

Production of L-Lactic Acid from Spent Grain, a By-Product of Beer Production

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ABSTRACT

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L-lactic acid production from spent grain with immobilized lactic acid bacteria was investigated. Spent grains were liquefied by a steam explosion treatment to obtain liquefied sugar. When 1 kg of wet spent grain was treated under the 30 kg/cm² pressure for 1 min using a 5-L steam explosion reactor, 60 g of total sugar was obtained from the liquefied spent grain. Furthermore, 1.3% (w/v) of glucose, 0.4% (w/v) of xylose, and 0.1% (w/v) of arabinose were produced when the liquefied spent grain was treated with glucoamylase, cellulase, and hemicellulase enzymes. When batch L-lactic acid production was carried out by *Lactobacillus rhamnosus* NBRC14710, 19.0 g/L L-lactic acid was produced from the Tween 80 liquefied spent grain after 5 days. Furthermore, during repeated batch production with immobilized *Lactobacillus rhamnosus* NBRC14710 from Tween 80 liquefied spent grain at 37°C, the productivity of L-lactic acid was maintained at a 10 time higher level over a period of 40 days.

Key words: Biodegradable plastic, L-lactic acid, spent grain.

INTRODUCTION

Lactic acid is an important chemical that has both food and industrial applications. The advantages of lactic acid polymers are their biodegradability, bioenvironmental compatibility, fabric ability, thermo plasticity, and high strength. These polymers have potentially large markets in commodity packaging, fabrication of prosthetic devices, and controlled delivery of drugs in humans. The substitution of existing synthetic polymers by biodegradable ones would also significantly alleviate waste disposal problems. Several studies on the production of lactic acid from various viable feedstocks have been reported^{2,4–8,10,13}.

Breweries generate one million tons of spent grain every year, and about 20% of the spent grain is recycled in Japan. Therefore, it is environmentally and economically significant to consider the production of lactic acid using the spent grain from the brewing industry. The present paper describes the hydrolysis of cellulose from spent grain by explosion and the subsequent conversion to L-lactic acid, a precursor of biodegradable plastic.

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A variety of biodegradable plastics have recently been developed. However, their high production costs are an important issue the industry must tackle to promote the use of biodegradable plastics. Thus, there is an urgent need to find some means to reduce production costs. Achieving a low production cost for L-lactic acid is dependent primarily on what is chosen for the raw material⁵. If spent grain can be efficiently used as a raw material for the production of polylactic acid, then a considerable reduction in costs would be possible.

MATERIALS AND METHODS

Raw materials

Spent grain was collected from local breweries with a percent water of 78.5% (w/w).

Steam explosion treatment

Preparation of liquefied spent grain was carried out using a 5-L steam explosion reactor (Tukishima Kikai Co. Ltd., Japan). The steam pressure was adjusted to achieve the desired reaction temperature. The pressure was controlled until 30 kg/cm² and residence times were varied for each experiment. Each treatment experiment used 1,000 g of wet spent grain. The treated solids blown from the reactor for each experiment were collected separately. The slurry was centrifuged at 12,000 g for 20 min and the supernatant was sterilized at 105°C for 20 min.

Microorganisms and enzymes

Lactobacillus rhamnosus NBRC3532 and NBRC14710 were purchased from the National Institute of Technology and Evaluation (Japan). *Lactobacillus vaccinosteraeus* JCM1716 was purchased from the Japan Collection of Microorganisms.

Medium

The lactic acid bacteria were cultured on Leichmani liquid medium (0.85% yeast extract, 0.85% peptone, 1.1% glucose, 0.2% potassium dihydrogenphosphate, 0.37% tomato powder, 0.1% polysorbet 80, and 1.0% calcium carbonate in distilled water, Nissui Pharmaceutical Co. Ltd. Japan) and Leichmani agar medium (with 1.5% agar added).

