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Production of Lactic Acid from Food Wastes

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Abstract

Conversion of food wastes into lactic acid by simultaneous saccharification and fermentation (SSF) was investigated. The process involves saccharification of the starch component in food wastes by a commercial amylolytic enzyme preparation (a mixture of amyloglucosidase, α-amylase, and protease) and fermentation by *Lactobacillus 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%: 27 g/L of lactic acid from 60 g/L of food waste. The overall performance of the SSF, however, was not significantly affected by the elimination of mineral supplements.

Index Entries: Food waste; lactic acid; simultaneous saccharificaiton and fermentation; *Lactobacillus delbrueckii*; amyloglucosidase.

Introduction

Approximately 5,000,000 t of food wastes are generated annually in South Korea. Most of the food wastes are landfilled or incinerated, and groundwater contamination and emission of noxious gases and dioxins have been frequently cited. Food waste management has therefore been an important issue from the standpoint of environmental protection and conservation of resources.

Among the major components in food wastes are carbohydrates (starch and cellulose) and proteins. The starch and cellulosic components of the food waste can be hydrolyzed into monomeric sugars. The sugars from the food wastes can be utilized as substrates for fermentative production of a variety of chemicals including lactic acid. Lactic acid is widely used in the

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food and chemical industries. Current demand for lactic acid in production of biodegradable polymers (polylactic acid) *(1)* is bringing its status from a specialty chemical to a commodity chemical.

Traditionally, hydrolysates of corn or potato starch have been used as the feedstock for the fermentative lactic acid production process *(2)*. Lignocellulosic biomass has been currently tested as an alternative feedstock for lactic acid production *(3–5)*. Municipal solid wastes have also been evaluated as feedstock for lactic acid *(6,7)*. Recently, Lee et al. *(8)* and Loh et al. *(9)* studied lactic acid production from food wastes and reported a product concentration of 20–40 g/L from direct fermentation of food wastes lasting 4 to 5 d. Amylolytic lactobacillus strains and other lactic acid–producing organisms, such as *Rhizopus oryzae (10,11)*, can directly metabolize starch to produce lactic acid. However, they do so with a very low fermentation rate giving a relatively low product yield, and low product concentration *(12)*. Enzymatic hydrolysis of starch and fermentation of glucose to lactic acid are well-established industrial processes. Separate enzymatic and microbial processing of food wastes can improve lactic acid production over that of direct microbial conversion. In the present study, we were interested in applying the enzymatic hydrolysis and fermentation for conversion of food wastes to lactic acid in order to achieve high product yield and production rate. The hydrolysis and fermentation can be carried out separately or simultaneously. Simultaneous saccharification and fermentation (SSF) has been extensively investigated in connection with ethanol production from starch or cellulosic feedstock. Recently, it has been evaluated as a means of producing lactic acid *(13)*. Our research was undertaken to assess the technical feasibility of an SSF process based on starch-hydrolyzing enzymes and lactic acid bacteria for production of lactic acid from food wastes.

Materials and Methods

Substrates

Food wastes were obtained from a university cafeteria (Kyungwon University, Korea). They consisted mainly of cooked rice, flour products, vegetables, fishes, and meat. They were ground and kept in a refrigerator before. The water content of the food waste was about 80% (w/w). The wet food waste sample was used directly as a substrate. The food waste was analyzed for its chemical composition; typical composition is given in Table 1. Total carbon, nitrogen, hydrogen, and oxygen contents were analyzed by an elemental analyzer (CHN-1000). Mineral elements such as P, K, Ca, Mg, and Na were analyzed by ICP-AES (JY ULTRACE 138).

Enzymes

A commercial enzyme mixture, SAN Super 240L (Novo Nordisk), was used. This enzyme preparation consists of mainly amyloglucosidases and a balanced amount of α-amylases and proteases. The activity of SAN Super

Table 1
Chemical Composition of Food Wastes[a]

pH	T-C (wt%)	T-N (wt%)	T-H (wt%)	T-O (wt%)	Ash (wt%)	P (wt%)	K (wt%)	Ca (wt%)	Mg (wt%)	Na (wt%)
4.8	41.9	2.14	6.40	42.4	1.06	0.23	0.58	0.37	0.07	2.3

[a]T-C, total carbon; T-N, total nitrogen; T-H, Total hydrogen; T-O, total oxygen.

