African Journal of Biotechnology Vol. 8 (17), pp. 4175-4178, 1 September, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Production of lactic acid from corn cobs hydrolysate through fermentation by *Lactobaccillus delbrukii*

Zulfiqar Ali*, Faqir Muhammad Anjum and Tahir Zahoor

Institute of Food Science and Technology, University of Agriculture, Faisalabad.

Accepted 12 June, 2006

This study describes several essential factors for indirect and effective lactic acid production from food wastes by strains of *Lactobaccillus delbrukii* using corn cob hydrolysates. The fermentation conditions considered were glucose concentration (1 - 5%), temperature (34 - 40°C), time (0 - 8 days) and pH (5 - 6). After pretreatment of corn cobs by dilute acid, the lignocellulosic residue was used as raw materials for the saccharification and followed by lactic acid fermentation. The best fermentation conditions were 40°C temperature, after 84 h of fermentation time. In the saccharification and subsequently fermentation process, the final concentration of lactic acid reached 17.73 g/L based on the glucose (reducing sugars) extracted from the saccharified corn cobs at pH 5-6.

Key words: Corn cobs, reducing sugars, *Lactobacillus delbrukii*, fermentation, lactic acid.

INTRODUCTION

Organic solid wastes, such as food wastes, are high in moisture and rich in carbon. Annual generation rate of organic wastes in Japan is around 19.4 million tons (Ministry of Environment, 2000). Lignocellulosic residue was used after pretreatment of corn cob by dilute acid, as raw materials for the simultaneous saccharification and lactic acid fermentation (SSLF). Because of the same optimal temperature and pH requirement as well as the anaerobic condition, the lactic acid fermentation is perfectly compatible with enzymatic hydrolysis of cellulosic materials. In the SSLF processes, the final concentration of lactic acid reached 33.97 g/l with a conversion ratio of 79% based on the consumed cellulose. A mathematical model to simulate the SSLF process with good agreement has been proposed (Luo et al., 1997).

Lactic acid (2-hydroxy propanoic acid) and its derivatives have found many applications in connection with foods, fermentations, pharmaceutical, plastics and the chemical industries. It is an important candidate for making the biodegradable polymers (Lipinsky and Sinclair, 1986). Lactic acid production is more than 50,000 metric tons worldwide per year. In the U.S. 85% of the lactic acid is made via synthetic routes. Improving microbial lactic acid production will help make lactic acid production from renewable resources more economical.

Whatever the fermentation scheme, the conversion of xylose and glucose to lactic acid requires the use of microorganisms suitable for each sugar. A large number of *Lactobacilli* and *Lactococci* were reported as xylose-fermenting lactic acid bacteria (Collins et al., 1984; Hujanen and Linko 1996). These lactic acid bacteria are heterofermentative and form approximately equimolar quantities of lactic acid and acetic acid from xylose, with a theoretical yield of lactic acid of 60% by weight of the consumed xylose. Garde et al. (2002) investigated lactic acid production from wheat straw hemicellulose hydrolysate by *Lactobacillus pentosus* and *L. brevis*. The authors reported that the concentrations of lactic acid produced by *L. pentosus* and *L. brevis* were 9 and 10 g/l, respectively, when 20 g/l of xylose were added to the medium containing the untreated hydrolysate. Tanaka et al. (2002) reported that the yield coefficient of lactic acid per mole of consumed xylose exceeded 1 mol/mol in cultivations at initial xylose concentrations of more than 50 g/l when *Lactococcus lactis* was used. As described above, in general, the yield of lactic acid obtained from xylose is much lower than that from glucose. Moreover, the fermentation rate of xylose by pentose-fermentative bacteria is lower than that of glucose by homofermentative lactic acid bacteria such as *L. casei* (Garde et al., 2002; Tanaka et al., 2002). However, a very interesting result was reported by Fukui et al. (1957) that *L.*

*Corresponding author. E-mail: zasafi@yahoo.com.

thermophilus produced lactic acid from xylose in a yield of 78% by weight of consumed xylose. They also showed that 93% of the consumed xylose was converted to lactic acid when intact cells of *L. thermophilus* were used.

In this study, we characterized the chemical composition of several food waste samples to evaluate their suitability as raw materials for lactic acid production. To achieve more rapid and cost-effective L(+)-lactic acid production from food wastes, we attempted direct fermentation using one of the *Lactobaccillus delbruekii* strains. We also examined the influence of initial pH, culture pH and minerals on productivity by this strain using starch as a model substrate.

MATERIAL AND METHODS

Raw materials

The corn cobs (red and white) were collected as a substrate for the production of lactic acid from Rafhan Maize Products (PVT) Ltd., Faisalabad. The samples were crushed with the help of grinder to small size and were passed through sieves of 2, 1 and 0.5 mm in order to get particle size of 2-1 mm, 1-0.5 mm and 0.5 mm. The chemicals used in the study were $(NH_4)_2HPO_5$, acetonitrile, H_3PO_4 and distilled water (HPLC-grade) from Sigma Chemical Co., St. Louis, MO, USA. The strains of *Lactobacillus delbrueckii* IFST-1 were obtained from the Microbiology and Biotechnology laboratory of Institute of Food Science and Technology, University of Agriculture, Faisalabad for the production of lactic acid through fermentation.

