

M 97052860

ANL/ES/CP--91789

CONF-9705112--2

**AN INTEGRATED BIOCONVERSION PROCESS FOR THE
PRODUCTION OF L-LACTIC ACID FROM STARCHY FEEDSTOCKS**

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Presented at the

19th Symposium on Biotechnology for Fuels and Chemicals

Colorado Springs, Colorado

May 4-8, 1997

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Keywords: Lactic acid, Starch, Simultaneous saccharification and fermentation

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AN INTEGRATED BIOCONVERSION PROCESS FOR THE PRODUCTION OF L-LACTIC ACID FROM STARCHY FEEDSTOCKS

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SUMMARY

The potential market for lactic acid as the feedstock for biodegradable polymers, oxygenated chemicals, and specialty chemicals is significant. L-lactic acid is often the desired enantiomer for such applications. However, stereospecific lactobacilli do not metabolize starch efficiently. In this work, Argonne researchers have developed a process to convert starchy feedstocks into L-lactic acid. The processing steps include starch recovery, continuous liquefaction, and simultaneous saccharification and fermentation. Over 100 g/L of lactic acid was produced in less than 48 h. The optical purity of the product was greater than 95%. This process has potential economical advantages over the conventional process.

INTRODUCTION

Lactic acid (2-hydroxypropionic acid), being an acid and alcohol, is a versatile organic chemical. In addition to its current uses (mostly in food and food-related applications), lactic acid has a huge potential market as a feedstock for the synthesis of specialty and commodity biodegradable plastics, oxychemicals, and "green" solvents (1). Lactic acid exists as two enantiomers: L(+)-lactic acid and D(-)-lactic acid. L-lactic acid is the natural form in human metabolism. D-lactic acid is metabolized differently by humans and has been reported to cause illness in infants (2). Lactic acid can be made chemically from hydrogen cyanide and acetaldehyde or via fermentation of carbohydrates. The chemical synthesis routes make only the

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racemic lactic acid, whereas a whole range of product optical purity (from nearly 100% D to racemic to nearly 100% L) can be made via fermentation (3), although the L-form is the desired product in most commercial lactic acid fermentation processes.

Carbohydrate metabolism of microorganisms for lactic acid production has been reviewed recently (4). Although lactobacilli use simple sugars (such as glucose and lactose) efficiently, the preferred industrial *Lactobacillus* strains do not metabolize starch effectively. Although amylolytic lactobacilli and other lactic acid organisms, such as *Rhizopus oryzae* and *Bacillus laevolacticus*, have been reported, they typically suffer from such problems as lower fermentation rates, lower product yields, lower product concentration, and undesirable product stereospecificity. When starch is to be used as the carbon source for lactic acid fermentation using lactobacilli, the starch can be hydrolyzed into glucose before fermentation by means of well-established processes, such as the two-step enzymatic hydrolysis process widely practiced by the corn wet milling industry at a very large scale to produce glucose syrup from corn starch. Such a process may not always be suitable for other starchy feedstocks, especially if a dedicated hydrolysis plant is needed at a smaller scale. In this work, an economical process to produce L-lactic acid from starchy feedstocks has been developed by using potato as a model starchy feedstock. This process consists of simplified starch-recovery steps, continuous liquefaction of the starch, and simultaneous saccharification and fermentation (SSF) with high-performance *Lactobacillus* species.

MATERIALS AND METHODS

Enzymes and Microorganisms

α -Amylase (G-ZYME G995) and glucoamylase (G-ZYME G-990) were obtained from Enzyme Bio-Systems (Englewood Cliffs, NJ). The *Lactobacillus* culture LBM5 has been described previously (3,5).

