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Bioconversion of Municipal Solid Waste to Lactic Acid by *Lactobacillus* Species

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ABSTRACT

Eight *Lactobacillus* species were screened for production of lactic acid from acid-hydrolyzed municipal solid waste (AHMSW). Screening criteria included carbohydrate utilization, lactic acid production, and the yield of lactic acid produced in modified Elliker broth and in MSW hydrolyzate. *Lactobacillus pentosus* B-227 metabolized the most carbohydrate (62%) and produced the highest concentration of lactic acid in AHMSW (21.2 mg/mL) containing 41.3 mg/mL carbohydrate. Fermentation parameters for the bioconversion of carbohydrates in MSW to lactic acid also were evaluated. Under optimum conditions, consisting of an initial pH of 7.6, 32°C, static fermentation, 1% v/v inoculum, and 5% calcium carbonate buffer, *L. pentosus* B-227 produced 65 mg/mL lactic acid from 100.6 mg/mL of carbohydrates in MSW hydrolyzate, an 87% yield based on carbohydrate utilization.

Index Entries: Municipal solid waste; lactic acid; fermentation; bioconversion; *Lactobacillus* spp.

INTRODUCTION

Approximately 181 million metric tons of municipal solid waste (MSW) are generated annually in the United States (*1*). Currently, landfills and incinerators are the primary means of MSW disposal, but these methods may harm the environment by contaminating water or emitting noxious

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gases. Concerns about conservation of resources, impact of MSW on landfills, and protection of human health have stimulated exploration of alternative strategies for MSW management.

MSW consists primarily of cellulosic materials in the forms of newsprint, wood, and cardboard (2), which contain sugar polymers that can be acid-hydrolyzed to monomeric sugars. Many of the sugars derived from acid-hydrolyzed newsprint can be fermented by microorganisms to a variety of chemicals that have market value. Recently, lactic acid production has received attention because of the development of polylactic acid (PLA) plastics, which are 100% degradable and have been approved for use by the FDA (3). PLA plastics can emulate the characteristics of many of the thermoplastics now used in packaging consumer goods and may become the basis of a family of environmentally benign polymers (4).

Strains of organisms used for commercial lactic acid fermentation are proprietary (5). However, the major lactic acid-producing bacteria include the following genera: *Streptococcus, Pediococcus, Leuconostoc,* and *Lactobacillus* (6). The most important *Lactobacillus* species for industrial use are homofermentative and produce principally lactic acid from carbohydrate.

Several carbohydrate materials have been evaluated for the production of lactic acid, including hydrolyzed corn and potato starches, and food processing wastes (7). However, the study of lactic acid production from MSW has not been reported. This study focuses on the evaluation and selection of bacterial cultures for lactic acid production and the optimization of fermentation parameters for the production of lactic acid from acid-hydrolyzed MSW.

METHODS

Microorganisms

Eight *Lactobacillus* cultures were provided by the USDA Northern Regional Research Laboratory, Peoria, IL. These were *L. arabinosus* B-787, *L. arabinosus* B-788, *L. arabinosus* B-813, *L. arabinosus* B-531, *L. pentosus* B-277, *L. pentosus* B-473, *L plantarum* USDA 422, and *L. xylosus* B-4449. The cultures were selected for their ability to metabolize pentoses as well as hexoses.

Fermentation Substrates

Three kinds of substrates were used in the fermentation studies:

