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Evaluation of Nitrogen Supplements for Bioconversion of Municipal Solid Waste to Lactic Acid

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ABSTRACT

Previous studies conducted by the authors showed that the ingredients of Elliker broth could be used as fermentation adjuncts for the bioconversion of municipal solid waste (MSW) acid hydrolysate to lactic acid. Five kinds of inexpensive organic nitrogen supplements were evaluated for their potential to replace more expensive nitrogen sources derived from tryptone and yeast extract, which are the nitrogenous components of Elliker broth. At a soluble nitrogen concentration of 0.28%, which is equivalent to that supplied by tryptone and yeast extract, soybean meal and cottonseed meal were the best alternative nitrogen sources and compared favorably to tryptone and yeast extract in terms of lactic acid production. Soybean meal nitrogen of 0.21% was the minimum nitrogen requirement for maximum production of lactic acid. When soybean meal was used as the nitrogen source, the addition of 1.91% phosphate as monobasic potassium phosphate significantly improved lactic acid production, but no improvement occurred below 1.91%. Ascorbic acid and sodium acetate are also components of Elliker broth, and the omission of these from the fermentation substrate did not affect lactic acid production. When 0.21% soluble soybean meal nitrogen and 0.4% sodium chloride were supplied to double-sugar MSW (DSMSW) hydrolysate, the amount of carbohydrate used, the percent of carbohydrate converted, the amount of lactic acid produced, and the percent yield after 3 d of fermentation were 78.3 mg/mL, 91%, 68.7 mg/mL, and 88%, respectively.

Index Entries: Municipal solid waste; fermentation; nitrogen supplements; MSW bioconversion; lactic acid production.

INTRODUCTION

The manufacture of 100% biodegradable polylactic plastics (PLP), requires large quantities of lactic acid *(1,2)*, and lactic acid production via fermentation has been the main source of lactic acid *(3)*. However, to be an economical source of lactic acid, the fermentation should employ a microorganism that produces high

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concentrations of lactic acid from inexpensive substrates. Previous studies have demonstrated the ability of *Lactobacillis pentosus* B-227 to ferment acid-hydrolysed municipal solid waste (MSW) to lactic acid, and optimum fermentation conditions have been reported *(4)*. These conditions include nutrient supplementation of MSW substrate with the ingredients of Elliker broth (tryptone, yeast extract, sodium chloride, sodium acetate, and ascorbic acid), an initial fermentation substrate pH of 7.6, substrate buffering with 5% calcium carbonate, 1% culture inoculation, and fermentation under static conditions at 32°C *(4)*. Tryptone and yeast extract are the most expensive components of the MSW fermentation substrate. In order to produce lactic acid on an industrial scale from MSW hydrolysate, a more economical nitrogen source is required to replace tryptone and yeast extract. A previous study indicated that cottonseed meal, soybean meal, and several other nitrogen sources have been used successfully in bioconversion processes *(5)*.

MATERIALS AND METHODS

Microorganism

The lactic acid producing bacterium *L. pentosus* B-227 was provided by the USDA Northern Regional Research Laboratory (Peoria, IL). The culture was serially transferred twice in Elliker broth (Difco, Detroit, MI) before it was used to inoculate the MSW fermentation substrate.

MSW Hydrolysate Substrate

MSW was "classified" in a commercial facility to recover recyclables to produce an organic fraction for hydrolysis. This organic fraction was comprised predominantly of papers, yard wastes, and food wastes. The classified MSW (organic fraction) was processed at the Tennessee Valley Authority's hydrolysis facility. Dilute sulfuric acid (2%) was mixed with the MSW and heated at 180°C for 20–40 min. These process conditions converted the cellulose and hemicellulose in the MSW to the monomeric sugars, glucose, mannose, xylose, and galactose, in that order of predominance. The hydrolyzed MSW was pumped through a filtration system to separate the hydrolysate from the solids. The hydrolysate was treated with calcium hydroxide as described in previous studies *(4,6)*. After treatment, the hydrolysate was used as the fermentation substrate.