Starch Recovery

Idaho potatoes were purchased from a local grocery store and were peeled and cut into small pieces (around 4 x 4 x 4 cm). Potato wastes were obtained from a potato processing plant. The potato wastes consisted of variable sizes of cut pieces and were partially gelatinized because of steam peeling in the plant. The raw potato materials were disintegrated by using a Waring blender (Model CB-3), a hammer-mill type disintegrator (Dynacrush Soil Crusher, Custom Laboratory Equipment Inc., Orange City, FL), or pestle and mortar. One to two liters of water were added to one kilogram of potatoes for disintegration in the Waring blender. The blended potato and water were screened through a 20-mesh sieve and transferred to a 4-L beaker for starch settling. Potato starch powder was prepared by drying the wet starch under vacuum ($p = 140$ mmHg) at 50°C overnight and crushing the material by using a pestle and mortar. The potato starch recovered in our laboratory was compared with a commercial potato starch (Sigma, Catalog No. S 4251) by using enzymatic hydrolysis, as described below. Starch solutions were prepared as 1.656% dry substance (DS) in phosphate buffer. Three hundred forty units of α -amylase were added to 3 mL of starch solution. Reaction conditions for liquefaction were pH 6.5 and 65°C for 3 min. For saccharification of the starch, the pH of the liquefied starch was adjusted to 5 by adding 0.5 N HCl. Four units of glucoamylase were added to the liquefied starch, and saccharification was carried out at 65°C for 1 h.

Starch Liquefaction

Continuous liquefaction experiments using either the commercial potato or the potato starch recovered in our laboratory from Idaho potatoes were performed in a continuously stirred, 500-mL tank reactor (working volume). One liter of starch solution containing 248.4 g/L DS supplemented with 70 mg/L CaCl_2 was prepared. The pH of the starch solution was adjusted to 6.5, and 675 units of α -amylase was added. The starch solution was pumped at a flow rate of 25 mL/min into a 4.5-mm-i.d. x 91-cm stainless-steel tube immersed in a heating medium at a

temperature of 102°C and subsequently transferred into the reactor. The residence time in the gelatinization tubing was 35 s. The reaction temperature and residence time for liquefaction were controlled at 95°C and 20 min, respectively.

In the plug flow liquefaction experiment, a jacketed glass column (2.5 cm i.d. x 30 cm) was used as the reactor instead of the stirred tank reactor. This reactor was operated with or without packing materials. A dilute starch slurry recovered from potato waste without centrifugation was used in this experiment. One liter of starch solution containing 120 g/L dry substance supplemented with 70 mg/L CaCl₂ was prepared. The pH of the starch solution was adjusted to 6, and 338 units of α -amylase was added. The starch solution was pumped at a flow rate of 7 mL/min into a 4.5-mm i.d. x 91-cm stainless-steel tube immersed in a heating medium at a temperature of 103.5°C and subsequently transferred into the reactor. The reaction temperature was controlled at 103.5°C.

Samples were collected from the reactor effluent. The samples were quenched in ice water and diluted (1:10) with 0.1 N HCl solution to deactivate enzymatic activities. Concentrations of total reducing sugar in the samples were measured. Dextrose Equivalent (DE), defined as the concentration of total reducing sugar (as glucose) as a percentage of dry substance, was calculated.

Simultaneous Saccharification and Fermentation

Precipitated starch granules recovered from potatoes as described above were used. A combination of 218 mL of water and 435 units of α -amylase were added to 363 g of a 40.2% dry substance (DS) potato starch suspension, in a 2-L BioFlo IIc fermentor (New Brunswick, Edison, NJ). The fermentor was autoclaved at 110°C for 40 min for liquefaction. Nutrient solution, which was autoclaved separately, was prepared by mixing 67.5 mL of corn steep liquor, 254.5 mL of water, and 11.5 mL of 10 N sodium hydroxide to adjust pH to 7.0. A 400 mL sterile water and nutrient solution were added to the fermentor containing the liquefied starch.

The *Lactobacillus* inoculum, LBM5, was grown overnight at 42°C. At the time of the inoculation and 24 h, 50 units each of glucoamylase was injected to the fermentor. The pH of the medium was 6.4 initially and later controlled at 5.5 by adding 5 N sodium hydroxide. The temperature and agitation speed were set at 42°C and 200 rpm, respectively.