The samples of corn cob were analyzed for moisture, ash, crude protein, crude fat, crude fiber and nitrogen free extract (NFE), cellulose, hemicellulose and lignin according to methods described in AACC (2000).

Hydrolysis

Six flasks (200 ml) were taken containing corn cob sample (1-10 g) in each. Different concentrations of sulphuric acid (5, 10 and 15%) were added. The flasks were closed tightly with cotton plugs and placed in an autoclave (SANYO MLS-3780) at different temperatures for different intervals of time. After hydrolysis the contents of hydrolysate were filtered through Whattman filter paper No.1. Then contents after filtration were neutralized with NaOH to bring the pH to 7 as outlined by Wang (2003).

Standardization of glucose in the corn cob hydrolysate

After the completion of hydrolysis, the glucose contents were standardized by concentrating the solution through evaporation process. Different glucose concentrations (1-5%) were prepared and were used in the fermentation.

Fermentation

Fermentation of corn cob hydrolysate under optimum conditions of temperature (34, 35, 36, 37, 38, 39 and 40ºC) pH (5 - 6), time (0 - 8 days), glucose concentration (1 - 5%) and strain composition was carried out for the production of lactic acid (Table 1). The fermentation media consists of 10 g peptone, 10 g Lab-Lemo meat extract, 5 g yeast extract, 1 g Tween 80, 2 g dipotassium hydrogen phosphate, 5 g sodium acetate, 2 g triammonium citrate, 0.2 g $MgSO_4.7H_2O$ and 0.5 g $MnSO_4.4H_2O$. The corn cob sample was added as required.

Table 1. Fermentation conditions.

Temperature (°C)	34, 35, 36, 37, 38, 39, 40
Time (days)	2, 3, 4, 5, 6, 7, 8
pH	5, 6
Glucose conc. (%)	1, 2, 3, 4, 5

Analysis of lactic acid by HPLC

The contents of lactic acid were analyzed by the High Pressure Liquid Chromatography (HPLC) technique by following the procedure described by Bevilacqua and Califano (1989).

Statistical analysis

The data obtained for each parameter was subjected to statistical analysis to determine the level of significance by following the methods described by Steel et al. (1997).

RESULTS AND DISCUSSION

The lactic acid was produced from the hydrolysate containing glucose with the different substrate, glucose, pH and temperature. The corn cobs were utilized as substrate and *L. delbruekii* was used as fermentatitive organism for lactic acid production. The data containing different parameters like pH, glucose and lactic acid was analyzed statistically and discussed here. The lactic acid production relative to glucose concentration and temperature by are given in the Table 2. The effect of glucose concentration on lactic acid production was significant. While the temperature also exhibited exceptional effects on the lactic acid production. 4% glucose concentration at 5°C showed highest lactic acid recovery (13.19 g/L). The effects of glucose concentration and time on lactic acid production are shown in the Table 3. The most effective treatment was at 84 h that gave lactic acid recovery (25.62 g/L). The outcome from the present research is similar to that of Kim et al. (2003) who demonstrated the conversion of food wastes into lactic acid by simultaneous saccharification and fermentation. Their process involves saccharification of the starch component in food wastes by a commercial amylolytic enzyme preparation (a mixture of amyloglucosidase, alpha-amylase, and protease) and fermentation by *L. delbrueckii*. The highest observed overall yield of lactic acid in the SSF was 91% of theoretical. Lactic acid concentration as high as 80 g/L was attainable in 48 h of the SSF. The optimum operating conditions for the maximum productivity were found to be 42°C and pH 6.0. Without supplementation of nitrogen-containing nutrients, the lactic acid yield in the SSF decreased to 60%. The overall performance of the SSF, however, was not significantly affected by the elimination

Table 2. Mean values for interactive effect of standardized glucose concentration and temperature on lactic acid production (g/l) from corn cob white and corn cob red for 84 h.

Glucose (%)	Temperature (°C)						
	34	**35**	**36**	**37**	**38**	**39**	**40**
1	4.46[a]	4.93	4.79	4.75	4.11	5.33	5.03
2	8.23	9.61	9.53	9.03	9.56	10.73	9.50
3	9.76	10.50	12.08	9.32	10.44	9.79	10.74
4	13.12	13.19	12.01	12.11	13.82	12.77	14.74
5	12.64	10.94	11.23	8.94	10.14	11.73	12.11

[a]Lactic acid concentration (g/l).

Table 3. Mean values for interactive effect of standardized glucose concentration and time on lactic acid production (g/l) from corn cob white and corn cob red at 39°C.

