

# Production of Lactic Acid from Soybean Straw Using Immobilized *Lactobacillus casei* and Batch or Repeated-batch Fermentation

Ming Gao,<sup>a,b</sup> Juan Wang,<sup>a,b</sup> Zhong Xu,<sup>c</sup> Wenyu Zhang,<sup>a</sup> Chuanfu Wu,<sup>a,b,\*</sup> and Qunhui Wang<sup>a, b</sup>

Approximately 18 million tons of soybean straw are produced annually in China, with its disposal becoming an increasingly serious problem. Moreover, the Chinese government has banned the open burning of agricultural straw for environmental reasons. One potential solution is to use soybean straw for lactic acid (LA) production, avoiding open burning and waste. In this study, a Ca-alginate-immobilized *Lactobacillus casei* strain was used to produce LA from soybean straw enzymatic hydrolysate. Optimized conditions for the production of LA were initially established, consisting of Ca-alginate beads made from 2.5% sodium alginate with a diameter of 2 mm and a fermentation process using a 10% inoculum at 30 °C for 30 h. Lactic acid productivity was increased with only a slight decrease in overall yield by raising the initial sugar concentration from 8 g L<sup>-1</sup> to 35 g L<sup>-1</sup>. Finally, immobilized cells could be reused at least 10 times without a noticeable performance decrease. These results indicated that production of LA from soybean straw enzymatic hydrolysate is a sustainable and feasible method to utilize these resources.

*Keywords:* Lactic acid; Soybean straw; Cell immobilization; *Lactobacillus casei*; Repeated-batch fermentation

*Contact information:* a: Department of Environmental Engineering, University of Science and Technology Beijing, Beijing 100083, China; b: Beijing Key Laboratory on Disposal and Resource Recovery of Industry Typical Pollutants, University of Science and Technology Beijing, Beijing 100083, China; c: Department of Environmental Engineering, Harbin University of Commerce, Harbin 150028, Heilongjiang, China; \*Corresponding author: wuchuanfu83@163.com

## INTRODUCTION

Lactic acid (LA) is an important organic acid with a wide range of applications across the chemical, food, cosmetic, and pharmaceutical industries (Abdel-Rahman *et al.* 2011). Lactic acid has also attracted attention over the past two decades as a raw material for the manufacture of polylactic acid (PLA), a promising biodegradable plastic that can be used as an environmentally friendly alternative to petrochemical-derived plastics (Hassan and Idris 2016). Annual PLA production (0.45 million tons) remains a small proportion of total annual plastic production (200 million tons) in 2007 (Christensen *et al.* 2008). Further, in 2012, PLA contributed to more than 35% of the bioplastic market. However, the cost of PLA is still comparatively higher than synthetic plastics, thus becoming a primary restraining factor for the market growth (Kwan *et al.* 2018). This is partially because PLA production is restricted by the price of LA. This price is constantly increasing because LA is traditionally produced from glucose or starchy food materials (*e.g.*, potato, wheat, rice, cassava, and sorghum) (Juturu and Wu 2015). Lactic acid production therefore competes with the food and feed supply industries for raw materials,

contributing to the overall production cost. Indeed, it has been reported that the cost of raw material accounted for more than 34% of the total cost of LA production *via* fermentation (Åkerberg and Zacchi 2000). Therefore, utilizing less expensive raw materials and techniques has become a major focus for optimizing LA production. Previously, several studies have reported that it is possible to produce LA from waste or renewable materials, such as kitchen waste (Wang *et al.* 2009; Liu *et al.* 2013), agricultural residues (Zhang *et al.* 2014a,b; Akao *et al.* 2015), or industrial byproducts (Kulozik and Wilde 1999; Mazumdar *et al.* 2010; Shi *et al.* 2015). Using these waste materials to produce LA would not only lower the cost of production but also address the pressing environmental problems caused by the constant buildup of such waste.

Soybean is one of the major crops grown in China today, with approximately 12 million tons produced in 2014. Alongside the production of soybean, 18 million tons of soybean straw is also generated annually. Soybean straw is typically poorly utilized after production, and up to two thirds is burned. Apart from representing a large waste of resources, the open burning of soybean straw causes a substantial amount of air pollution and has therefore been banned by the Chinese government. Consequently, the disposal of increasing amounts of soybean straw has become a serious issue that has yet to be resolved. However, recent research has suggested that soybean straw can be used to produce useful materials. For example, soybean straw has been used to produce biochar for heavy metal adsorption (Tong *et al.* 2011; Qian *et al.* 2013) and to generate activated carbon for phenol adsorption (Miao *et al.* 2013). Like other lignocellulosic materials, soybean straw can also be hydrolyzed into soluble sugar and fermented to produce valuable chemicals or fuels, including bio-hydrogen (Han *et al.* 2012) and LA (Wang *et al.* 2015). Previous studies have shown that soybean straw can be hydrolyzed with ammonia solution, with the hydrolysate then fermented with *Lactobacillus casei* to produce LA. Using this preliminary system, a maximum LA yield of 0.8 g g<sup>-1</sup> and a productivity of 0.61 g L<sup>-1</sup> h<sup>-1</sup> were established (Wang *et al.* 2015). However, LA production using free cells in a batch fermentation process often suffers from low productivity due to the lag and exponential phases of cell growth. To improve this process and increase productivity, immobilizing cells has been suggested as a possible method to increase cell concentrations in fermenters and raise efficiency (Abdel-Rahman *et al.* 2013a).