240L was specified to be 240 AGU/mL by the supplier, in which 1 AGU is defined as the amount of enzyme hydrolyzing 1 μmol of maltose/min at 25°C and pH 4.3. It also contains controlled amounts of fungal amylase and protease activities.

Microorganisms and Inoculumn Preparation

Homofermentative *Lactobacillus delbrueckii* NRRL B-445 (KCCM 40069, renamed as *Lactobacillus rhamnosus*) was obtained from Korean Culture Center of Microorganisms (KCCM). The lyophilized culture was transferred to plates of solid Elliker broth (Difco) medium. The plates were incubated at 37°C for 36 h. The grown colonies either were used to prepare inoculum or were stored at 4°C for later use. The microorganism on agar slants was transferred to a liquid Elliker broth medium and cultivated at 37°C for 36 h to be used as inoculum in lactic acid fermentation experiments.

Enzymatic Hydrolysis of Food Wastes

The ground food waste sample was diluted with water to a final concentration between 65 and 150 g/L (dry wt), which was the extent of selected experimental conditions used. SAN Super 240L was added and the mixture was placed in an incubator shaker maintained at a given temperature. Samples were withdrawn periodically and boiled for 1 min to arrest enzyme action. Reducing sugars and glucose were measured. Experiments were carried out to determine the effect of enzyme dosage on the glucose yield.

Glucose Fermentation

Glucose fermentation was performed to determine the lactic acid productivity of the strain used. Fermentation medium contained 15 g/L of yeast extract, 0.5 g/L of dipotassium hydrogen phosphate, 0.5 g/L of potassium dihydrogen phosphate, 1.0 /L of sodium acetate trihydrate, 0.5 g/L of magnesium sulfate heptahydrate, 0.05 gL of manganese sulfate monohydrate, 1 g/L of ammonium citrate, and 0.03 g/L of iron sulfate heptahydrate. Three weight percent of glucose was added. The fermentation medium was sterilized at 121°C for 20 min. A 10% (v/v) inoculum was used in this fermentation. The fermentation was carried out at 42°C and 150 rpm in a shake flask. An excess of $CaCO_3$ powder was used to neutralize the lactic acid produced.

Simultaneous Saccharification and Fermentation

The food wastes prepared as described above were used as substrates in SSF experiments. The same level of nutrient supplements as used in glucose fermentation was used in this SSF experiment. The SSF solution was autoclaved at 121°C for 20 min. It was then inoculated and SSF was carried out after adding SAN Super 240L enzyme (1.8 mL/100 g of dry substrate). All experiments, with the exception of the pH optimization studies, were carried out in 250-mL shake flasks with a 100-mL working volume at a temperature of 42°C and an agitation rate of 150 rpm, unless specified otherwise. The initial pH was adjusted to 6.0 and controlled by the addition of $CaCO_3$ powder. An oxygen-free environment was maintained by initially sparging CO_2 through a 0.3-μm filter. A New Brunswick Bioflo model III fermentor was used for the pH optimization studies. It was operated with a pH control and at a 1000 mL working volume. The pH was controlled with 5 N NaOH. Samples were boiled for 1 min to eliminate any bacterial activity and then stored at 4°C for further analysis.

Analytical Methods

Samples taken from the hydrolysis and fermentation experiments were analyzed for lactic acid and glucose by a high-performance liquid chromatography equipped with an refractive index detector. A Bio-Rad-HPX-87H column was used at 65°C with a 0.005 *M* H_2SO_4 mobile phase at a flow rate of 0.6 mL/min. The total carbohydrate content was determined by hydrolyzing the food waste to sugars followed by measuring the concentration (i.e., reducing sugars) of the resulting sugars. Hydrolysis was performed by a primary 72% H_2SO_4 hydrolysis and a secondary dilute-acid hydrolysis, as described in ref. *14*. Reducing sugars were determined according to Nelson *(15)* using glucose as a standard. The yield was expressed as a percentage based on initial available sugars.