Enzymes

Alpha-amylase (TC-3 from *Bacillus subtilis*, 27 U/mg) was obtained from the Daiwa Kasei Co. Ltd., Japan. Glucoamylase (Gluczyme NL4.2 from *Aspergillus oryzae*, 4.2 U/mg) was obtained from the Amano Enzyme Inc.,

Japan. Cellulase (Y-NC from *Aspergillus niger*; 36 U/mg) and hemicellulase (Macerozyme R-10 *Rhizopus* sp., 3 U/mg) were obtained from the Yakult Pharmaceutical Co. Ltd., Japan.

Immobilization

Immobilized lactic acid bacteria were prepared under aseptic conditions using glass beads (Siran beads, Schott GmbH, Germany). The seed cultures were prepared by inoculating a loopful of cells grown on Leichmani agar into 300 mL conical flasks containing 50 mL of Leichmani liquid medium, followed by incubation at 37°C for 3 days under static conditions. The 50 mL lots of the seed cultures were transferred to 1000 mL conical flasks containing 500 mL Leichmani liquid medium and 200 mL of glass beads. For the growth of lactic acid bacteria and their immobilization onto glass beads, the mixture was incubated at 37°C for 3 days under static conditions. Free lactic acid bacteria grew on the carrier.

Fermentation

Fermentation by the free lactic acid bacteria was as follows. The seed cultures were prepared by inoculating a loopful of cells grown on a Leichmani agar plate into a 15 mL test tube containing 10 mL of the Leichmani liquid medium, followed by incubation at 37°C for 3 days under static conditions. One millilitres of the seed culture was transferred to a 300 mL conical flask containing 100 mL of the liquefied spent grain and fermented at 37°C under static conditions.

Fermentation by immobilized lactic acid bacteria was as follows: 100 mL of immobilized lactic acid bacteria was placed into a 300 mL conical flask containing 70 mL of liquefied spent grain that contained the commercial enzyme. The mixture was incubated at 37°C under static conditions.

Repeated batch production of L-lactic acid was examined with liquefied spent grain at 37°C. The liquefied spent grain solution was replaced after 4 days, the immobilized lactic acid bacteria washed twice with 50 mL of sterilized water at 15°C and pressed to extract liquid from the carrier. Liquefied spent grain solution was then added to the immobilized lactic acid bacteria.

Analyses

Total sugar concentration was measured by the phenol-sulphuric acid method¹. Sugars were quantified by high performance liquid chromatography with pulsed amperometric detection using a DIONEX Bio-CL gradient pump and a DIONEX CarboPac PA-1 column equipped with a DIONEX Model PAD 2 detector. Organic acids were measured by high-performance liquid chromatography (HPLC). The HPLC apparatus was a Waters (Fraction-Lynx System, Massachusetts). The effluent for organic acid analysis was monitored at a wavelength of 210 nm for the detection of carboxyl groups. Organic acids were separated on a 300 × 7.8 mm HPLC organic acid column (HPLC). The column temperature was kept at 40°C. The HPLC system also included an ion exclusion microguard column that was connected to the analytical column to remove sample contaminants. Analyses were performed isocratically at a flow rate 1.0 mL/min at 20°C. The mo-

bile phase was 0.1% H₃PO₄. L-lactic acid and D-lactic acid concentration were measured by enzymatic assay (F-kit, R-Biopharm GmbH, Germany), respectively.

RESULTS AND DISCUSSION

Preparation of liquefied spent grain

Steam explosion generally refers to the process of exposing fibres to high-pressure steam and then explosively discharging the product to atmospheric pressure¹⁴. Wet spent grain was liquefied by this steam explosion treatment. To clarify the effect of steam explosion conditions on the production of sugars, wet spent grain was treated under various pressures and times. The percent water content of the spent grain was 78.5% (w/w) and the concentration of total sugars extracted from the wet spent grain was 0.5% (w/v). Fig. 1 shows the effect of the pressure treatment on the production of soluble sugars from wet spent grain. One kg of wet spent grain was treated under the various pressures for 10 min. As shown in this figure, the highest total soluble sugar quantities were achieved with experiments performed under the 20 kg/cm² of pressure for 10 min. However, with an increase in the pressure of steam from 10 kg/cm² to 30 kg/cm², the recovery liquid volume also increased. Fig. 2 shows the effect of treat-