1. Modified Elliker broth (MEB);
2. Acid-hydrolyzed MSW (AHMSW); and
3. Double-sugar, acid-hydrolyzed MSW (DSMSW).

MEB was prepared from the basic ingredients of Elliker Broth (Difco cat. no. 0974-01), and was supplemented with the carbohydrates of interest at concentrations found in MSW to determine their preferential utilization and bioconversion to lactic acid. The sugar and nitrogen sources comprising MEB were combined after being heat-sterilized separately. AHMSW was prepared from newsprint hydrolyzed with 2% (w/w) of sulfuric acid (1:10 solid-to-liquid ratio) at 124°C for 15 min. The concentrations of carbohydrates resulting from the hydrolysis are shown in Table 1 (untreated hydrolyzate). The hydrolyzate was supplemented with nitrogen sources based on the concentration in Elliker broth (tryptone, 20.0 g/L and yeast extract, 5.0 g/L), and the mixture was filter-sterilized through 0.45-μm Millipore™ membrane filters. DSMSW was prepared in the same manner as AHMSW, except additional carbohydrate was added to the AHMSW to approximately double the concentration of the individual carbohydrates present in AHMSW. This was done to evaluate the fermentation potential of cultures in MSW containing a higher concentration of sugars. The pH of all media was adjusted to 6.8–7 with concentrated sulfuric acid or 50% (w/v) sodium hydroxide.

MSW Hydrolyzate Treatments

Untreated AHMSW was prepared by adjusting the hydrolyzate to pH 7 with 50% (w/v) sodium hydroxide followed by filtration through Whatman #42 filter paper to remove insolubles. Calcium hydroxide-treated AHMSW was prepared by adjusting MSW hydrolyzate to pH 11 with calcium hydroxide powder, agitating for 15 min, and then filtering through Whatman #42 filter paper to remove insolubles. The filtrate was adjusted to pH 4 with concentrated sulfuric acid, filtered, then adjusted to pH 7 with 50% (w/v) sodium hydroxide, and filtered again. Calcium hydroxide and charcoal-treated AHMSW was made according to the same procedure as for the calcium hydroxide treatment with the following modification: after pH adjustment to 7, 5% (w/v) charcoal was added to the filtrate; the mixture was agitated for 30 min and filtered through Whatman #42 filter paper.

Treatments to Remove Inhibitors in MSW Hydrolyzate

Components in AHMSW that inhibit microbial activity include sulfate ion, phenolic compounds, and furfural (*8–10*). Concentrations of these inhibitors were measured in AHMSW, calcium hydroxide-treated AHMSW, and calcium hydroxide and charcoal-treated AHMSW. Sulfate ion was precipitated in an acetic acid solution with barium chloride to form barium sulfate, which was measured spectrophotometrically (*11*). Total phenolic compounds in AHMSW were determined by a colorimetric procedure

Table 1
Concentrations of Phenolic Compounds, Furfural, Sulfate Ion, and Carbohydrates in Untreated and Treated AHMSW[a]

Treatments,	mg/100 mL		Sulfate ion, mg/mL	mg/mL			
	Phenolic compounds	Furfural		Glucose	Xylose	Galactose	Mannose
Untreated	143.5	66.9	24.3	14.8	5.4	3.7	13.0
Calcium hydroxide	78.0	20.6	1.2	16.1	6.5	4.3	13.7
Calcium hydroxide + charcoal	4.4	2.3	1.4	16.4	6.5	4.4	14.0

[a]Hydrolyzate volume was reduced 8% following calcium hydroxide treatment and 15% after both calcium hydroxide and charcoal treatment.

that used Folin-Ciocalteu reagent in alkaline solution (*12*). Furfural was measured colorimetrically with aniline-acetic acid reagents (*13*). The effect of the hydrolyzate treatments on the production of lactic acid was determined by cultivating *L. xylosus* B-4449 in untreated and treated AHMSW. Screw-capped tubes (25 × 150 mm) containing 40 mL of substrate were inoculated with 1 mL of a 1:100 dilution of *L. xylosus* B-4449 and incubated at 32°C for 3 d. Analyses for bacterial growth and lactic acid and sugar concentrations were conducted daily during the fermentation. Bacterial growth was determined by measuring the increase in optical density of the medium at 610 nm, and sugar and lactic acid concentrations were determined using a Waters Sugars II HPLC as described by Yongsuwan (*14*).