Results and Discussion

Enzymatic Hydrolysis of Food Wastes

Food wastes were hydrolyzed enzymatically and yields of glucose were examined under various hydrolysis conditions. From 100 g/L of food waste (dry basis), 75 g/L of reducing sugars was produced by acid hydrolysis. This reducing sugar concentration was used as the initial available sugar concentration.

Figure 1 shows the time course of the saccharification of 65 g/L of food waste at 55°C and pH 6.0 with varying enzyme (SAN Super 240L) concentrations. The amyloglucosidase activity of this enzyme was 240 AGU/mL, as mentioned earlier. The highest glucose yield, about 67%, was obtained in 24 h with at least 1.8 mL of the enzyme mixture/100 g of food waste. The overall optimal working conditions for SAN Super 240L are a temperature of 40–55°C and a pH of 4.5–6.0, as determined by the supplier. The hydrolysis and the SSF experiments were therefore performed at these conditions.

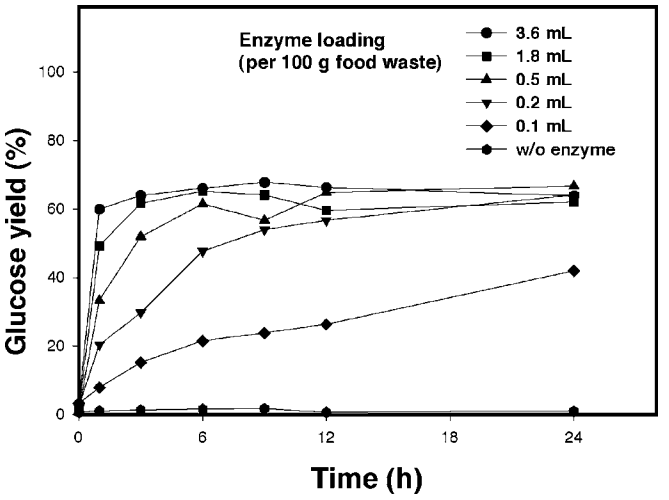

Fig. 1. Effect of enzyme loading on the enzymatic hydrolysis of food waste (65 g/L) at 55°C and pH 5.5.

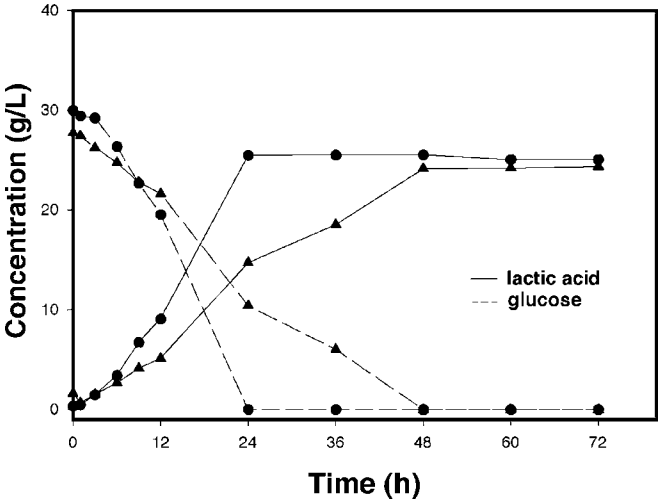

Fig. 2. Time course of lactic acid fermentation with pure glucose (30 g/L) by *L. delbrueckii* at 42°C and pH 6.0 using two different nitrogen supplements: (●) 15 g/L of yeast extract; (▲) 15 g/L of peptone.

SSF of Food Wastes

Glucose fermentations were performed to determine the lactic acid production capability of the strain *L. delbrueckii*. As shown in Fig. 2, 25.5 g/L of lactic acid was produced from 30 g/L of glucose in 24 h in the fermentation carried out at 42°C and pH 6.0. When peptone, instead of yeast extract, was used, the lactic acid production rate decreased significantly, but almost the same level of the final lactic acid concentration was obtained.