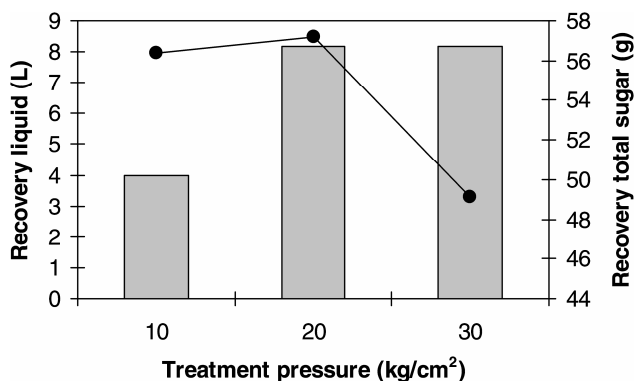


Fig. 1. Effect of treatment pressure on production of soluble sugar from wet spent grain: ●, Recovery total sugar; filled column, Recovery liquid.

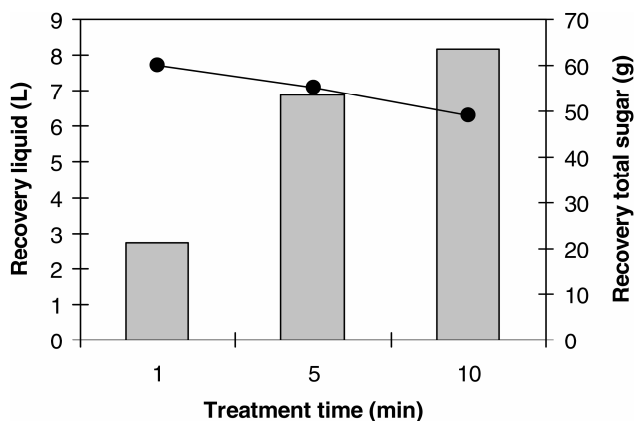


Fig. 2. Effect of treatment time on production of soluble sugar from wet spent grain: ●, Recovery total sugar; filled column, Recovery liquid.

Table I. Effect of various enzymes on production of sugars using liquefied spent grain.

Run no.	Enzymes	Glucose (w/v %)	Xylose (w/v %)	Arabinose (w/v %)
1	Cellulase	0.49	0.43	0.3
2	Hemicellulase	0.48	0.23	0.27
3	Glucoamylase	1.1	0.23	0.27
4	α -Amylase	0.2	0.15	0.23
5	Cellulase, hemicellulase	0.86	0.44	0.3
6	Cellulase, hemicellulase, glucoamylase	1.5	0.5	0.3
7	No addition	0.2	0.1	0.2

Table II. Production of L-lactic acid with lactic acid bacteria and saccharifying enzymes.

Lactic acid bacteria	L-Lactic acid (g/L)	D-Lactic acid (g/L)	Acetic acid (g/L)
<i>Lactobacillus vaccinostraeus</i> JCM 1716	8.3	4.2	7.2
<i>Lactobacillus rhamnosus</i> NBRC 3532	8.2	0.2	1.6
<i>Lactobacillus rhamnosus</i> NBRC 14710	14.5	0.2	1.6
Control ^a	N.D. ^b	0.2	1.6

^a Control; liquefied spent grain

^b N.D.; Not detected

ment time on production of soluble sugars from wet spent grain. These experiments were carried out independently. As shown in Fig. 2, 60 g of total sugar was obtained when 1 kg of wet spent grain was treated under 30 kg/cm² pressure for 1 min. On the other hand, when the wet spent grain was treated under the 30 kg/cm² pressure for 10 min, the total sugar recovery was reduced compared to the 1 min treatment. In the present experiments, an increase in the treatment time from 1 min to 10 min, also increased the recovery liquid volume. It was noted that the moisture content was higher under the high pressure in the reactor compared to the low pressure, so final liquid recovery volume was higher. The optimal steam explosion treatment was 30 kg/cm² for 1 min.