Culture Screening

The ability of the cultures to ferment glucose, mannose, galactose, and xylose, the principal carbohydrates of AHMSW, to lactic acid was determined in screw-capped tubes (16 × 125 mm) containing 40 mL of MEB inoculated with 1% (v/v) of culture. Prior to inoculation, the cultures were serially transferred three times in MEB. The carbohydrate fermentations were conducted under static incubation conditions at 32°C for 3 d. The cultures also were screened for ability to produce lactic acid in AHMSW. Screw-capped tubes (16 × 125 mm) containing 40 mL of calcium hydroxide-treated AHMSW were inoculated with 1% (v/v) of culture previously transferred three times in MEB. The inoculated AHMSW was incubated for 3 d at 32°C under static fermentation conditions.

Optimum Conditions for Lactic Acid Production

Incubation temperature, inoculation rate, initial substrate pH, and addition of calcium carbonate buffer to the AHMSW were evaluated in order to establish optimum fermentation conditions.

The effect of incubation temperature on the production of lactic acid was determined in 250-mL Erlenmeyer flasks containing 100 mL of MEB supplemented with 41.3 mg/mL of carbohydrate comprised of the following: 16.4 mg/mL glucose, 14.0 mg/mL mannose, 6.5 mg/mL xylose, and 4.4 mg/mL galactose. These concentrations represented the levels of sugars in AHMSW. The flasks of MEB were inoculated with 1% (v/v) of *L. pentosus* B-227 and incubated for 3 d at 25, 32, and 37°C under static fermentation conditions. Sugar and lactic acid concentrations were determined daily. The optimum inoculum size was determined using Erlenmeyer flasks (250 mL) containing 150 mL calcium hydroxide-treated AHMSW inoculated with 1, 5, or 10% (v/v). *L. pentosus* B-227. The fermentation was conducted under static conditions at 32°C for 3 d.

The effect of initial substrate pH on the production of lactic acid was determined using culture *L. pentosus* B-227. MEB substrate containing 41.3 mg/mL total carbohydrate, comprised of four monosaccharides as described above, was adjusted to pH 4.1, 5.0, 6.0, 6.8, 7.6, 8.4, and 9.3

with concentrated sulfuric acid or 50% w/v sodium hydroxide. One hundred milliliters of substrate in 250-mL Erlenmeyer flasks were inoculated with 1 mL of a 1:100 dilution of culture and incubated at 32°C for 3 d under static conditions. Growth of the culture and lactic acid production were determined at the various substrate pH values.

Addition of buffer was evaluated to assess means to increase lactic acid production from acid-hydrolyzed MSW. Three levels of calcium carbonate, 1, 3, and 5% (w/v), were added to calcium hydroxide-treated DSMSW, which initially contained 35.6 mg/mL glucose, 30.6 mg/mL mannose, 13.3 mg/mL xylose, and 9.4 mg/mL galactose. The DSMSW was dispensed in 100-mL aliquots in 250-mL Erlenmeyer flasks, and inoculated with 1% (v/v) of *L. pentosus* B-227, and incubated at 32°C under static fermentation conditions for 5 d.

Fermentation Kinetics of *L. pentosus* B-227 in DSMSW

A second fermentation of calcium hydroxide-treated DSMSW was conducted using a 10.9 mg/mL higher sugar level than the previous study. Initial carbohydrate concentration was 100.6 mg/mL, which was comprised of 40.0 mg/mL glucose, 37.5 mg/mL mannose, 14.1 mg/mL xylose, and 9.0 mg/mL galactose. One liter of calcium hydroxide-treated DSMSW was sterilized by filtering through 0.45-μm membrane filters and buffered by adding 5% (w/v) of calcium carbonate powder. The 1-L aliquots of DSMSW were dispensed into 2-L Erlenmeyer flasks, and inoculated with 1% (v/v) of a 2-d-old culture of *L. pentosus* B-227, and incubated at 32°C for 5 d under static conditions.