Cell immobilization technology has been applied across numerous industries to protect cells from unfavorable environments, promoting continuous production. There are generally four types of immobilization techniques: adsorption, entrapment, containment, and self-aggregation (Pilkington *et al.* 1998). Entrapment using alginate gels is currently a popular technique due to several attractive characteristics, including high biocompatibility and porosity but low toxicity and leakage (Behera *et al.* 2012). More specifically, cell immobilization using Ca-alginate gel beads has been widely used to produce many important materials, such as ethanol (Duarte *et al.* 2013), xylitol (Carvalho *et al.* 2004), and LA (Boyaval and Goulet 1988). To date, LA production from renewable biomass has gained much attention around the world, especially from starchy and lignocellulosic residues. However, few researches have ever reported using raw soybean straw as feedstock for LA production. The aim of this study is to establish whether cell immobilization technology could be applied to improve the fermentation of soybean straw. This study used Ca-alginate gel beads to immobilize *L. casei* for LA production from soybean straw enzymatic hydrolysate. Several parameters were investigated to optimize fermentation conditions, including alginate concentration, Ca-alginate bead size, temperature, inoculum size, and initial sugar concentration. Finally, the study established

how many times alginate beads could be used repeatedly to assess their stability for LA production. This study therefore describes a system with the potential to efficiently produce economically important LA from soybean straw waste. This will contribute to increasing the raw materials available for the production of biodegradable plastic and also aid in avoiding the environmental issues that arise from the accumulation of soybean straw waste.

## EXPERIMENTAL

### Materials

#### *Preparation of soybean straw hydrolysate*

Soybean straw was supplied by a local processing factory (Nengjing food processing plant, Heihe, China), which contained 45.5% (w/w, based on dry weight) glucan, 12.1% (w/w, based on dry weight) xylan, and 19.0% (w/w, based on dry weight) klason lignin. Additionally, the moisture content of the raw soybean straw was 7.40% (w/w, based on wet weight). Raw soybean straw was firstly dried at 80 °C to a constant weight (no residual water). Dry soybean straw was milled and sieved (mesh size: 0.106 mm × 0.106 mm) into fine powder; then it was pre-treated with 10% ammonia (solid to liquid rate, 1:10 (w v<sup>-1</sup>)) at room temperature for 24 h. The mulch was then filtered through gauze, and the remaining residue was dried at 80 °C to a constant weight (no residual water). This material was used as the substrate for all subsequent enzymatic hydrolysis experiments. The hydrolysis of soybean straw was performed in a 250-mL conical flask containing 100 mL 0.05 mol L<sup>-1</sup> citric acid-sodium citrate buffered solution (pH 4.8) and 5% (w v<sup>-1</sup>) pre-treated soybean straw. Cellulase (15000 filter paper units (FPU) g<sup>-1</sup>; Wuxi Enzyme Factory, Jiangsu, China) was added to the flask with 50 FPU g<sup>-1</sup> straw, and hydrolysis was carried out at 50 °C for 36 h. The main reducing sugars present in the hydrolysate were glucose 10.59 g L<sup>-1</sup>, xylose 2.01 g L<sup>-1</sup>, and cellobiose 0.97 g L<sup>-1</sup>, with a total reducing sugar content of 0.271 g g<sup>-1</sup> straw. Reducing sugar concentrations were determined using a 3,5-dinitrosalicylic acid method (Miller 1959).

#### *Preparation of immobilized cells*

The *Lactobacillus casei* CGMCC 1.6 used in the study was obtained from the China General Microbiological Culture Collection Center, Beijing, China, and was maintained in de Man, Rogosa, and Sharpe (MRS) medium (Abdel-Rahman *et al.* 2013b). After pre-culturing on MRS medium for 24 h, *L. casei* cells were separated from the broth by centrifugation at 14000 g for 10 min at 4 °C, washed with sterile water three times, and then re-suspended using phosphate-buffered saline (PBS; 10<sup>10</sup> cells mL<sup>-1</sup>). Bacterial suspensions were added to the sodium alginate solution, stirred for 5 min, and then dropped into a sterile, continuously stirred 2% CaCl<sub>2</sub> solution using a syringe. During the process, the alginate drops solidified upon contact with CaCl<sub>2</sub>, forming beads that immobilized the bacterial cells. The beads were allowed to harden for 1 h at 37 °C and then washed with sterile saline solution. To increase the activity of the immobilized cells, the beads were inoculated in enrichment medium at 15% (w v<sup>-1</sup>) and cultured at 30 °C for 24 h. The enrichment medium consisted of 30 g L<sup>-1</sup> glucose, 15 g L<sup>-1</sup> yeast extract, 10 g L<sup>-1</sup> peptone, 5 g L<sup>-1</sup> potassium phosphate monobasic, and 20 g L<sup>-1</sup> calcium carbonate dissolved in water adjusted to pH 6.0. After enrichment, the number of *L. casei* cells increased from 1.07 × 10<sup>9</sup> to 6.21 × 10<sup>12</sup> g<sup>-1</sup> in the Ca-alginate beads.

### *Fermentation conditions*

The fermentation medium was composed of 5 g L<sup>-1</sup> peptone, 5 g L<sup>-1</sup> yeast extract, 0.5 g L<sup>-1</sup> magnesium sulfate heptahydrate, 0.5 g L<sup>-1</sup> monopotassium phosphate, and 0.1 g L<sup>-1</sup> sodium chloride in 1 L of soybean straw enzymatic hydrolysate. The initial pH of the medium was adjusted to 6.0. Before fermentation, the flasks were flushed with nitrogen gas and sealed with rubber stoppers to maintain anaerobic conditions. The medium was sterilized at 115 °C for 15 min. Lactic acid fermentation was conducted in 250-mL conical flasks containing 100 mL sterilized fermentation medium and different quantities of immobilized cells in an incubator shaker (HZB-200, Yuejin instruments Corporation, Shanghai, China) with a rotational speed of 140 r min<sup>-1</sup>.

Immobilization and fermentation conditions of LA production from soybean straw hydrolysate were optimized by using Ca-alginate immobilized *L. casei* cell beans. The effects of immobilization conditions, such as sodium alginate concentrations and bead diameters, on LA production were conducted from the range of 1.5% to 3.0% (w v<sup>-1</sup>) and 1 to 3 mm in batch fermentation modes (30 °C; inoculum size, 10% (v v<sup>-1</sup>)), respectively. Further, the effect of fermentation conditions, such as temperature, inoculum size, and initial reducing sugar concentration, on LA production were investigated from the range of 25 °C to 40 °C, 5% to 20%, and 8.00 to 35.0 g L<sup>-1</sup> in batch fermentation modes (immobilized cell beads: sodium alginate, 2.5% (w v<sup>-1</sup>); diameter, 2 mm), respectively. To evaluate the reusable stability of Ca-alginate cell beads, repeated-batch fermentation was performed by using soybean straw hydrolysate as substrate (containing approximately 12.5 g L<sup>-1</sup> reducing sugar) under optional immobilization and fermentation conditions.