Effect of various enzymes on production of sugar

In order to improve the productivity of sugars from the liquefied spent grain, the effect of enzyme treatment on sugar production was further investigated. Liquefied spent grain was prepared under the optimum conditions by steam explosion treatment and the concentration of total sugars was 3.6% (w/v) in this experiment. Additional various enzymes were added to the liquefied spent grain solution and incubated at 30°C for 3 days. Each enzyme preparation was added to the liquefied spent grain solution at a concentration of 0.1% (w/v). As shown in Table I, additional enzymes produced glucose, xylose and arabinose from the liquefied spent grain. Of note was the 1.1% (w/v) glucose produced when liquefied spent grain was treated with glucoamylase (TC-3). The TC-3 hydrolysed the starch that remained in the spent grain and also hydrolysed cellulose. It was speculated that TC-3 included a side activity such as cellulase because the enzyme preparation was crude. Furthermore, glucose, xylose, and arabi-

nose were produced, 1.5%, 0.5%, and 0.3% (w/v) respectively, when the liquefied spent grain was treated with glucoamylase, cellulase, and hemicellulase.

Production of L-lactic acid with lactic acid bacteria and saccharifying enzyme

L-lactic acid production was investigated with various lactic acid bacteria using saccharifying enzymes from liquefied spent grain. L-lactic acid was produced by simultaneous saccharification and fermentation. The glucoamylase, cellulase, and hemicellulase were used as saccharifying enzymes in this investigation and three types of lactic acid bacteria were used. The batch production of L-lactic acid was carried out under the following conditions: total sugar concentration of liquefied spent grain was 4.0% (w/v); temperature was 30°C; glucoamylase, cellulase, and hemicellulase preparation were added to the liquefied spent grain solution at a concentration of 0.1% (w/v) respectively. The enzymes were added simultaneously as the Lactic acid bacteria were inoculated. As shown in Table II, *Lactobacillus rhamnosus* NBRC 14710 produced more L-lactic acid compared to the other Lactic acid bacteria. *Lactobacillus vaccinostraeus* JCM 1716 produced not only L-lactic acid but also produced D-lactic acid and acetic acid. On the other hand, *Lactobacillus rhamnosus* NBRC 14710 produced only L-lactic acid. This lactic acid bacteria was isolated from the silage of sweet sorghum (*Sorghum bicolor*)^{11,12}. Lactic acid can exist as one of two different chemical structures, called the L- and D-forms. They are mirror images of each other. Fermentation with *L. rhamnosus* NBRC14710 was a cost-effective way of producing high-grade lactic acid only in the L-form. Thus *L. rhamnosus* NBRC 14710 was chosen as the bacteria of choice for lactic acid production from spent grains due to these desirable characteristics.

Effect of temperature on production of L-lactic acid

The effect of temperature on the production of L-lactic acid was investigated with liquefied spent grain. The batch production of L-lactic acid was carried out under the following conditions: the total sugar concentration of liquefied spent grain was 4.0% (w/v); the temperature

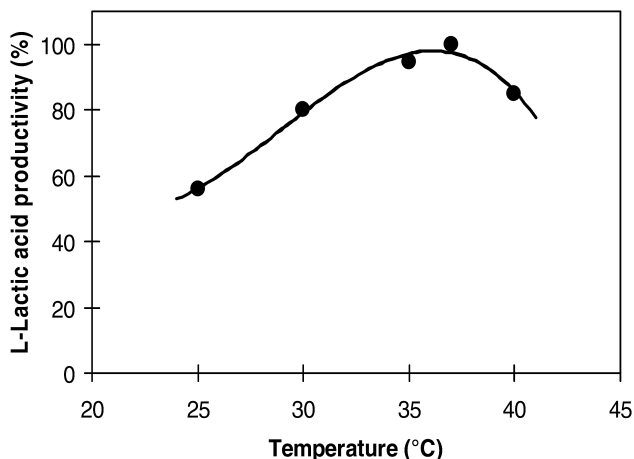


Fig. 3. Effect of temperature on production of L-lactic acid.

was varied from 25°C to 40°C; glucoamylase, cellulase, and hemicellulase preparations were added to the liquefied spent grain solution at a concentration of 0.1% (w/v), respectively. As shown in Fig. 3, L-lactic acid productivity was increased when the temperature increased from 25°C to 37°C. However, when the temperature was increased beyond 37°C, L-lactic acid productivity gradually decreased. The optimum temperature of each enzyme was over 45°C. The optimum temperature for L-lactic acid production by *L. rhamnosus* NBRC 14710 was reported previously as 37°C¹¹. It was considered that this temperature was the optimal temperature range for L-lactic acid production by these bacteria.

