) who optimized the production of lactic acid by *L. coryniformis* from glucose in the medium increasing the duration and obtained maximum lactic acid concentration (58.9 g/l) at 96 h. Lactic acid production enhanced from 0.98 g/l at the beginning of the study to 15.42 g/l at the end. Highest production was observed at pH 6 after 8 days of fermentation (Table 1). The optimum pH for the production of lactic acid was found to be pH 6 at which 15.73 g/l of lactic acid was produced at the end of the fermentation.

In the medium containing 1 to 5% glucose, utilization of reducing sugars increased with progress of time and ranged from 7.06 to 20.68 g/l with maximum utilization of 20.68 g/l at 5% glucose level.

Lactic acid production increased from 3.45 g/l to 11.60 g/l with the increase in glucose concentration (1% to 5%) (Table 2).

The temperature was found to have non-significant effect on lactic acid production in the present study (Table 3). The highest amount of lactic acid was produced at temperature of 38 °C with 5% glucose concentration. The present findings are in agreement with those of Yanez *et al.* (2003) who produced D (-)-lactic acid from cellulose using *Lactobacillus coryniformis*.

It is concluded that high yield of lactic acid can be obtained using corn cob hydrolysate through batch fermentation with *Lactobacillus delbruekii*, and the yield can be further enhanced by standardization of glucose contents in the fermentation medium. Corn cob is a cheap raw material for industrial lactic acid production; however, it is necessary to further study the effect of inhibition mechanism of toxic chemicals in corn cob hydrolysate on lactic acid fermentation.

Table 1. Interactive effect of pH and days on the lactic acid production (g/l) during fermentation

pH	Lactic acid (g/l)									Mean
	D_0	D_1	D_2	D_3	D_4	D_5	D_6	D_7	D_8	
pH 5	0.00p	0.65o	2.23m	4.59k	7.80i	11.13g	12.89e	14.60c	15.12b	7.67b
pH 6	0.00p	1.30n	2.88l	5.64j	8.80h	12.07f	13.75d	15.44ab	15.73a	8.40a
Mean	0.00i	0.98h	2.56g	5.11f	8.30e	11.60d	13.32c	15.02b	15.42a	

figures carrying same letters (a - p) in a column and row are not significantly different; D_1 = day one; D_2 = day 2 and so on.

Table 2. Interactive effect of glucose and days on the lactic acid production (g/l) during fermentation

Glucose level	Lactic acid (g/l)									Mean
	D_0	D_1	D_2	D_3	D_4	D_5	D_6	D_7	D_8	
G_1 (1%)	0.00q	0.68p	2.04n	3.86m	4.70l	4.96l	4.95l	4.94l	4.93l	3.45e
G_2 (2%)	0.00q	0.68p	2.05n	3.86m	6.61k	9.66j	10.08j	10.77i	10.67i	6.04d
G_3 (3%)	0.00q	0.68p	2.04n	3.85m	6.60k	9.66j	13.92g	16.38f	16.70f	7.76c
G_4 (4%)	0.00q	1.42o	3.33m	7.00k	11.79h	16.85f	18.46e	20.93c	22.09b	11.32b
G_5 (5%)	0.00q	1.41o	3.33m	6.99k	11.79h	16.85f	19.20d	22.09b	22.72a	11.60a
Mean	0.00i	0.98h	2.56g	5.11f	8.30e	11.60d	13.32c	15.02b	15.42a	

D_1 = day one; D_2 = day 2 and so on; figures carrying same letters in a column and row are not significantly different.

Table 3. Interactive effect of temperature and glucose on the lactic acid production (g/l) during fermentation

Temperature(°C)	Lactic acid (g/l)					Mean
	G_1 (1%)	G_2(2%)	G_3(3%)	G_4(4%)	G_5(5%)	
34	3.43	6.01	7.72	11.25	11.54	7.99a
35	3.39	5.95	7.68	11.25	11.51	7.96a
36	3.43	6.01	7.74	11.29	11.56	8.01a
37	3.47	6.07	7.77	11.33	11.61	8.05a
38	3.50	6.12	7.82	11.40	11.66	8.10a
39	3.49	6.10	7.81	11.36	11.64	8.08a
40	3.46	6.04	7.78	11.36	11.65	8.06a
Mean	3.45e	6.04d	7.76c	11.32b	11.60a	

G_1 to G_5 denote glucose percentage in the medium.

References

AOAC. 1990. *Official Methods of Analysis of the Association of Analytical Chemists*, 15th edition, Arlington, Virginia, USA.

Bevilacqua, A.E., Califano, A.N. 1989. Determination of organic acids in dairy products by high performance liquid chromatography. *J. Food Sci.* **54:** 1076-1079.

Bustos, G., Moldes, A.B., Cruz, J.M., Dominguez, J.M. 2004. Formulation of low-cost fermentative media for lactic acid production with *Lactobacillus rhamnosus* using vinification lees as nutrients. *J. Agric. Food Chem.* **52:** 801-808.

Gadgil, N.J., Daginwala, H.F., Chakarbarti, T., Khanna, P. 1995. Enhanced cellulose production by a mutant of *Trichoderma reesei. Enzymes Microbiol. Technol.* **17:** 942-946.

Krishna, G., Ranjhan, S.K. 1980. *Laboratory Manual for Nutrition Research*, Vikas Publishing House, New Delhi, India.

Nakasaki, K., Adachi, T. 2003. Effect of intermittent addition of cellulase for the production of L-lactic acid from wastewater sludge by simultaneous saccharification and fermentation. *Biotechnol. Bioeng.* **82:** 263-270.

Steel, R.G.D., Torrie, J.H., Dickey, D.A. 1997. *Principles and Procedures of Statistics. A Biometric Approach*, 3rd edition, McGraw Hill Book Inc., New York, USA.

Tsay, S.S. 1983. Studies on the factors influencing the digestion of rice straw by Cellomonas.*Zhonghua Min Guo Wei Sheng Wu Ji Mian Yi xue Za Zhi.* **16:** 64-70.

Vishnu, C., Seenayya, G., Reddy, G. 2000. Direct conversion of starch to L(+)-lactic acid by amylase producing *Lactobacillus amylophiles* GV6. *Bioprocess Bioeng.* **23:** 155-158.

Wang, N.S. 2003. *Cellulose Degradation*, Department of Chemical & Biomolecular Engineering, University of Maryland, College Park, Maryland, USA.

Yanez, R., Moldes, A.B., Alonso, J.L., Parajo, J.C. 2003. Production of D(-)-lactic acid from cellulose by simultaneous saccharification and fermentation using *Lactobacillus coryniformis* subsp. *Torquens. Biotechnol. Lett.* **25:** 1161-4.