## **Methods**

### *Analysis of raw soybean straw composition*

The glucan, xylan, klason lignin content of the raw soybean straw were determined according to the National Renewable Energy Laboratory Analytical Procedure (Sluiter *et al.* 2008)

### *Analysis of LA production and yield*

The amount of LA was determined *via* high-performance liquid chromatography (HPLC; Shimadzu LC-20AT, Shimadzu Corporation, Kyoto, Japan) with Agilent SB-Aq columns (4.6 mm × 250 mm, 5 μm; Agilent Technologies, Santa Clara, CA, USA) and a Shimadzu SPD-20 ultraviolet absorption detector (flow rate of 0.7 mL min<sup>-1</sup> at 22 °C with 0.01 mol L<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub> as the mobile phase and a wavelength of 210 nm; Shimadzu Corporation, Kyoto, Japan). The lactic acid yield (g g<sup>-1</sup>) was defined as the ratio of LA produced (g L<sup>-1</sup>) to the reducing sugar that was consumed (g L<sup>-1</sup>). Lactic acid productivity (g L<sup>-1</sup> h<sup>-1</sup>) was calculated as the ratio of the highest LA concentration (g L<sup>-1</sup>) to the indicated fermentation time (h).

## **RESULTS AND DISCUSSION**

### **Optimization of Immobilization Conditions for LA Production**

#### *The effect of sodium alginate concentration on LA production*

As the concentration of sodium alginate used for preparing the beads may affect sugar consumption and subsequent LA production, the study first established the effects of different concentrations of sodium alginate to produce the beads.

It was hypothesized that there would likely be an important effect because different concentrations would alter the cross-linked structure of the Ca-alginate beads, affecting nutrient diffusion. In this study, four different sodium alginate concentrations were compared to determine the optimal concentration for LA fermentation. As shown in Table 1, a sodium alginate concentration of 2.5% led to the highest LA production ( $6.77 \text{ g L}^{-1}$ ), the highest yield ( $0.795 \text{ g g}^{-1}$ ), and highest productivity ( $0.226 \text{ g L}^{-1} \text{ h}^{-1}$ ). One hypothesis to explain the differences was that beads prepared from 1.5% and 2.0% sodium alginate were much softer and therefore more easily broken, leading to a leakage of the bacteria from the beads.

Conversely, beads produced at higher sodium alginate concentrations were likely too hard, with a more densely cross-linked structure. This would have consequent effects on the diffusion of nutrients and substrates to the *L. casei* cells. The beads prepared using 2.5% sodium alginate produced the highest concentrations of LA because their cross-linked structures facilitated the diffusion of nutrients. These results were consistent with those obtained by Idris and Suzana (2006) in which liquid pineapple waste was fermented to produce LA using immobilized *Lactobacillus delbrueckii*, although in that case the optimal sodium alginate concentration was 2.0%.

**Table 1.** The Effects of Sodium Alginate Concentration on LA Production <sup>a</sup>

Sodium Alginate (%)	Initial Sugar ( $\text{g L}^{-1}$ )	Residual Sugar ( $\text{g L}^{-1}$ )	LA ( $\text{g L}^{-1}$ )	LA Productivity ( $\text{g L}^{-1} \text{ h}^{-1}$ )	LA Yield ( $\text{g g}^{-1}$ )
1.5	10.4	3.75	3.06	0.102	0.460
2.0	11.2	3.02	4.25	0.142	0.520
2.5	10.0	1.48	6.77	0.226	0.795
3.0	10.7	2.96	4.13	0.138	0.534

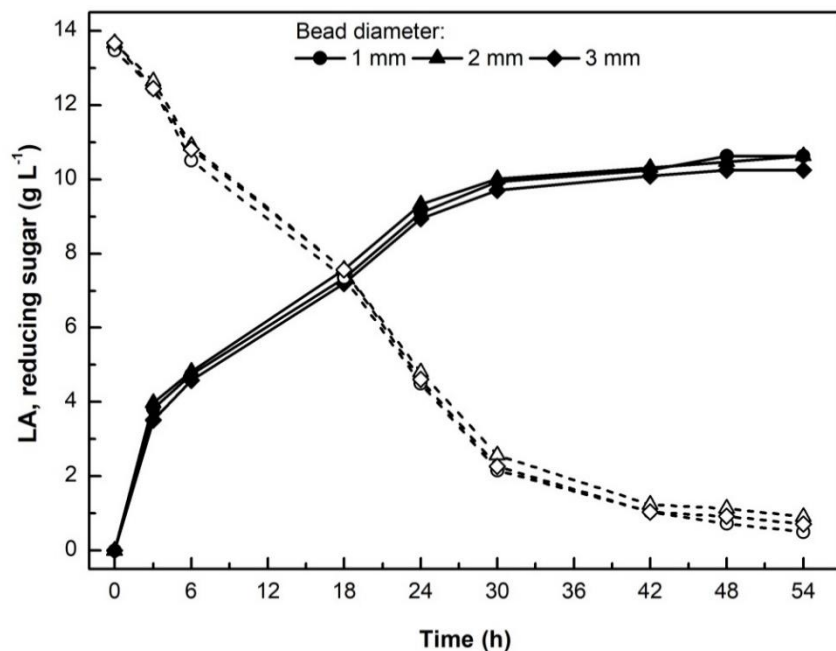
<sup>a</sup>: Each batch culture of immobilized cells was fermented at  $30 \text{ }^{\circ}\text{C}$  for 54 h in soybean straw hydrolysate medium containing approximately  $10 \text{ g L}^{-1}$  sugar, with initial pH 6.0.