Effect of additional Tween 80 on production of L-lactic acid

In order to stimulate the productivity of L-lactic acid from liquefied spent grain, the effect of additional Tween 80 on the production of L-lactic acid was investigated. Tween 80 is known to be growth factor for lactic acid bacteria⁹. The batch production of L-lactic acid was carried

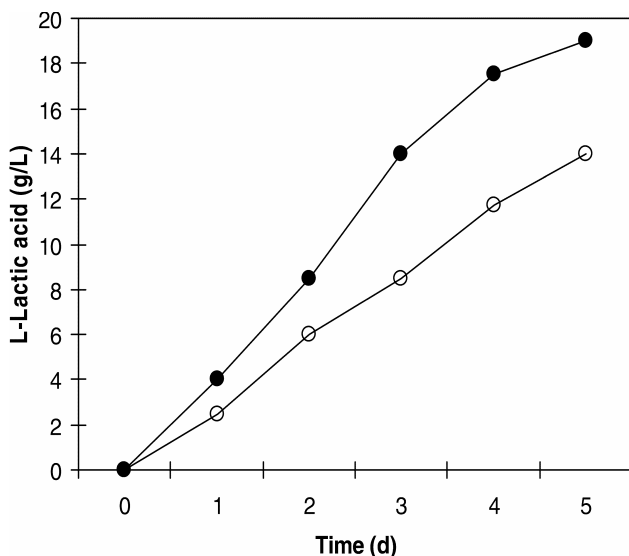


Fig. 4. Effect of additional Tween 80 on production of L-lactic acid: ●, additional Tween 80 liquefied spent grain; ○, normal liquefied spent grain.

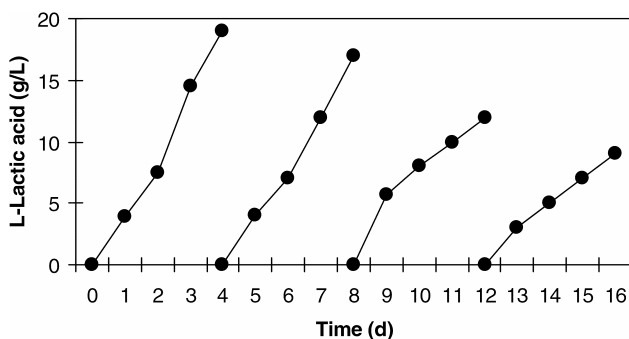


Fig. 6. Repeated batch production of L-lactic acid with immobilized *Lactobacillus rhamnosus* NBRC14710 from normal liquefied spent grain.

out under the following conditions: the total sugar concentration of liquefied spent grain was 4.0% (w/v); the temperature was 37°C; glucoamylase, cellulase, and hemicellulase preparation were added to the liquefied spent grain solution at a concentration of 0.1% (w/v), respectively, the concentration of Tween 80 was 0.5% (w/v). As shown in Fig. 4, when *Lactobacillus rhamnosus* NBRC 14710 was incubated in additional Tween 80 medium, the L-lactic acid production rate was higher compared to the normal medium. Furthermore, the amount of L-lactic acid produced was 1.4-fold higher in the additional Tween 80 liquefied spent grain compared to the normal liquefied spent grain after 4 days. Oh *et al.*⁹ reported that the growth of *L. casei* YIT 9018 was strongly affected by Tween 80. Goldberg *et al.*³ reported that the antibiotic cerulenin