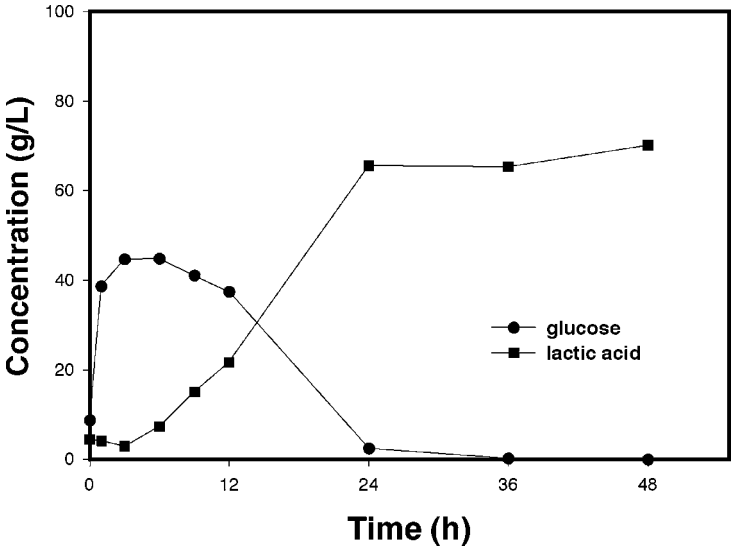

Fig. 3. Time course of batch SSF run with food waste (120 g/L) at 42°C.

The typical time course of lactic acid production from food waste by SSF with SAN SUPER 240L enzyme and *L. debrueckii* is illustrated in Fig. 3. During the SSF process, the food waste was saccharified to glucose, and the glucose was then metabolized by the microorganism and converted into lactic acid. Accumulation of glucose was seen in the initial phase of the SSF. The fermentation then proceeded at a rate comparable with the fermentation rate of lactic acid by the same organisms under similar conditions using glucose (Fig. 2). At the given SSF conditions, 70.1 g/L of lactic acid was produced from 120 g/L of food waste in about 48 h, giving a yield of 78%. From this preliminary experiment, it was clear that food wastes can be used as a resource for lactic acid production and that SSF could be an effective bioprocess for producing lactic acid from the food wastes. To further develop an SSF process for lactic acid production from food waste, the effect of various operating parameters on performance was examined. The parameters studied were pH, temperature, substrate concentration, and nitrogen and mineral supplements.

Effect of pH and Temperature

In the SSF process, both bioreactions of carbohydrate hydrolysis by enzymes and glucose fermentation by microorganisms occur simultaneously. Thus, optimum conditions of both bioreactions should be coincident for effective SSF operation. Generally, the SSF process requires operating conditions that represent a compromise between the optimum conditions of the enzyme and the microorganisms.

The optimum temperature of the *L. delbrueckii* was reported to be 37°C by the supplier (KCCM). On the other hand, the optimum temperature for

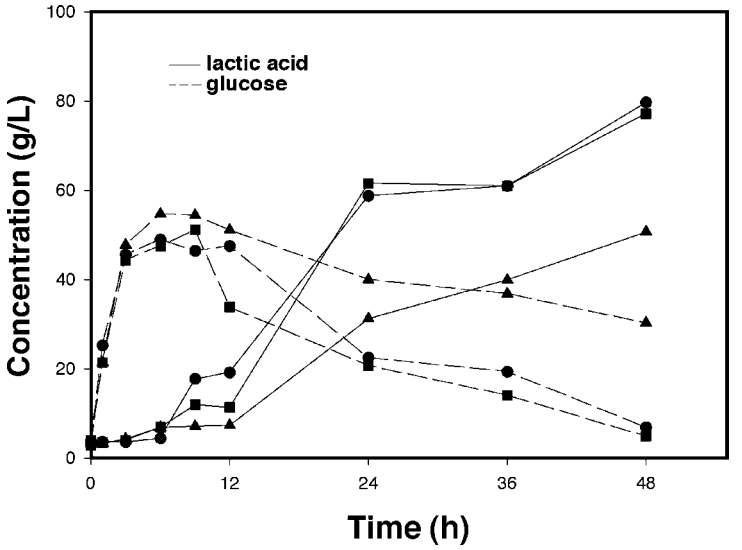

Fig. 4. Effect of temperature on batch SSF of food waste (145 g/L): (●) 35°C; (■) 45° C; (▲) 55°C.