Time (h)	Glucose (%)				
	1	**2**	**3**	**4**	**5**
0	1.30[a]	2.14	1.45	2.57	2.04
12	2.26	3.24	3.26	3.92	3.38
24	3.18	5.25	7.34	6.37	7.53
36	4.12	7.07	9.03	10.20	8.05
48	4.85	10.00	10.83	12.28	10.92
60	5.58	12.12	12.61	15.65	13.60
72	6.94	13.65	14.77	18.28	15.88
84	7.91	16.55	18.06	25.62	20.52
96	6.85	15.09	16.05	23.09	18.06

[a]Lactic acid concentration (g/l).

Table 4. Mean values for interactive effect of temperature and time on lactic acid production (g/l) from corn cob white and corn cob red using 4% glucose.

Time (h)	Temperature (°C)						
	34	**35**	**36**	**37**	**38**	**39**	**40**
0	1.29[a]	1.69	1.56	1.58	2.11	2.50	2.59
12	2.23	2.99	4.08	2.38	3.07	3.63	4.08
24	8.52	5.69	6.60	4.05	5.44	5.44	5.80
36	7.99	8.17	8.31	5.86	7.84	7.24	8.44
48	9.96	10.27	10.15	7.96	10.24	9.78	10.07
60	11.54	12.29	12.38	10.76	11.92	12.43	4.61
72	13.45	14.24	13.86	13.29	14.47	14.47	4.12
84	17.23	17.23	17.12	17.49	17.32	18.44	3.32
96	14.59	15.92	15.32	16.13	15.15	16.73	3.75

[a]Lactic acid concentration (g/l).

of mineral supplements.

The end product of time and temperature are presented in Table 4. 39°C at 84 h gave the maximum yield for lactic acid production (18.44 g/L). The results are in conformity with Yanez et al. (2003) who produced D(-)-Lactic acid from cellulose by simultaneous saccharification and fermentation (SSF) in media containing cellulolytic enzymes and *Lactobacillus coryniformis* subsp. *torquens* ATCC 25600 at 39°C and pH 5.4, though no L(+)-lactic acid was found in the medium.

REFERENCES

AACC (2000). Approved Methods of American of Association of Cereal Chemist. The American Association of Cereal Chemists, Inc.St/Paul.Minnesota. acid in mixed-acid fermentation by the lactic acid bacterium *Lactococcus lactis*. Appl. Microbiol. Biotechnol. 60: 160-167.

Bevilacqua AE, Califano AN (1989). Determination of organic acids in dairy products by high performance liquid chromatography. J. Food Sci. 54: 1076-1079.

Collins MD, Jones D, Farrow AAE, Kilpper-BR, Schleifer KH (1984). *Enterococcus avium* nom. rev., comb. nov.; *E. casseliflavus* nom. rev., comb. nov.; *E. durans* nom. rev., comb. nov.; *E. gallinarum* comb. nov.; and *E. malodoratus* sp. nov. Int. J. Syst. Bacteriol. 34: 220-223

Fukui S, Oi A, Obayashi A, Kitahara K.1957. Studies on the pentose metabolism by microorganisms. I. A new type-lactic acid fermentation of pentose by lactic acid bacteria. J. Gen. Appl. Microbiol. 3: 258-268

Garde A, Jonsson G, Schmidt AS, Ahring BK (2002). Lactic acid production from wheat hydrolysate by *Lactobacillus pentosus* and Lactobacillus brevis. Bioresour. Technol. 81: 217-223.

Kim KI, Kim WK, Seo DK, Yoo IS, Kim EK, Yoon HH (2003). Production of lactic acid from food wastes. Appl. Biochem. Biotechnol. 105-108: 637-647.

Lipinsky ES, Sinclair RG (1996). Is lactic acid a commodity chemical? Chem. Eng. Progr. 82 (Aug), 26.

Luo J, Xia L, Lin J, Cen P (1997).Kinetics of simultaneous saccharification and lactic acid fermentation processes. Biotechnol. Prog. 13(6): 762-767.

Ministry of Environment (2000). Quality of the Environment in Japan (in Japanese). Gyosei, Tokyo.

Steel RGD, Torrie JH, Dickey DA (1997). Principles and Procedures of statistics. a biometric approach 3rd ed. McGraw Hill Book. Inc., New York.

Tanaka K, Komiyama A, Sonomoto K, Ishizaki A, Hall SJ, Stanbury PF (2002). Two different pathway for D-xylose metabolism and the effect of xylose concentration on the yield coefficient of Llactic

Yanez R, Moldes AB, Alonso JL, Parajo JC (2003). Production of D(-)-lactic acid from cellulose by simultaneous saccharification and fermentation using *Lactobacillus coryniformis* subsp. torquens. Biotechnol. Lett. L. 25(14): 1161-1164.

Wang NS. 2003. Cellulose degradation. Department of Chemical Engineering, University of Maryland, College Park.