RESULTS AND DISCUSSION

Treatments to Remove Fermentation Inhibitors from AHMSW

Sulfate ion, phenolic compounds, and furfural were present in untreated AHMSW at concentrations of 24.3, 143.5, and 66.9 mg/mL, respectively (Table 1). Calcium hydroxide treatment substantially reduced the concentration of inhibitors. Calcium hydroxide followed by charcoal was very effective in removing phenolic compounds and furfural, but it did not further reduce the sulfate ion concentration. The slight increase in carbohydrate levels in hydrolyzate following the treatments was the result of an approx 8% vol loss following calcium hydroxide treatment, and about 15% loss following both calcium hydroxide and charcoal treatment. Thus, the volume losses contributed to an increased concentration of carbohydrates in the treated hydrolysate.

Untreated AHMSW did not support good growth or lactic acid production by *L. xylosus* B-4449 (Table 2). Only 4.4 mg/mL sugar were used

Table 2
Growth and Lactic Acid Production by *L. xylosus* B-4449
in Untreated and Treated AHMSW[a] under Static Conditions after 3 Days at 32°C

Treatments	OD_{610}	Carbohydrate used, mg/mL	Carbohydrate conversion,[b] %	Lactic acid, mg/mL	Acid yield,[c] %
Untreated	0.36	4.4	10.7	0.3	7
Calcium hydroxide	0.62	14.0	33.9	6.8	49
Calcium hydroxide + charcoal	1.20	17.1	41.4	6.9	40
Modified Elliker broth (MEB)	1.27	11.2	27.1	7.1	63

[a] All fermentation substrates were adjusted to pH 6.8–7.0 and initially contained 41.3 mg/mL total carbohydrates composed of 16.4 mg/mL glucose, 14.0 mg/mL mannose, 6.5 mg/mL xylose, and 4.4 mg/mL galactose.
[b] mg carbohydrate used/mg initial carbohydrate × 100.
[c] mg lactic acid/mg carbohydrate used × 100.

and 0.3 mg/mL lactic acid was produced, compared to 11.2 mg/mL sugar used and 7.1 mg/mL lactic acid produced when MEB was fermented. Maximum growth and production of lactic acid should occur in MEB because it contains no inhibitors. Treatment of AHMSW with calcium hydroxide reduced the concentration of inhibitors and improved lactic acid production, resulting in 6.8 mg/mL of acid from 14.0 mg/mL sugar. Calcium hydroxide followed by charcoal treatment did not improve lactic acid production vs calcium hydroxide treatment alone, but culture growth was improved (Table 2). Further studies did not employ charcoal treatment of AHMSW because the minor benefit of the treatment did not warrant its added cost. Carbohydrate conversion was greater in the treated hydrolyzate than in the MEB because the former had a higher buffer capacity following calcium hydroxide treatment. This observation was confirmed in studies reported later in this article. However, the yield of lactic acid from carbohydrate was greater in MEB, suggesting that the carbohydrates in the hydrolyzate were metabolized to other products in addition to lactic acid.

Culture Screening

Glucose, mannose, and galactose, added at 10 mg/mL into MEB, were readily fermented by all eight cultures of lactic acid bacteria (Table 3). However, differences were apparent in the amount of lactic acid produced and sugar fermented. Lactic acid produced from 10 mg/mL glucose ranged from 5.2 to 7.3 mg/mL, and yield of acid from glucose used ranged from 76 to 97%. Slightly higher concentrations of lactic acid (5.7 to 8.3 mg/mL) were produced from 10 mg/mL of mannose, but yields (78 to 93%) were not improved over those from glucose. The bioconversion of 10 mg/mL of galactose resulted in lactic acid concentrations ranging from 2.8 to 7.4 mg/mL, and lactic acid yields for the eight cultures ranged from 54 to 88%. Five of the eight cultures evaluated did not ferment xylose, and those that did produced <2.0 mg/mL of lactic acid (Table 3). Preliminary studies (not shown here) indicated that the cultures used glucose first, followed by mannose and galactose, and finally xylose.