the SAN Super 240L enzyme was specified to be 40–55°C, as stated earlier. We therefore selected 35, 45, and 55°C for the optimization study. As shown in Fig. 4, at 35 and 45°C, 79.7 (yield 73%) and 77.2 g/L (yield 71%) of lactic acid, respectively, were produced from 145 g/L of food waste in 48 h. At 55°C, however, the fermentation rate was significantly reduced. The final lactic acid concentration was reduced to 50.7 g/L (yield 47%), leaving a higher residual glucose concentration within the 48-h experiment. From these studies, the optimum temperature would appear to be between 35 and 45°C. An operating temperature of 42°C was chosen for subsequent SSF experiments. Note that this optimum temperature might vary with different enzyme loadings and thus needs further study.

The optimum pHs for both the enzyme and the microorganisms were similar, at about 6.0. The optimum pH of the SSF process was therefore expected to be about 6.0. Figure 5 depicts the effect of pH on the lactic acid production rate and yield. Lactic acid concentrations produced from 120 g/L of the food waste in 48 h were 11.5, 54.2, 63.4, and 57.6 g/L at the operating pHs of 4.0, 5.0, 6.0, and 6.5, respectively. The corresponding lactic acid yields were 13, 60, 71, and 64%. Maximum lactic acid production rate was indeed observed at pH 6.0. In the SSF at pH 4.0, glucose was accumulated throughout the reaction time, and lactic acid was formed very slowly. This indicates that the SSF process was limited by the fermentation, probably owing to suppressed cell growth at this pH. In this set of experiments, the pH was continuously controlled with 5 *N* NaOH in a 1.0-L (working volume) fermentor. Interestingly, a slightly higher lactic acid yield (78%) was produced in the shake-flask operation in which pH was controlled by

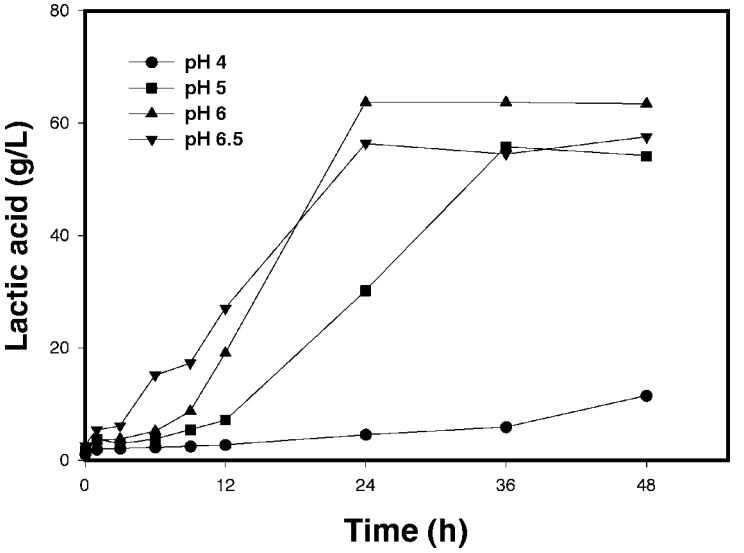

Fig. 5. Effect of pH on batch SSF of food waste (120 g/L) at 42°C.

$CaCO_3$, as shown in Fig. 3, than in the bioreactor controlled at similar pH. This result could not be explained clearly and needs to be investigated further. However, since there was no significant difference in lactic acid production and because of its ease of operation, the shake-flask system was used throughout the optimization study.