#### *The effect of bead diameter on LA production*

It was also likely that the sizes of the Ca-alginate beads would affect LA production by altering the surface area and permeability. In this experiment, Ca-alginate beads were prepared with 2.5% sodium alginate, and fermentation was performed at  $30 \text{ }^{\circ}\text{C}$  with an inoculum size of 10% and an initial reducing sugar concentration of  $13.7 \text{ g L}^{-1}$ . As shown in Fig. 1, an increase in bead size (from 1 mm to 2 mm) was matched by an increase in final LA concentrations (from  $10.6 \text{ g g}^{-1}$  to  $10.7 \text{ g g}^{-1}$ ). Moreover, lowest final LA concentration ( $10.2 \text{ g g}^{-1}$ ) was observed with bead size of 3 mm.

A previous report (Givry *et al.* 2008) suggested that more LA is produced when using beads of smaller diameter. Idris and Suzana (2006) also reported decreased LA production when the bead diameter was 5 mm. These apparent differences may be due to the relatively narrow diameter range used in this study. Despite this, beads 1 to 3 mm in size had enough surface area and permeability to facilitate the diffusion of substrates, nutrients, and products.

As the 2 mm diameter beads had a slightly higher LA yield ( $0.902 \text{ g g}^{-1}$ ) compared with that of the other sizes tested ( $0.879 \text{ g g}^{-1}$  for 1 mm beads and  $0.851 \text{ g g}^{-1}$  for 3 mm beads), 2 mm beads were chosen as the optimal size.



**Fig. 1.** The effects of Ca-alginate bead diameter on soybean straw hydrolysate LA production. Solid lines with solid symbols represent LA concentrations, whereas dotted lines with open symbols represent reducing sugar concentrations.

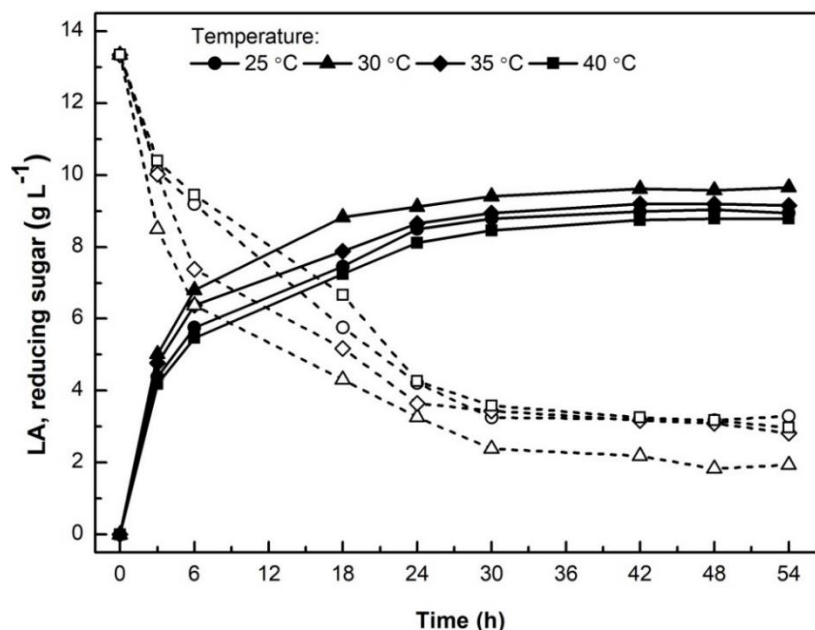
## Optimization of Fermentation Conditions for LA Production

### *The effect of temperature on LA production*

As temperature has major effects on the growth and metabolic activities of microorganisms, the study next established the effects of temperature on LA production using immobilized *L. casei*. In this study, Ca-alginate beads were prepared with 2.5% sodium alginate. After cell immobilization, the main-cultures were performed at 25 °C, 30 °C, 35 °C, and 40 °C, respectively, with 10% inoculum size of cell beads and an initial reducing sugar concentration of 13.3 g L<sup>-1</sup>. Initially, this was investigated using a starting reducing sugar concentration of 13.3 g L<sup>-1</sup>. The highest LA production was obtained at 30 °C, with a final concentration of 9.40 g L<sup>-1</sup> and a yield of 0.857 g g<sup>-1</sup> (Fig. 2). However, while fermentation at 30 °C resulted in the highest LA concentration and productivity, it did not achieve the highest overall LA yield. This result supports a prior study by Yumoto and Ikeda (1995) that reported increased yield but decreased productivity as temperature increased when producing LA from soluble starch using *Lactobacillus amylophilus*. However, because the highest productivity was obtained at 30 °C, this was selected as the optimal temperature.

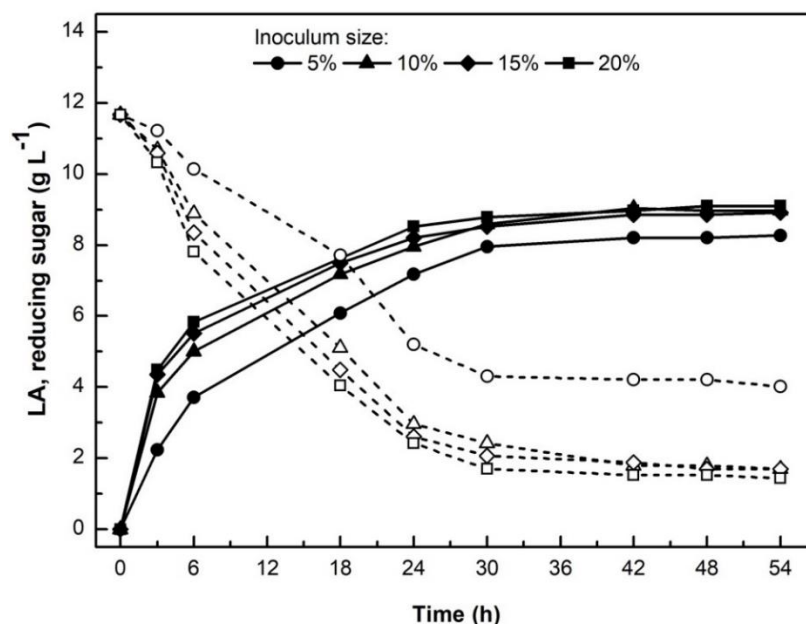
### *The effect of inoculum size on LA production*

As the number of cells inoculated into the fermentation system could affect subsequent growth dynamics, the study next investigated the effects of using different inoculum sizes. This was likely an important factor, as the time needed for microorganisms to reach the logarithmic phase shortened as inoculum size increased (within at least 5%). Additionally, with higher growth rates, more microbial cells could be able to be involved in LA production. In this experiment, Ca-alginate beads were prepared with 2.5% sodium alginate.