Nitrogen Supplements

The nitrogen sources shown in Table 1 were dried at 60°C for 2 d and ground through a 2-mm mesh screen before they were evaluated as nitrogen adjuncts to the MSW substrate.

Fermentation Substrate

The total and water-soluble nitrogen contents of each nitrogen supplement were determined, and an equivalent amount of dry supplement was added to the MSW hydrolysate to obtain 0.28% soluble nitrogen. This percent is equivalent to the nitrogen content supplied by tryptone and yeast extract in Elliker broth. The nitrogen-supplemented MSW was then filtered through four layers of cheesecloth. The sugar content was increased by the addition of sugars rather than by concentrating the sugars in the hydrolysate to simulate hydrolysate with a higher

Table 1
Nitrogen Sources Evaluated and Approximate Costs

Nitrogen source	Price $/kg approx	Soluble N % of DM	Amt. for ESN,[a] g/L
Soybean meal[b]			
41% protein	0.19	1.62	173.0
Cottonseed meal			
40% protein	0.15	0.93	301.1
Feather meal			
75% protein	0.17	1.02	274.5
Fish meal			
57% protein	0.42	1.99	140.7
Meat and bone meal			
58% protein	0.21	3.03	92.4
Tryptone			
74% protein	70.00	11.75	23.8
Yeast extract			
61% protein	99.00	9.70	28.9

[a]Equivalent soluble nitrogen (ESN) is based on 0.28% soluble nitrogen in Elliker broth.
[b]Crude protein determined as %N × 6.25. Tryptone and yeast extract were obtained from Difco Laboratories, and all other nitrogen supplements were animal feed ingredients obtained from local feed companies. Approximate price was calculated from quantity discounts.

content of fermentable sugar. The following sugars (mg/mL), glucose (16.4), mannose (14.0), xylose (6.5), and galactose (4.4), were added to the MSW filtrate to double the concentrations of sugar monomers naturally in the hydrolyzed MSW, henceforth designated as double-sugar MSW (DSMSW). Sodium chloride, sodium acetate, and ascorbic acid were added to the substrate at the same concentrations as in Elliker broth. The substrate was sterilized at 121°C for 15 min and buffered with 5% (w/v) sterile calcium carbonate *(4)*.

Fermentation Conditions

The fermentation was conducted in 250-mL Erlenmeyer flasks containing 100 mL substrate. The flasks were inoculated with 1% (v/v) of *L. pentosus* B-227 broth culture, which had been serially transferred twice in Elliker broth. The fermentation was conducted under static conditions for 3 d at 32°C, and each treatment was replicated three times.

Evaluation of the Effects of Nutrients

The effects of different nitrogen supplements on lactic acid production were evaluated in DSMSW substrate. The nitrogen supplement was added to give a soluble nitrogen content of 0.28% in the fermentation substrate, which is equivalent to the nitrogen content supplied by tryptone and yeast extract in Elliker broth.

Based on the fermentation conditions described, soybean meal was used to make 0.28, 0.21, and 0.14% water-soluble nitrogen in the DSMSW substrate to determine the minimum nitrogen supplement required for maximum production of lactic acid. Calcium carbonate was used as a fermentation buffer and may have caused phosphate deficiency in the fermentation substrate owing to the formation of insoluble calcium phosphate. To determine the effect of phosphate addition on lactic acid production, monobasic potassium phosphate was added to give phosphate concentrations of 0.64, 1.28, and 1.91% in the DSMSW substrate, supplemented with 0.21% soluble soybean meal nitrogen. The concentration of monobasic potassium phosphate used was based on the 1.80% solubility of $Ca(H_2PO_4)_2$ *(7)*.

Elliker broth, which is a good substrate for lactic acid production, also contains 0.05% ascorbic acid and 0.15% sodium acetate. To determine if the adjuncts are beneficial for the bioconversion of DSMSW to lactic acid, the addition or omission of the adjuncts on lactic acid production from DSMSW with 0.21% soluble soybean nitrogen was determined. The adjuncts were added to DSMSW at the same levels as in Elliker broth.