Effect of Nitrogen and Mineral Supplements

One of the major costs in lactic acid fermentation is the consumption of large quantities of expensive nitrogen sources, such as yeast extract. They are required as the growth nutrients and can affect the lactic acid productivity. Thus, the effect of nitrogen supplements on lactic acid production from food wastes was studied. A series of SSF experiments was conducted using yeast extract or peptone as the nitrogen source. An SSF run without nitrogen supplement was also performed. As shown in Fig. 6, 33.5 (yield 75%) and 30.3 g/L (yield 67%) of lactic acid were produced after 2 d of SSF with 60 g/L of food waste when 15 g/L of yeast extract and peptone, respectively, were used as the nitrogen supplement. Without supplementation of nitrogen sources, 27.2 g/L (yield 60%) of lactic acid was produced from 60 g/L of food waste. Since the food waste used contained nitrogen compounds, as indicated in Table 1, it was thought that those nitrogen compounds in the food waste were used as the growth nutrients' sources. Proteins contained in food wastes can be hydrolyzed to nutrients for growth and lactic acid production of *L. delbrueckii* by the enzyme that contains a controlled amount of protease activity. This result indicates a minimal requirement of nitrogen supplements in lactic acid production from food wastes using SSF.

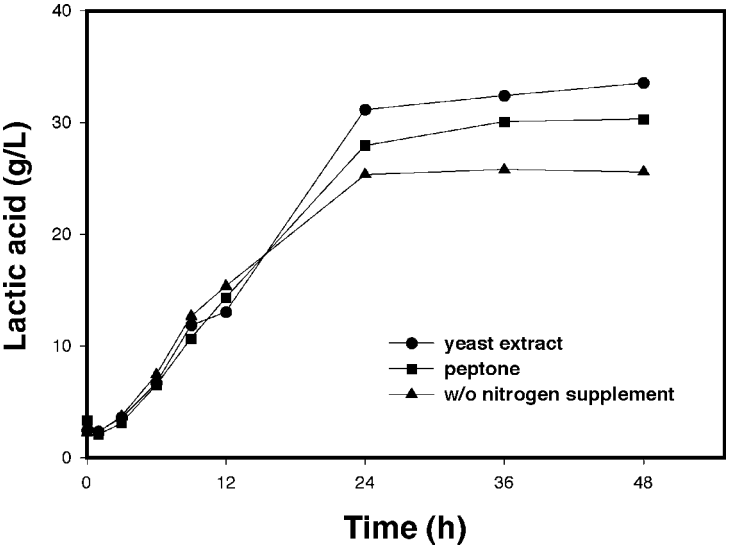

Fig. 6. Effect of nitrogen sources on batch SSF of food waste (60 g/L) at 42°C.

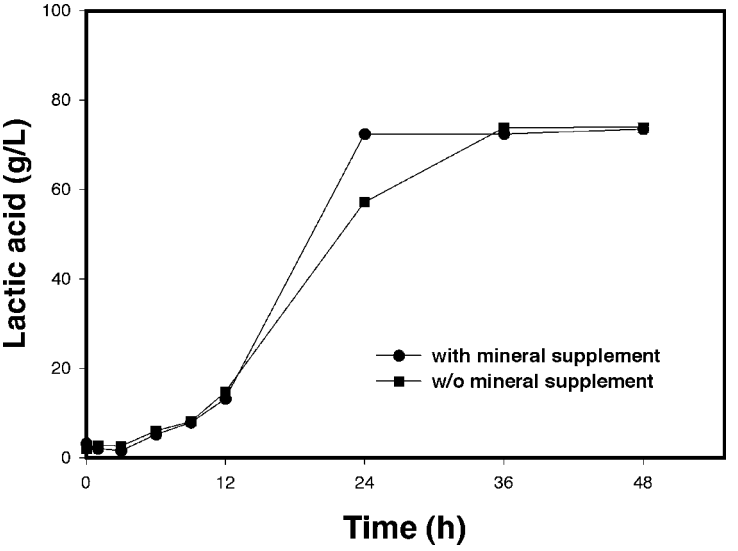

Fig. 7. Effect of mineral supplements on batch SSF of food waste (130 g/L) at 42°C.