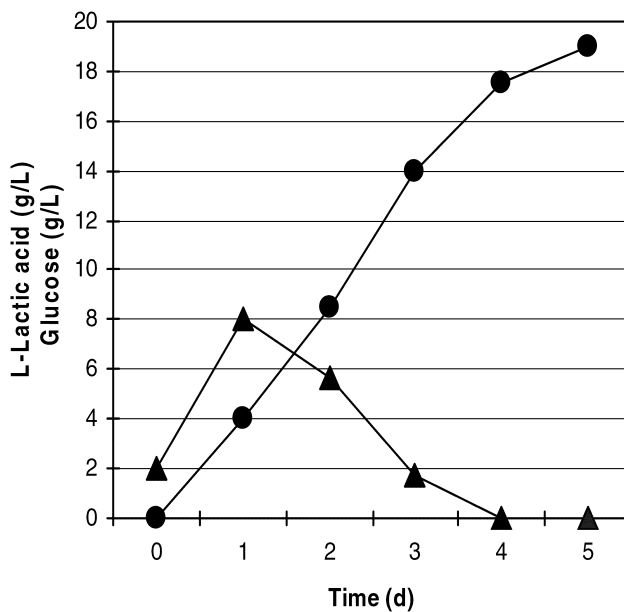


Fig. 5. Time course of lactic acid production and glucose consumption from additional Tween 80 liquefied spent grain: ● - L-lactic acid concentration; ▲ glucose concentration.

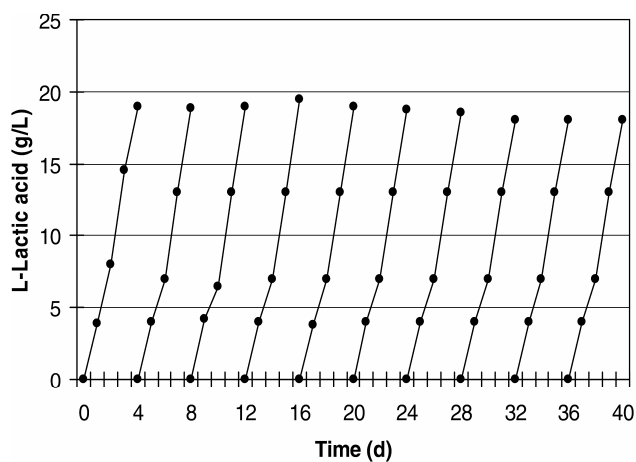


Fig. 7. Repeated batch production of L-lactic acid with immobilized *Lactobacillus rhamnosus* NBRC14710 from additional Tween 80 liquefied spent grain.

markedly inhibited the growth of lactic acid bacteria in tomato juice medium, but had almost no effect on the growth of the bacteria in tomato juice medium containing Tween 80. It was observed from experiments reported in this paper that L-lactic acid productivity of *L. rhamnosus* NBRC 14710 from liquefied spent grain was strongly affected by the presence of Tween 80. Fig. 5 shows the time course of lactic acid production and sugar consumption. By day 5, 19 g/L L-lactic acid was produced. Glucose concentration increased from 2 g/L to 8 g/L during 1 day. However, glucose concentration decreased sharply after 1 day since the rate of lactic acid production was faster than the rate of production of glucose.

Repeated batch production of L-lactic acid with immobilized *Lactobacillus rhamnosus*

Repeated batch production of L-lactic acid was examined with liquefied spent grain at 37°C. As shown in Fig. 6, during the first batch of production, 19.0 g/L of L-lactic acid was produced after 4 days. In the third batch production, however, the production rate started to slow during the latter half of incubation, while in the fourth run the production rate was markedly slower. On the other hand, repeated batch production was carried out using liquefied spent grain with 0.5% (w/v) of Tween 80. As shown in Fig. 7, during repeated batch production, the productivity of L-lactic acid was maintained at a level that was 10 times higher over a period of 40 days. It was concluded that continuous production of L-lactic acid could be stably obtained for more than 40 days using this system. It was observed that the Tween 80 was necessary for the stable production of L-lactic acid with immobilized *L. rhamnosus* NBRC 14710.

It can therefore be concluded that the L-lactic acid production system with immobilized *L. rhamnosus* NBRC 14710, using an explosion treatment, is a useful method for the production of lactic acid from a food waste.

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