**Fig. 2.** The effects of temperature on LA production from soybean straw hydrolysate. Solid lines with solid symbols represent LA concentrations, whereas dotted lines with open symbols represent reducing sugar concentrations.

After cell immobilization, the batch cultures with different inoculation sizes (5% to 20%) of cell beads were performed at 30 °C with an inoculum size of 10% and an initial reducing sugar concentration of 11.7 g L<sup>-1</sup>. A summary of the effects of inoculum size based on the wet weights of the beads is shown in Fig. 3. These data revealed that a 5% inoculum size resulted in a lower LA concentration, likely due to the lower growth rate of the cells (Fig. 3).

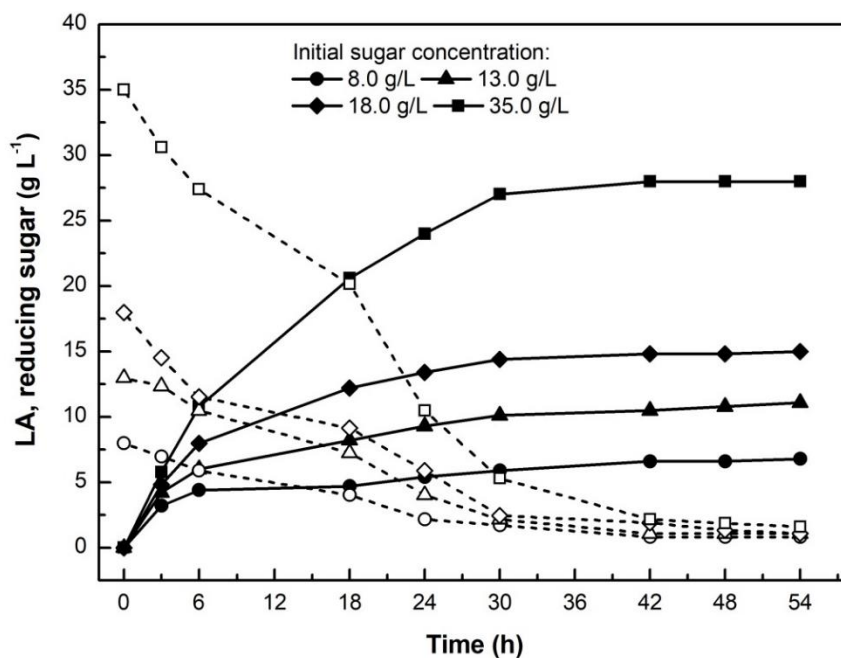


**Fig. 3.** The effects of inoculum size on LA production from soybean straw hydrolysate. Solid lines with solid symbols represent LA concentrations, whereas dotted lines with open symbols represent reducing sugar concentrations.

As more residual sugar ( $4.02 \text{ g L}^{-1}$ ) remained in the medium with the 5% inoculum, more LA would be produced if the remaining sugar was fully utilized by additional bacteria. This lower sugar utilization might also have been due to a remarkable decrease in pH (Zheng *et al.* 2017) that resulted from LA accumulation, as only the initial pH was adjusted to 6.0, and no additional pH adjustment was performed during the fermentation process. As there were markedly fewer bacteria in the medium with the 5% inoculum, they would be more vulnerable to the pH decrease. Lactic acid production showed no obvious increase at inoculum sizes greater than 10%, and thus the optimal inoculum size was considered 10%.

#### The effect of initial sugar concentration on LA production

The total reducing sugar concentration of the soybean straw hydrolysate solution was  $12.9 \text{ g L}^{-1} \pm 0.8 \text{ g L}^{-1}$ . As reducing sugars were the main carbon source for the *L. casei* cells, the available concentration of such sugars could affect fermentation. Therefore, the starting solution was condensed or diluted to obtain various reducing sugar concentrations (8 to  $35 \text{ g L}^{-1}$ ) to investigate the effects on LA production. In this study, Ca-alginate beads (2 mm) were prepared with 2.5% sodium alginate, and batch fermentations were performed at  $30^\circ \text{C}$  with an inoculum size of 10%. High concentrations of sugar is considered to cause inhibition of LA production due to carbon repression (Wang *et al.* 2014), while low concentrations are not economically favorable due to low productivity and high costs. As shown in Fig. 4, LA concentration increased during the first 30 h of fermentation but then only slightly increased during the next 24 h. Therefore, the optimal fermentation time was 30 h. There was also a clear link to higher LA production and productivity as the initial reducing sugar concentration increased (Fig. 4).



**Fig. 4.** The effects of initial reducing sugar concentration on LA production with soybean hydrolysate. Solid lines with solid symbols represent LA concentrations, whereas dotted lines with open symbols represent reducing sugar concentrations.



LA yield decreased only slightly from 0.941 g g<sup>-1</sup> to 0.909 g g<sup>-1</sup> as the sugar concentrations increased from 8 g L<sup>-1</sup> to 35 g L<sup>-1</sup> (Table 2). Moreover, initial LA production rate was drastically stimulated from 1.07 g L<sup>-1</sup> h<sup>-1</sup> to 1.93 g L<sup>-1</sup> h<sup>-1</sup> with increasing initial substrate concentration (Table 2). These results indicated that no substrate inhibition was observed within the tested sugar concentration range (8 g L<sup>-1</sup> to 35 g L<sup>-1</sup>). This could be attributable to diffusion resistance across the beads that made the sugar concentration of the inner regions lower than that of the medium. Based on these results, it is likely that initial sugar concentration can be further increased to achieve higher final LA concentrations and productivity. However, sugar concentrations in the soybean straw hydrolysate were still relatively low compared with those of other starting materials, and concentrating the liquor is uneconomical (Modenbach and Nokes 2012). Further effort is therefore required to increase the sugar concentration of the starting hydrolysate.