Assays

The DS of the starch products was measured as % (w/w) solid remained after drying overnight at 110°C in this and following experiments. Total reducing sugars (TRS) were measured by the dinitrosalicylic acid method (6) and expressed as glucose. Methods for measuring glucose, lactic acid, and L-lactic acid were described previously (3).

RESULTS AND DISCUSSION

Starch Recovery

Results of starch recovery by using the Waring blender for disintegration are summarized in Table 1. Gelatinization was found to affect starch recovery. Without gelatinization, most of the starch precipitated as starch granules in 1 to 2 h, forming a bottom layer of about 40% (w/w) dry substance. When gelatinization was significant, either before or during potato disintegration, starch did not settle quickly, and instead of forming starch granules, a 10—15% (w/w) dry-substance starch slurry was formed in the settling tank. This starch slurry could be concentrated to 25—45% (w/w) dry substance by using a centrifuge. Also, the mechanical energy input during disintegration was found to be an important process parameter. An optimum range of the energy input, at 0.02 to 0.03 kWh/kg potato for the blender, led to complete disintegration and a high settling rate. When energy input was too low, disintegration was incomplete; when energy input was too high, gelatinization occurred and the starch did not settle quickly.

Blending 1 kg of fresh cut and peeled potato and 2 L of water at high speed for 5 min (i.e., energy input = 0.053 kWh/kg) resulted in partial gelatinization, and no starch granules were precipitated after 2 h of settling. Instead, 1.6 L of a dilute starch slurry was obtained, which was concentrated to 0.58 L of starch mud at 30% (w/w) dry substance after centrifugation at 1,400 G for 15 min. When the same potato and water mixture was blended for 1 min at low speed and for 2 min at medium speed (i.e., total energy input = 0.024 kWh/kg), the blended mixture settled and formed three layers. The top layer (2 L) was a dark brown liquid (including some floats). The middle layer (1 L) was a dark brown slurry (10—12 % [w/w] dry substance). The middle layer seemed to contain proteins and a gelatinized portion of starch. The bottom layer contained white starch granules (40% [w/w] dry substance). The volume of the starch layer was 175 mL, and its density was 1.2 g/mL. When the starch product from the bottom layer was diluted with water to 25% (w/w) dry substance, the slurry could be pumped by a peristaltic pump. The starch recovery yield from the bottom layer was 62%, assuming 12% starch in potatoes. More starch could be recovered from the middle layer as slurry.

When 1 kg of potato waste was blended with 1 L of water for 1 min at low speed followed by 1 min at medium speed (i.e., total energy input = 0.015 kWh/kg), in two hours of settling, 1,500 mL of slurry was obtained. In the subsequent centrifugation of the slurry at 2,500 rpm (1,400 G) for 30 min, 800 g of the concentrated starch mud was recovered. The dry substance of the starch mud was measured as 25% (w/w). When the same potato waste and water mixture was blended for 1 min at low speed and then for 2 min at medium speed (i.e., total energy input = 0.024 kWh/kg), in two hours of settling, 1,200 mL of potato slurry was obtained. In the subsequent centrifugation at 2,500 rpm (1,400 G) for 30 min, 658 g of the concentrated starch mud was recovered. The dry substance of the starch mud was measured as 26.0% (w/w). After enzymatic hydrolysis, the starch samples obtained from potato waste at 0.015 kWh/kg of blending energy input yielded less glucose indicating insufficient disintegration. The hydrolysis results of all other samples of recovered starch were comparable with those of the commercial potato starch.