To determine the effect of mineral supplements on the lactic acid production, a set of SSF runs was carried out with and without mineral supplements. The composition of mineral supplements was indicated in the fermentation medium as described earlier. As shown in Fig. 7, in the early stages of SSF up to 24 h, 72.4 g/L of lactic acid was produced in the SSF conducted with mineral supplements. A lower lactic acid concentration of 57.2 g/L was produced in the SSF conducted without mineral supplements.

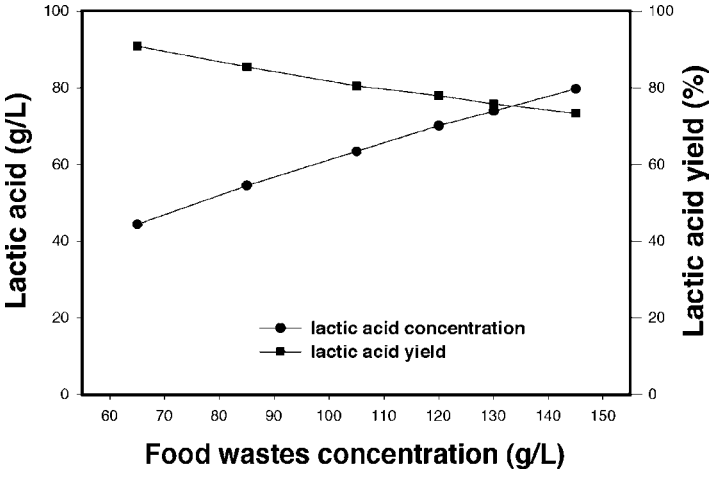

Fig. 8. Effect of food waste concentration on lactic acid yield in batch SSF at 42°C.

However, after 36 h of SSF operation, an almost identical level of lactic acid concentration (72.5 g/L, yield 74%) was produced from 130 g/L of food waste in both SSF runs. Since the food waste contained some minerals and phosphates, as shown in Table 1, which might be adequate for bacterial growth and lactic acid production, additional supplements may not be required. This experimental result along with the aforementioned results from the nitrogen supplements study demonstrates a high economic feasibility of lactic acid production from food waste.

Effect of Substrate Concentration

The effect of initial food waste concentration on the final concentration of lactic acid produced was examined. The initial food waste concentrations were varied from 65 to 145 g/L in the SSF runs. As shown in Fig. 8, the final lactic acid levels increased with each increase in food waste level, but, the conversional yield decreased; during the 2-d SSF, the lactic acid yields decreased from 91 to 73%. The highest yield of 91% was obtained from 65 g/L of food waste with a final lactic acid concentration of 44.3 g/L. On the other hand, the highest lactic acid concentration of 79.7 g/L was obtained from 145 g/L of food waste with the least yield of 73%. This result may be owing to incomplete hydrolysis of food waste, glucose and lactic acid inhibition on the microorganisms' activity for a higher level of food waste, and/or formation of other byproducts. Further work is needed to clarify this observation.

Conclusions

We have demonstrated that lactic acid production from food wastes by way of SSF is technically feasible. The highest yield of lactic acid on the

basis of available carbohydrates in the food waste was 91% of theoretical: 44.3 g/L of lactic acid from 65 g/L of food waste. A total lactic acid concentration of 80 g/L was attainable from 145 g/L of food waste within 48 h of the SSF, which is remarkably higher than the lactic acid concentration of 20–40 g/L obtained in 4–5 d in a direct fermentation of food wastes *(8,9)*. Within the scope of our study, the optimum operating conditions for the production of lactic acid were 42°C and pH 6.0. Without supplementation of nitrogen sources, the yield of lactic acid in the SSF decreased to 60%: 27 g/L of lactic acid from 60 g/L of food waste. Elimination of all the noncarbon nutrients other than yeast extract from the SSF medium did not adversely affect lactic acid production. Yeast extract therefore appears to be the only additional nutrient required to maintain optimum performance of the SSF. This clearly indicates that the food waste used in this investigation contains a sufficient amount of mineral sources needed for bacterial growth and lactic acid production, which would be one of the economic benefits associated with food waste. Although further, detailed economic analysis is needed, the preliminary results of our study demonstrate that lactic acid production using food wastes can be considered a promising way of food waste management.

Acknowledgment

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