Acid-hydrolyzed MSW contained the four carbohydrates described above in the following concentrations: 16.4 mg/mL glucose, 14.0 mg/mL mannose, 6.5 mg/mL xylose, and 4.4 mg/mL galactose. The bioconversion of this carbohydrate mixture to lactic acid was determined for the eight *Lactobacillus* cultures. All cultures except *L. xylosus* B-4449 produced 17.4 mg/mL or more of lactic acid from 41.3 mg/mL of carbohydrate in AHMSW (Table 4). *Lactobacillus pentosus* B-227 produced the highest concentration of lactic acid (21.2 mg/mL) and achieved the highest carbohydrate conversion (62%). On this basis, the culture was selected for further studies.

Optimum Conditions for Lactic Acid Production

When the fermentation temperature was increased from 25 to 37°C, the yield of lactic acid by *L. pentosus* B-227 in MEB was reduced. Yield of

Table 3
Lactic Acid Production and Sugar Conversion by *Lactobacillus* Cultures in MEB Containing 10 mg/mL Glucose, Mannose, Galactose, or Xylose under Static Conditions after 3 Days at 32°C

	Glucose			Mannose			Galactose			Xylose		
Culture	Acid mg/mL	CC[a] %	AY[b] %	Acid mg/mL	CC %	AY %	Acid mg/mL	CC %	AY %	Acid mg/mL	CC %	AY %
B-787	6.2	73	85	6.6	71	93	4.0	49	82	<1	6	0
B-788	6.0	68	88	7.3	81	90	4.9	56	88	<1	4	0
B-813	7.3	75	97	8.3	89	93	4.7	56	84	<1	8	0
B-531	5.4	61	89	5.7	67	85	3.7	44	84	<1	2	0
B-227	6.0	68	88	6.8	83	82	7.4	87	85	<1	2	0
B-473	6.9	85	81	7.4	89	83	5.9	82	72	1.4	24	58
USDA 422	5.2	66	79	6.2	89	78	3.1	57	54	1.3	23	57
B-4449	5.6	74	76	5.8	74	78	2.8	46	61	1.8	36	50

[a]Carbohydrate conversion (CC) as mg carbohydrate used/mg initial carbodrate × 100.
[b]Acid yield (AY) as mg lactic acid produced/mg carbohydrate used × 100.

Table 4
Lactic Acid Production by *Lactobacillus* Cultures
in Calcium Hydroxide-Treated AHMSW[a]
after 3 Days Fermentation at 32°C under Static Conditions

Culture	Lactic acid, mg/mL	Carbohydrate conversion,[b] %	Lactic acid yield,[c] %
B-787	17.4	53	79
B-788	19.0	57	81
B-813	17.7	50	86
B-531	17.9	53	82
B-227	21.2	62	83
B-473	17.6	51	84
USDA 422	17.8	50	86
B-4449	6.6	18	91

[a]AHMSW contained 41.3 mg/mL carbohydrate composed of 16.4 mg/mL glucose, 14.0 mg/mL mannose, 6.5 mg/mL xylose, and 4.4 mg/mL galactose.
[b]mg carbohydrate used/mg initial carbohydrate × 100.
[c]mg lactic produced/mg carbohydate used × 100.

lactic acid after 3 d of fermentation was 80, 72, and 42% at 25, 32, and 37°C, respectively. Lactic acid concentration in the MEB was 5.9, 6.7, and 4.6 mg/mL, at 25, 32, and 37°C, respectively. Since the highest concentration of lactic acid occurred at 32°C and yield was not substantially less than at 25°C, 32°C was selected as the optimum temperature for lactic acid production. A lower concentration of lactic acid (6.7 mg/mL) was produced from MEB fermented at 32°C compared to calcium hydroxide-treated MSW (21.2 mg/mL; Table 4) because the latter substrate had a higher buffer capacity for lactic acid production.