**Table 2.** LA Production at Different Initial Sugar Concentrations <sup>a</sup>

Initial Sugar (g L <sup>-1</sup> )	LA (g L <sup>-1</sup> )	LA Yield <sup>b</sup> (g g <sup>-1</sup> )	LA Productivity <sup>b</sup> (g L <sup>-1</sup> h <sup>-1</sup> )	Initial LA Production Rate <sup>c</sup> (g L <sup>-1</sup> h <sup>-1</sup> )
8.02	5.90	0.941	0.197	1.07 (0–3 h)
13.3	10.1	0.931	0.337	1.40 (0–3 h)
18.6	14.4	0.927	0.480	1.60 (0–3 h)
35.1	27.1	0.909	0.903	1.93 (0–3 h)

<sup>a</sup>: Each batch culture of immobilized cells was fermented at 30 °C for 54 h in soybean straw hydrolysate medium containing reducing sugar at the indicated concentrations, with initial pH 6.0;

<sup>b</sup>: Parameters were calculated based on LA production obtained during a 30 h fermentation;

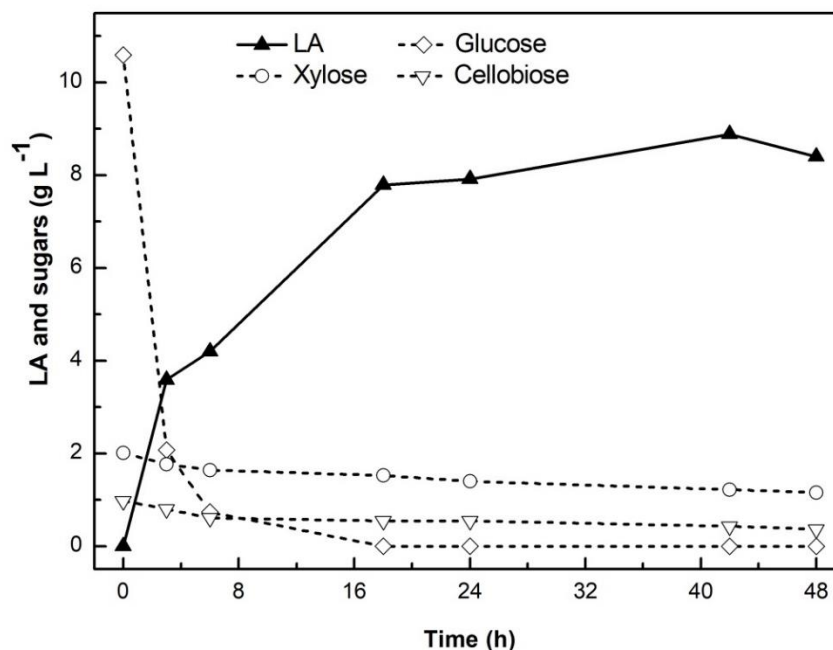
<sup>c</sup>: Initial production rate was calculated based on LA production obtained during initial 3 h fermentation.

### LA Production and Sugar Consumption at Optimal Conditions

As previous research indicates, lactic acid bacteria are similar to many other microorganisms in that they tend to preferentially use carbon sources that are more rapidly metabolized and avoid less favored carbon sources. This phenomenon is termed carbon catabolite repression (CCR) (Abdel-Rahman *et al.* 2015). The current study found that the soybean straw hydrolysate contained a starting sugar mixture of glucose (10.6 g L<sup>-1</sup>), xylose (2.01 g L<sup>-1</sup>), and cellobiose (0.97 g L<sup>-1</sup>). The consumption patterns of each sugar in the hydrolysate under optimal conditions were next investigated to establish which carbon sources were being utilized.

For the experiment, *L. casei* cells immobilized in 2-mm Ca-alginate beads were prepared with 2.5% sodium alginate solution and fermented at 30 °C with an inoculum size of 10%. As predicted, glucose was rapidly utilized with a consumption rate of 0.588 g L<sup>-1</sup> h<sup>-1</sup> and completely consumed after 18 h fermentation (Fig. 5). However, xylose and cellobiose were utilized at a much lower rate, with only 42.4% of the xylose and 62.5% of the cellobiose consumed after 48 h fermentation. This result indicated that CCR occurred during the LA fermentation process. These low xylose and cellobiose consumption rates, even after glucose was completely exhausted, may be due to the fact that the fermentation rates for xylose by homofermentative LA bacteria are lower than those for glucose (Taniguchi *et al.* 2004).

Alternatively, as the fermentation process was performed without pH adjustment, xylose, and cellobiose utilization may be inhibited by low pH due to acid accumulation. Therefore, LA production and sugar consumption might be further improved by controlling the pH of the medium, selecting a bacterial strain that can metabolize glucose, xylose, and cellobiose simultaneously, or co-fermenting with different LA bacteria.



**Fig. 5.** Lactic acid production and sugar consumption from soybean straw hydrolysate at optimal conditions.

### Lactic Acid Production Across Repeated Fermentation Batches

To investigate the stability of the Ca-alginate beads, repeated fermentations were performed with recycled beads for 11 runs at sugar concentrations of approximately 12.5 g L<sup>-1</sup> at 30 °C.

The LA concentrations that were obtained in the first and last batches were 8.73 g L<sup>-1</sup> and 9.11 g L<sup>-1</sup> (Table 3), respectively. The average LA concentration of the 11 batches was 9.29 g L<sup>-1</sup> ± 0.26 g L<sup>-1</sup>, and no remarkable changes in LA production were observed during the 11 successive batches. The steady rate of LA production across different batches indicated that the Ca-alginate-immobilized *L. casei* cells retained their catalytic activity for long periods.