Normal starch granules have a helical structure, which permits individual molecules to be packed together closely and accumulated as a concentrated deposit in water. This behavior, called retrogradation, is a desired property for the separation and concentration of starch granules. Starch is gelatinized when it absorbs excess water at a high temperature. Gelatinized starch is soluble in water and cannot be recovered as starch granules. Mechanical degradation of starch is known to result in the formation of branched starch, which increases the amorphous region of the granules and decreases the retrogradation capability. Besides possible mechanical degradation, the high mechanical energy can increase temperature, which may increase gelatinization and thereby decrease starch recovery in the starch-separation stage. Adding water, from 1 to 2 L/kg potato, to the blender improved the starch recovery yield, compared with blending without additional water. Water probably worked as a lubricating agent and helped to dissipate the heat generated by mechanical energy.

Using the hammer-mill-type crusher and grinding the potato with pestle and mortar both produced comparable starch yields and concentrations, but with two apparent advantages: (1) foaming was reduced and no antifoam was needed in the settling tank, and (2) the crushed or ground potato passed through the sieve faster than the blended potato with effective removal of fibrous materials.

Starch Liquefaction

For continuous liquefaction in the stirred tank reactor, a steady state was achieved using the commercial potato starch in 140 min (obtaining 25 g/L TRS or 10 DE), whereas by using the potato starch prepared in our laboratory, the steady-state sugar concentration was 20 g/L TRS or 8 DE. For continuous liquefaction of starch recovered from potato waste in the plug flow reactor (PFR), the use of packing material (ceramic Berl saddle or Rashig ring) partially clogged the reactor with gelatinized starch. Even without packing material, bubbles of the boiling starch solution helped achieve efficient mixing. Continuous liquefaction was, thus, performed without packing material, and a liquefied starch of 10 DE was produced at the steady state. In this

experiment, the feed (starch slurry recovered from potato wastes) contained greater than 10^5 colony-forming-unit/mL microbial contaminants that were able to grow at 37°C and 42°C on *Lactobacilli* MRS (Difco, Detroit, MI) plates. The liquefied starch (a residence time of 20 min in the reactor at 103.5°C) contained some microbes that grew at 37°C , but no colony growth was observed at 42°C on MRS plates. These findings suggest that the liquefied starch prepared by using the above procedure can be used for lactic fermentation at 42°C without further sterilization.

Simultaneous Saccharification and Fermentation

The time course of the simultaneous saccharification and fermentation is shown in Figure 1. During the run, the liquefied starch was saccharified to glucose by glucoamylase, and the glucose was metabolized by microorganisms and converted into lactic acid. Initially, the saccharification rate was greater than the fermentation rate, resulting in an accumulation of sugars. The fermentation ended at around 30 h, at which time the concentration of glucose was lower than 0.5 g/L and the lactic acid concentration was 100 g/L. The fermentation rate was comparable with those of lactic acid by the same organisms under similar conditions using purer glucose. Using the crude starch prepared in this study seemed to yield a slightly higher fermentation rate. The starch recovered by blending and settling in this work contained about 0.025 g protein/g starch. This finding suggests that the simplified starch-recovery process, which generates a starch product that retains some of the potato proteins, may be more suitable for providing the carbon substrate for lactic acid fermentation than the typical intensive starch-recovery processes that make highly purified starch.

The lactic acid yield from starch was essentially 100% of theoretical yield, as predicted from the dry substance of the raw material, assuming that the corn steep liquor contained 25% (dry basis) lactic acid initially. The optical purity of lactic acid at the end of fermentation was 95% L-lactic acid. This LBM5 culture has routinely produced greater than 98% L-lactic acid in

our laboratory in fermentations using a well-defined medium not containing corn steep liquor (3). The D-lactic acid introduced by the corn steep liquor used in the fermentation medium caused the slight decrease in product stereospecificity in this run.

The glucoamylase used in this process had an optimal pH at 4.3 and is typically used at 60°C in industrial dextrose production processes. These conditions are considerably different from the optimal pH, 5.5, and temperature, 42°C (7), for lactic acid fermentation by the LBM5 culture. Nevertheless, the glucoamylase exhibited satisfactory activity at the process conditions. Furthermore, the glucoamylase activity was not appreciably inhibited by the constituents of corn steep liquor or impurities in the recovered crude starch. Although the total glucoamylase dosage in this run was 0.56 unit/g•lactic acid produced, the second dosage of glucoamylase added at 24 h could be reduced or eliminated.