The amount of culture inoculum (1, 5, or 10% v/v) added to calcium hydroxide-treated AHMSW did not affect lactic acid production by culture B-227. Following a 3-d fermentation at 32°C under static conditions, the culture produced 22.2 mg/mL of acid when a 1% inoculum was used, 24.7 mg/mL for the 5% inoculum, and 22.8 mg/mL of lactic acid for the 10% inoculation rate. The carbohydrate-to-lactic-acid conversion was similar for the 1 and 5% inocula (86 and 87%, respectively) and lowest for the 10% inoculum (64%).

The initial pH of the substrate had an effect on growth and lactic acid production by culture B-227 (Table 5). Growth and lactic acid production were best at pH 7.6; however, good growth and lactic acid production occurred over the pH range of 6.8 to 8.4. *Lactobacillus pentosus* B-227 did not grow at pH 4.1 or 9.3.

Calcium carbonate was an effective buffer for lactic acid production from DSMSW containing 88.9 mg/mL of carbohydrate (Table 6). The addition of calcium carbonate substantially increased lactic acid production

Table 5
Effect of Initial pH on Growth and Lactic Acid Production by *L. pentosus* B-227 in MEB[a] after 3 Days at 32°C under Static Conditions

Initial pH	OD_{610}	Lactic acid, mg/mL
4.1	0.00	0.0
5.0	1.52	4.6
6.0	1.58	5.7
6.8	1.89	6.5
7.6	2.08	8.3
8.4	1.86	7.2
9.3	0.01	0.0

[a]MEB contained 41.3 mg/mL carbohydrate composed of 16.4 mg/mL glucose, 14.0 mg/mL mannose, 6.5 mg/mL xylose, and 4.4 mg/mL galactose.

Table 6
The Effect of Calcium Carbonate Addition on pH, Carbohydrate Utilization, Lactic Acid Production, and Carbohydrate Conversion by *L. pentosus* B-227 in DSMSW during 3 Days Incubation at 32°C under Static Conditions

Addition of $CaCO_3$, %	Day	pH[a]	mg/mL		Carbohydrate conversion[c] %	Lactic acid yield,[d] %
			Carbohydrate used[b]	Lactic acid		
0	1	4.5	10.6	7.5	12	71
	2	3.9	20.9	16.2	24	78
	3	3.7	26.2	20.0	29	76
1	1	4.8	17.7	13.3	20	75
	2	4.2	41.3	33.0	46	80
	3	4.0	47.3	38.3	53	81
3	1	5.2	11.9	10.1	13	85
	2	4.7	50.2	43.1	56	86
	3	5.0	56.9	57.1	64	87
5	1	5.2	13.8	10.1	16	73
	2	5.0	56.3	48.1	63	85
	3	5.5	67.3	58.0	73	86

[a]Initial pH was 6.8 for all treatments.
[b]Initial carbohydrate concentration was 88.9 mg/mL, which was comprised of 35.5 mg/mL glucose, 30.6 mg/mL mannose, 13.3 mg/mL xylose, and 9.4 mg/mL galactose.
[c]mg carbohydrate used/mg initial carbohydrate × 100.
[d]mg lactic acid produced/mg carbohydate used × 100.