**Table 3.** LA Production from Soybean Straw Hydrolysate Across Repeated Fermentation Batches <sup>a</sup>

Run Number	1	2	3	4	5	6	7	8	9	10	11
LA (g L <sup>-1</sup> )	8.73	8.96	9.27	9.32	9.39	9.43	9.46	9.61	9.53	9.38	9.11

<sup>a</sup>: The batch culture of immobilized cells was repeated over 11 rounds using soybean straw hydrolysate medium containing approximately 12.5 g L<sup>-1</sup> sugar, with initial pH 6.0

## CONCLUSIONS

1. Immobilized *Lactobacillus casei* was used to produce LA from soybean straw enzymatic hydrolysate under optimal conditions: Ca-alginate beads made from 2.5% sodium alginate with a diameter of 2 mm, fermentation process using a 10% inoculum at 30 °C for 30 h.
2. Lactic acid production and productivity increased when the initial reducing sugar concentrations were increased from 8 g L<sup>-1</sup> to 35 g L<sup>-1</sup>, with only a slight decrease in LA yield.
3. Immobilized cells could be reused at least 10 times without a noticeable performance decrease.

## ACKNOWLEDGMENTS

This research was financially supported by the China Postdoctoral Science Foundation (2017M610774), the Fundamental Research Funds for the Central Universities (FRF-TP-16-061A1), and the Key Laboratory for Solid Waste Management and Environment Safety of the Education Ministry of China (SWMES2017-05).

## REFERENCES CITED

- Abdel-Rahman, M. A., Tashiro, Y., and Sonomoto, K. (2011). "Lactic acid production from lignocellulose-derived sugars using lactic acid bacteria: Overview and limits," *J. Biotechnol.* 156(4), 286-301. DOI: 10.1016/j.jbiotec.2011.06.017
- Abdel-Rahman, M. A., Tashiro, Y., and Sonomoto, K. (2013a). "Recent advances in lactic acid production by microbial fermentation processes," *Biotechnol. Adv.* 31(6), 877-902. DOI: 10.1016/j.biotechadv.2013.04.002
- Abdel-Rahman, M. A., Tashiro, Y., Zendo, T., and Sonomoto, K. (2013b). "Improved lactic acid productivity by an open repeated batch fermentation system using *Enterococcus mundtii* QU 25," *RSC Adv.* 3(22), 8437-8445. DOI: 10.1039/C3RA00078H
- Abdel-Rahman, M. A., Xiao, Y., Tashiro, Y., Wang, Y., Zendo, T., Sakai, K., and Sonomoto, K. (2015). "Fed-batch fermentation for enhanced lactic acid production from glucose/xylose mixture without carbon catabolite repression," *J. Biosci. Bioeng.* 119(2), 153-158. DOI: 10.1016/j.jbiosc.2014.07.007
- Akao, S., Nagare, H., Maeda, M., Kondo, K., and Fujiwara, T. (2015). "Combined use of sugars and nutrients derived from young maize plants for thermophilic L-lactic acid fermentation," *Ind. Crop. Prod.* 69, 440-446. DOI: 10.1016/j.indcrop.2015.02.055
- Åkerberg, C., and Zacchi, G. (2000). "An economic evaluation of the fermentative production of lactic acid from wheat flour," *Bioresource Technol.* 75(2), 119-126. DOI: 10.1016/S0960-8524(00)00057-2
- Behera, S., Mohanty, R. C., and Ray, R. C. (2012). "Ethanol fermentation of sugarcane molasses by *Zymomonas mobilis* MTCC 92 immobilized in *Luffa cylindrica* L. sponge discs and Ca-alginate matrices," *Braz. J. Microbiol.* 43(4), 1499-1507. DOI: 10.1590/S1517-838220120004000034

- Boyaval, P., and Goulet, J. (1988). "Optimal conditions for production of lactic acid from cheese whey permeate by Ca-alginate-entrapped *Lactobacillus helveticus*," *Enzyme Microb. Tech.* 10(12), 725-728. DOI: 10.1016/0141-0229(88)90116-0
- Carvalho, W., Santos, J. C., Canilha, L., Silva, J. B. A., Felipe, M. G. A., Mancilha, I. M., and Silva, S. S. (2004). "A study on xylitol production from sugarcane bagasse hemicellulosic hydrolysate by Ca-alginate entrapped cells in a stirred tank reactor," *Process Biochem.* 39(12), 2135-2141. DOI: 10.1016/j.procbio.2003.11.021
- Christensen, C. H., Rass-Hansen, J., Marsden, C. C., Taarning, E., and Egeblad, K. (2008). "The renewable chemicals industry," *ChemSusChem* 1(4), 283-289. DOI: 10.1002/cssc.200700168
- Duarte, J. C., Rodrigues, J. A. R., Moran, P. J. S., Valença, G. P., and Nunhez, J. R. (2013). "Effect of immobilized cells in calcium alginate beads in alcoholic fermentation," *AMB Express* 3, 31-39. DOI: 10.1186/2191-0855-3-31
- Givry, S., Prevot, V., and Duchiron, F. (2008). "Lactic acid production from hemicellulosic hydrolyzate by cells of *Lactobacillus bifementans* immobilized in Ca-alginate using response surface methodology," *World J. Microb. Biot.* 24(6), 745-752. DOI: 10.1007/s11274-007-9534-0
- Han, H., Wei, L., Liu, B., Yang, H., and Shen, J. (2012). "Optimization of biohydrogen production from soybean straw using anaerobic mixed bacteria," *Int. J. Hydrogen Energ.* 37(17), 13200-13208. DOI: 10.1016/j.ijhydene.2012.03.073
- Hassan, N., and Idris, A. (2016). "Simultaneous saccharification and fermentation of lactic acid from empty fruit bunch at high solids loading," *BioResources* 11(2), 3799-3812. DOI: 10.15376/biores.11.2.3799-3812
- Idris, A., and Suzana, W. (2006). "Effect of sodium alginate concentration, bead diameter, initial pH and temperature on lactic acid production from pineapple waste using immobilized *Lactobacillus delbrueckii*," *Process Biochem.* 41(5), 1117-1123. DOI: 10.1016/j.procbio.2005.12.002
- Juturu, V., and Wu, J. C. (2015). "Microbial production of lactic acid: The latest development," *Crit. Rev. Biotechnol.* 36(6), 967-977. DOI: 10.3109/07388551.2015.1066305
- Kulozik, U., and Wilde, J. (1999). "Rapid lactic acid production at high cell concentrations in whey ultrafiltrate by *Lactobacillus helveticus*," *Enzyme Microb. Tech.* 24(5-6), 297-302. DOI: 10.1016/S0141-0229(98)00122-7
- Kwan, T. H., Hu, Y., and Lin, C. S. K. (2018). "Techno-economic analysis of a food waste valorisation process for lactic acid, lactide and poly (lactic acid) production," *J. Clean Prod.* 181, 72-87. DOI: 10.1016/j.jclepro.2018.01.179
- Liu, J., Wang, Q., Zou, H., Liu, Y., Wang, J., Gan, K., and Xiang, J. (2013). "Glucose metabolic flux distribution of *Lactobacillus amylophilus* during lactic acid production using kitchen waste saccharified solution," *Microb. Biotechnol.* 6(6), 685-693. DOI: 10.1111/1751-7915.12046
- Mazumdar, S., Clomburg, J. M., and Gonzalez, R. (2010). "*Escherichia coli* strains engineered for homofermentative production of D-lactic acid from glycerol," *Appl. Environ. Microb.* 76(13), 4327-4336. DOI: 10.1128/AEM.00664-10
- Miao, Q., Tang, Y., Xu, J., Liu, X., Xiao, L., and Chen, Q. (2013). "Activated carbon prepared from soybean straw for phenol adsorption," *J. Taiwan Inst. Chem. E.* 44(3), 458-465. DOI: 10.1016/j.jtice.2012.12.006