Analyses

Total nitrogen was determined with 1 g of dry supplement by the semimicro-Kjeldahl method *(8)*. Water-soluble nitrogen was extracted from 5 g of dry supplement soaked in 100 mL of distilled water overnight at 25°C, and then filtered through four layers of cheesecloth. The filtrate was then used to determine water-soluble nitrogen by the semimicro-Kjeldahl method *(9)*. Water-soluble phosphate was determined on the filtrate by the vanadomolybdophosphoric acid colorimetric method *(8)*. Additional filtrate was centrifuged and filtered through 0.45-μm membrane filter, and carbohydrate concentration of the filtrate was determined by high-performance liquid chromatography (HPLC) *(4)*.

During fermentation, lactic acid and carbohydrate concentrations of the fermentation substrate were determined as follows: about 15 mL of fermentation substrate were collected, centrifuged at 12,000*g* for 15 min, and the supernatant was filtered through a 0.45-μm filter. Lactic acid and carbohydrate concentrations in the filtrate were determined by HPLC *(4)*. The data were analyzed by least-squares test of variance using the SAS GLM procedures *(10)*.

RESULTS AND DISCUSSION

Nitrogen and Phosphate Contents

Seven organic nitrogen sources, including tryptone and yeast extract, were evaluated as nitrogen adjuncts for the bioconversion of MSW to lactic acid (Table 1). Tryptone and yeast extract are highly refined sources of nitrogen that are used principally as nitrogen adjuncts for laboratory microbiological media. Because of the high cost of tryptone and yeast extract, less costly nitrogen supplements derived from animal protein feeds were evaluated as possible nitrogen substitutes (Table 1). An advantage of using the animal feed protein supplements is their low cost, but a disadvantage (because they are not refined) is their lower content of soluble nitrogen. For example, data in Table 1 indicate that 173.0 g of 41% protein soybean meal would be required to achieve 0.28% soluble nitrogen, which is the amount contained in Elliker broth. If tryptone were used, 23.8 g or about one-seventh of the amount would be required. However, because technical grade tryptone and yeast extract are valued at over 300 times the cost of soybean meal, the latter nitrogen source is more economical, provided that the nitrogen of soybean meal can substitute for the nitrogen of tryptone and yeast extract for lactic acid production.

Table 2
Nitrogen and Phosphate Contents of Nitrogen Supplements

Nitrogen supplement	TKN, %	WSN, % of TKN	WSP, mg/100 g	WEC, mg/mL
Soybean meal	6.6	24.5	26	<1
Cottonseed meal	6.4	14.6	16	<1
Feather meal	12.0	8.5	6	<1
Fish meal	8.9	22.3	177	<1
Meat and bone meal	9.3	32.5	117	<1
Tryptone	11.8	100	303	<1
Yeast extract	9.7	100	146	<1

TKN, total Kjeldahl nitrogen.
WSN, water-soluble nitrogen as a percentage of TKN.
WSP, water-soluble phosphate.
WEC, water-extractable carbohydrate as hexose monomers.

The nitrogen and water-soluble nitrogen contents of the supplements are shown in Table 2. Feather meal had the highest nitrogen content (12.0%), which was slightly higher than tryptone (11.8%) and yeast extract (9.7%). Soybean meal (6.6%), cottonseed meal (6.4%), fish meal (8.9%), and meat and bone meal (9.3%) all contained less nitrogen than tryptone and yeast extract. However, nitrogen must be soluble to be utilized by microorganisms. Meat and bone meal contained the most water-extractable nitrogen (32.5%), followed by soybean meal (24.5%), fish meal (22.3%), cottonseed meal (14.6%), and feather meal (8.5%). Water-soluble phosphate content (mg/100 g), was highest in fish meal (177) and meat and bone meal (117), and least in soybean meal (26), cottonseed meal (16), and feather meal (6).