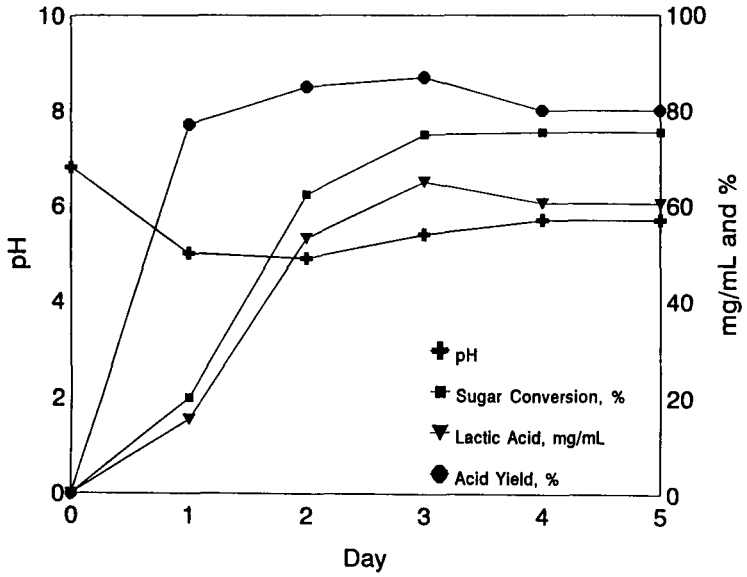

Fig. 1. Fermentation kinetics of *L. pentosus* in DSMSW with 100.6 mg/mL total carbohydrate and 5% calcium carbonate at 32°C under static incubation conditions.

after 3 d, from 20.0 mg/mL with no added calcium carbonate, to 38.3, 57.1, and 58.0 mg/mL for 1, 3, and 5% calcium carbonate, respectively. The higher levels of calcium carbonate (3 and 5%) increased lactic acid production by more than a 1% addition, although yield was not different. Although differences between 3 and 5% additional calcium carbonate were small after 3 d of incubation, 5% calcium carbonate would be more effective in moderating pH change if the fermentation time was increased beyond 3 d, or if the substrate contained higher carbohydrate levels, which potentially would result in increased production of lactic acid.

Fermentation Kinetics of *L. pentosus* B-227 in DSMSW

To determine the lactic acid productivity of *L. pentosus* B-227 in MSW hydrolyzate containing a higher level of carbohydrate, a DSMSW substrate was prepared as described earlier. The total carbohydrate concentration of the DSMSW was 100.6 mg/mL comprised of the following carbohydrates: 40.0 mg/mL glucose, 37.5 mg/mL mannose, 14.1 mg/mL xylose, and 9.0 mg/mL galactose.

Under optimum fermentation conditions consisting of an initial pH of 7.6, 32°C, 1% inoculum, 5% added calcium carbonate, and static fermentation conditions, *L. pentosus* B-227 produced 65 mg/mL of lactic acid after 3 d, an 87% conversion based on carbohydrate utilization (Fig. 1). However, the volumetric productivity of lactic acid at 3 d, 0.9 g* $(h*L)^{-1}$, was lower than that after 2 d fermentation, 1.1 g* $(h*L)^{-1}$. Lactic acid concentration remained steady throughout the remainder of the 5-d fermentation.

CONCLUSIONS

Five of the *Lactobacillus* cultures screened were suitable for lactic acid production from MSW hydrolyzate, *L. arabinosus* B-787, *L. arabinosus* B-788, *L. arabinosus* B-813, *L. pentosus* B-473, and *L. pentosus* B-227. *Lactobacillus pentosus* B-227 showed the greatest potential for lactic acid production from acid-hydrolyzed MSW based on carbohydrate conversion and lactic acid produced. Calcium hydroxide treatment was very effective in removing microbial inhibitors from MSW hydrolyzate, which improved culture growth and lactic acid production. Optimum lactic acid production occurred at 32°C under static fermentation conditions. The pH of the substrate had significant effects on the production of lactic acid. The best initial pH for fermentation was 7.6. Addition of 5% calcium carbonate increased the buffer capacity of the DSMSW, and increased lactic acid production to 58 mg/mL, almost three times that produced without added calcium carbonate (20 mg/mL). There was little difference in lactic acid production when calcium-hydroxide-treated AHMSW was inoculated with 1, 5, or 10% (v/v) of culture. Maximum production of lactic acid, 65 mg/mL, occurred when 1% (v/v) of *L. pentosus* B-227 was inoculated into DSMSW containing 100.6 mg/mL of total carbohydrate, 5% added calcium carbonate, and incubated for 3 d at 32°C under static conditions.

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