Commercial-Scale-Process Scheme

On the basis of the laboratory results, an integrated process suitable for commercial-scale production of L-lactic acid from starchy feedstocks was designed; the process flow diagram is shown in Figure 2. The process consists of starch recovery, continuous liquefaction, and simultaneous saccharification and fermentation. Although the process described below involves using potatoes, other root and tuberous, starchy crops, such as cassava, could also be used.

The potato raw material is fed into a disintegrator (such as a ball mill or a hammer mill). When a high-fiber potato feed is used, a Rietz mill (a type of hammer mill) is preferred for separation of a large portion of fibrous materials as a secondary discharge. The disintegrated potato is screened to remove large particles on a mechanically vibrated woven wire or plastic texture, with the Tyler equivalent from 10 to 100 mesh. Water is sprayed over the screen to entrain starch. The starch is concentrated from the dilute starch suspension in a settling tank or a centrifuge. While a settling tank is used for precipitating starch granules from normal (unprocessed) potato starch in a 1—2-h holding time, a centrifuge is used for concentrating the

starch slurry from gelatinized starch wastes. A centrifuge can be used even for the normal potato starch to enhance the separation rate. Most of the supernatant water is recycled to the screen, and part of it is purged. If wet milling is desired, the make-up water is fed into the mill. The starch concentrator (i.e., settling tank or centrifuge) generates a crude starch product at a dry substance concentration from 10 to 45% (w/w) as needed. The starch products are diluted to 15—25% dry substance for gelatinization and liquefaction.

Complete gelatinization is achieved at a minimal ratio of water to starch of 4. Gelatinization causes the change of the retrograded starch structure, which is resistant to enzyme attack, into an amorphous pattern that is accessible to the enzymes required for degradation. Gelatinization is not completed for a starch concentration exceeding 25% (w/w dry substance). If the pH of the starch is far from the optimum pH of the α -amylase to be used for liquefaction, the pH should be adjusted before gelatinization. The recovered starch is mixed with α -amylase and then preheated at 90—120°C for no longer than one minute in a line-mixer. Gelatinization occurs during the preheating. Mixing and preheating can be practiced in a single unit, but the fluid must not be heated before mixing is completed to ensure that α -amylase is distributed uniformly. The gelatinized starch is fed into a liquefaction reactor, which can be a PFR or a continuous stirred tank reactor (CSTR) (preferably a CSTR). The residence time for starch liquefaction is from 15 min to 3 h; preferably 20 min when 3 units α -amylase/g dry substance are used. Calcium concentrations of 30—75 ppm on a dry-substance basis are required for α -amylase use. The optimum residence time varies with the dosage of the enzyme. With a suitable residence time, the continuous liquefaction process should generate a solution of short-chain starch molecules, with a typical DE value of 10. The liquefied starch is cooled or heat-exchanged with the feed and fed into the fermentor without further sterilization.

The fermentor is operated in a batch mode. Initially, the fermentor is filled with the liquefied starch, sterilized corn steep liquor, and additional water if the total carbohydrate

concentration needs to be adjusted. Inoculum culture (at 5—10% of the working volume of the production fermentor) and corn steep liquor (ranging from 10 to 50 g/L [dry basis], preferably 10g/L) are added. The higher concentration of corn steep liquor increases the fermentation rate, but it also increases the concentration of impurities in the fermentation broth, which increases the purification cost in the downstream process. Suitable microorganisms include *Lactobacillus delbrueckii* subsp. *lactis*, the LBM5 culture used in this work, and many other L-specific lactobacilli reported in the literature. Fermentation can be carried out at 37—45°C (preferably at 42°C). The pH of the medium is adjusted at 6.3 initially. The pH is allowed to drop and is controlled at 5.5 by adding 5—10 N sodium hydroxide. Because of the high temperature and acidic pH, this fermentation is highly resistant to microbial contamination. At the time of inoculation, glucoamylase is added to the fermentor aseptically to effect simultaneous saccharification and fermentation. Glucoamylase dosage is typically 0.3 units/g starch. If necessary, more glucoamylase is added at timed intervals. The fermentation is completed in 25—40 h if 10% carbohydrates are present initially in the medium. Cell mass is separated from the fermentation broth in a filter or a centrifuge. The cell-free broth containing lactate and impurities is transferred to a downstream process where lactic acid is recovered and purified.