Equivalent Available Nitrogen Supplements

DSMSW substrates supplemented with equivalent amounts of soluble nitrogen (0.28% [w/v]) from the various sources supported growth of *L. pentosus* B-227, and lactic acid was produced in concentrations ranging from 17.9 to 65.3 mg/mL (Table 3). Based on the carbohydrate used, carbohydrate conversion rate, lactic acid produced, and yield, soybean meal, followed by cottonseed meal, was the best nitrogen supplement, compared to the control supplement of tryptone and yeast extract. After a 3-d fermentation, 65.3 mg/mL of lactic acid (yield 85%), was produced from 95 mg/mL of carbohydrate (conversion 81%) when soybean meal supplement was used. When cottonseed meal was used as the supplement, 61.1 mg/mL of lactic acid (yield 79%) was produced from 89 mg/mL of carbohydrate (conversion 88%). These lactic acid concentrations were not significantly ($p < 0.05$) different from that of the control supplement of tryptone and yeast extract, which produced 67.8 mg/mL of lactic acid (yield 83%) from 94 mg/mL of carbohydrate (conversion 87%). When feather meal, fish meal, and meat and bone meal were used as nitrogen supplements, lactic acid produced and carbohydrate used were significantly ($p < 0.05$) lower than for the tryptone and yeast extract supplements (Table 3).

The data in Table 4 indicate that different levels of soybean meal nitrogen supplement affect lactic acid production. A 0.28% soluble nitrogen content produced the highest level of lactic acid (65.3 mg/mL), with an 85% yield. When

Table 3
Effects of Nitrogen Supplements on Lactic Acid Production from DSMSW Hydrolysate by *L. pentosus* B-227

Nitrogen supplements	Carbohydrate[a] Used, mg/mL	Carbohydrate[a] Conversion,[b] %	Lactic acid Produced, mg/mL	Lactic acid Yield,[c] %
Soybean meal	77.1^{A}	81	65.3^{A}	85
Cottonseed meal	77.8^{A}	88	65.1^{A}	79
Feather meal	63.9^{B}	75	46.0^{B}	85
Fish meal	54.8^{C}	65	40.5^{C}	74
Meat and bone meal	27.5^{D}	33	17.9^{D}	65
Tryptone and yeast extract[d]	81.5^{E}	87	67.8^{A}	83

[a]Initial substrate carbohydrate concentrations were 95, 89, 85, 85, 84, and 94 mg/mL for soybean meal, cottonseed meal, feather meal, fish meal, meat and bone meal, and tryptone and yeast extract, respectively.
[b]Conversion = carbohydrate used/initial carbohydrate × 100.
[c]Yield = lactic acid produced/carbohydrate used × 100.
[d]Nitrogen supplementation is at a rate of 20 g/L tryptone and 5 g/L yeast extract.
$^{A-E}$Different superscript capital letters in a data column are significantly different ($p < 0.05$).

Table 4
Effects of the Levels of Soybean Meal Supplement (SBM) on Lactic Acid Production from DSMSW Hydrolysate by *L. pentosus* B-227

SBM supplement level, %	Carbohydrate[a] Used, mg/mL	Carbohydrate[a] Conversion, %	Lactic acid Produced, mg/mL	Lactic acid Yield, %
0.28	77.1^{A}	81	65.3^{A}	85
0.21	70.8^{B}	74	61.1^{A}	86
0.14	60.8^{C}	64	39.3^{B}	65

[a]Initial carbohydrate concentration was 95 mg/mL.
$^{A-C}$Different superscript capital letters in a data column are significantly different ($p < 0.05$).

the soluble nitrogen level was decreased from 0.28 to 0.14%, lactic acid production decreased from 65.3 to 39.3 mg/mL, carbohydrate used decreased from 77.1 to 60.8 mg/mL, and carbohydrate conversion also decreased from 81 to 64%. However, carbohydrate conversion, lactic acid produced, and yield did not differ significantly ($p < 0.05$) between the 0.28 and 0.21% soluble nitrogen levels supplied by the soybean meal. Therefore, the 0.21% soluble soybean meal nitrogen supplement was used for further studies.

Phosphate Supplement

The addition of phosphate as monobasic potassium phosphate to the DSMSW fermentation substrate showed some benefit on the production of lactic acid from MSW (Table 5). When phosphate addition increased from 0.64 to 1.91%,

Table 5
Effects of Phosphate Addition on Lactic Acid Production from DSMSW Hydrolysate by *L. pentosus* B-227

	Carbohydrate[a]		Lactic acid	
Phosphate addition, %	Used, mg/mL	Conversion, %	Produced, mg/mL	Yield, %
0	82.7^{A}	87	67.4^{B}	81
0.64	80.1^{A}	84	67.2^{B}	84
1.28	79.9^{A}	84	$71.1^{A,B}$	89
1.91	81.4^{A}	85	75.4^{A}	93

[a]Initial carbohydrate concentration was 95 mg/mL.
[A,B]Different superscript capital letters in a data column are significantly different ($p < 0.05$).

Table 6
Effects of Ascorbic Acid and Sodium Acetate Supplementation on Lactic Acid Production from DSMSW Hydrolysate by *L. pentosus* B-227

	Carbohydrate[a]		Lactic acid	
Substrate	Used, mg/mL	Conversion, %	Produced, mg/mL	Yield, %
Control[b]	77.2^{A}	90	69.4^{A}	90
Without ascorbic acid[c]	78.5^{A}	91	69.7^{A}	89
Without ascorbic acid and NaAC[d]	78.3^{A}	91	68.7^{A}	88

[a]Initial carbohydrate concentration was 86 mg/mL.
[b]DSMSW with 0.21% soybean meal nitrogen, 0.05% ascorbic acid, and 0.15% sodium acetate.
[c]Same as control without 0.05% ascorbic acid.
[d]Same as control without 0.05% ascorbic acid and 0.15% sodium acetate.
[A]Different superscript capital letters in a data column are significantly different ($p < 0.05$).

lactic acid production significantly increased from 67.2 to 75.4 mg/mL, and yield increased from 84 to 93%. However, phosphate addition had no effect on carbohydrate used or its conversion, which were the same for all treatments. There were two possible reasons why the phosphorus addition did not improve the utilization of carbohydrate or its conversion to lactic acid. First, the organic phosphate in soybean meal might be adequate for bacterial growth and lactic acid production. Second, most of the inorganic phosphorus added to the fermentation substrate might have combined with the calcium from the calcium carbonate buffer and precipitated as the calcium salt, making the phosphate unavailable.

Ascorbic Acid and Sodium Acetate Supplements

The omission of ascorbic acid or both ascorbic acid and sodium acetate from the fermentation substrate supplements had no effect on carbohydrate used, carbohydrate conversion, lactic acid produced, or yield of lactic acid (Table 6). After a 3-d fermentation, 69.4 mg/mL of lactic acid (90% yield) were produced in the control, which was not different ($p < 0.05$) from 69.7 mg/mL (yield 89%) for the omission of ascorbic acid and 68.7 mg/mL (yield 88%) for the omission of both ascorbic acid and sodium acetate. Although ascorbic acid and sodium acetate are ingredients in Elliker broth recommended for the cultivation of lactic acid bacteria, these compounds did not improve lactic acid production from DSMSW hydrolysate and, therefore, need not be used as nutrient adjuncts for DSMSW hydrolysate.

CONCLUSION

Soybean meal and cottonseed meal were suitable substitutes for tryptone and yeast extract as nitrogen supplements for lactic acid production from MSW. A minimum soluble soybean meal nitrogen content of 0.21% was required for maximum production of lactic acid. When soybean meal was used as the nitrogen supplement, the addition of 0.64% phosphate as monobasic potassium phosphate did not improve lactic acid production, but the addition of 1.91% phosphate gave a slight improvement. The omission of ascorbic acid, or both ascorbic acid and sodium acetate, from the fermentation substrate did not affect lactic acid production.

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