CONCLUSIONS

Simultaneous saccharification and fermentation have been used widely for ethanol production from starchy or cellulosic feedstocks (8). Recently, a simultaneous liquefaction, saccharification, and fermentation process was reported for L-lactic acid production from barley starch by using *Lactobacillus casei* and a medium containing yeast extract and peptone as nutrients (9). In this work, the integrated process covers starch recovery, continuous liquefaction, and simultaneous saccharification and fermentation. In addition, the feasibility of using an inexpensive nutrient source, corn steep liquor, for such a process is demonstrated, and a high optical purity (95% L) of the lactic acid product was obtained by using a stereospecific *Lactobacillus* culture. Other advantageous features of this process include the following:

- This process can use various types of potato feedstocks. Although starch recovery from the unprocessed potatoes is easier, the starch recovery steps in this process are capable of generating a suitable starch stream from partially gelatinized potato wastes.
- The simplified starch-recovery step used in this process, which generates a crude starch, is more suitable for supplying a low-cost carbohydrate for fermentation than the conventional potato starch manufacturing processes that involve many processing steps and generate highly purified starch (10,11). The crude starch prepared in this process is expected to be less expensive and can provide part of the fermentation nutrient requirements.
- The costs of enzyme required for this process are low — they are estimated at less than 0.5¢/lb of lactic acid produced. Although the α -amylase and glucoamylase are not used at their optimal conditions and the enzyme dosages used in this process are slightly higher than those used in typical corn wet milling operations, the costs of enzymes in this process are controlled at a reasonable level.
- This process uses proprietary *Lactobacillus* cultures that give a high fermentation rate (3 g/L·h), a high product concentration (over 100 g/L), a high product stereospecificity (95% L), and a high product yield (nearly 100%) in simultaneous saccharification and fermentation.
- This process is expected to be more economical by combining hydrolysis with fermentation. Instead of the conventional two-step hydrolysis of starch followed by fermentation, the liquefied starch is fed into the fermenter for simultaneous saccharification and fermentation, eliminating the sterilizer and the saccharification tank and reducing the overall process time, the capital investment, and the operating costs.

ACKNOWLEDGMENT

The work reported here was supported by the U.S. Department of Energy, Assistant Secretary for Energy Efficiency and Renewable Energy, under Contract W-31-109-Eng-38.

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Legends

Figure 1. Time course of a batch simultaneous saccharification and fermentation run.

Figure 2. Process flow diagram of the integrated process for L-lactic acid production from starchy feedstocks.

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Table 1. Results of Starch Recovery Using a Blender for Disintegration

Raw Materials	Fresh Potatoes		Potato Wastes		Commercial Potato Starch	
Energy Input during Blending (kWh/kg potato)	0.053	0.024	0.015	0.024	N/A	
Form of Recovered Starch	w/o centrifugation	Starch slurry at 11% (w/w)	Precipitated starch granules at 40% (w/w)	Starch slurry at 12% (w/w)	Starch slurry at 13% (w/w)	N/A
	w/ centrifugation	Starch mud at 30% (w/w)	N/A	Starch mud at 25% (w/w)	Starch mud at 26% (w/w)	N/A
Sugar Concentrations after Hydrolysis						
Glucose (g/L)	N/A	21.3	17.6	22.0	21.1	
TRS (g/L)	N/A	23.6	20.3	24.2	22.9	
Glucose/TRS	N/A	0.91	0.87	0.91	0.92	