- Miller, G. L. (1959). "Use of dinitrosalicylic acid reagent for determination of reducing sugar," *Anal. Chem.* 31(3), 426-428. DOI: 10.1021/ac60147a030
- Modenbach, A. A., and Nokes, S. E. (2012). "The use of high-solids loadings in biomass pretreatment—A review," *Biotechnol. Bioeng.* 109(6), 1430-1442. DOI: 10.1002/bit.24464
- Pilkington, P. H., Margaritis, A., Mensour, N. A., and Russell, I. (1998). "Fundamentals of immobilised yeast cells for continuous beer fermentation: A review," *J. I. Brewing* 104(1), 19-31. DOI: 10.1002/j.2050-0416.1998.tb00970.x
- Qian, W., Zhao, A., and Xu, R. (2013). "Sorption of As(V) by aluminum-modified crop straw-derived biochars," *Water Air Soil Poll.* 224, 1610-1618. DOI: 10.1007/s11270-013-1610-5
- Shi, S., Kang, L., and Lee, Y. Y. (2015). "Production of lactic acid from the mixture of softwood pre-hydrolysate and paper mill sludge by simultaneous saccharification and fermentation," *Appl. Biochem. Biotech.* 175(5), 2741-2754. DOI: 10.1007/s12010-014-1451-8
- Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., and Crocker, D. (2008). *Determination of Structural Carbohydrates and Lignin in Biomass. Laboratory Analytical Procedure (LAP)* (Report No. NREL/TP-510-42618), U.S. Department of Energy, National Renewable Energy Laboratory, Golden, CA.
- Taniguchi, M., Tokunaga, T., Horiuchi, K., Hoshino, K., Sakai, K., and Tanaka, T. (2004). "Production of L-lactic acid from a mixture of xylose and glucose by co-cultivation of lactic acid bacteria," *Appl. Microbiol. Biot.* 66(2), 160-165. DOI: 10.1007/s00253-004-1671-x
- Tong, X., Li, J., Yuan, J., and Xu, R. (2011). "Adsorption of Cu(II) by biochars generated from three crop straws," *Chem. Eng. J.* 172(2-3), 828-834. DOI: 10.1016/j.cej.2011.06.069
- Wang, J., Wang, Q., Xu, Z., Zhang, W., and Xiang, J. (2015). "Effect of fermentation conditions on L-lactic acid production from soybean straw hydrolysate," *J. Microbiol. Biotechn.* 25(1), 26-32. DOI: 10.4014/jmb.1405.05025
- Wang, X. Q., Wang, Q. H., Ma, H. Z., and Yin, W. (2009). "Lactic acid fermentation of food waste using integrated glucoamylase production," *J. Chem. Technol. Biot.* 84(1), 139-143. DOI: 10.1002/jctb.2007
- Wang, Y., Abdel-Rahman, M. A., Tashiro, Y., Xiao, Y., Zendo, T., Sakai, K., and Sonomoto, K. (2014). "L-(+)-lactic acid production by co-fermentation of cellobiose and xylose without carbon catabolite repression using *Enterococcus mundtii* QU 25," *RSC Adv.* 4(42), 22013-22021. DOI: 10.1039/C4RA02764G
- Yumoto, I., and Ikeda, K. (1995). "Direct fermentation of starch to L-(+)-lactic acid using *Lactobacillus amylophilus*," *Biotechnol. Lett.* 17(5), 543-546. DOI: 10.1007/BF00132025
- Zhang, Y., Chen, X., Luo, J., Qi, B., and Wan, Y. (2014a). "An efficient process for lactic acid production from wheat straw by a newly isolated *Bacillus coagulans* strain IPE22," *Bioresource Technol.* 158, 396-399. DOI: 10.1016/j.biortech.2014.02.128
- Zhang, Y., Chen, X., Qi, B., Luo, J., Shen, F., Su, Y., Khan, R., and Wan, Y. (2014b). "Improving lactic acid productivity from wheat straw hydrolysates by membrane integrated repeated batch fermentation under non-sterilized conditions," *Bioresource Technol.* 163, 160-166. DOI: 10.1016/j.biortech.2014.04.038

Zheng, J., Liu, Y., Sun, X., Wang, Q., Zou, H., Wang, J., and Gao, M. (2017). “Open fermentative production of L-lactic acid from distillers’ grains by *Lactobacillus casei* CICC 6056,” *BioResources* 12(1), 393-406. DOI: 10.15376/biores.12.1.393-406

Article submitted: March 15, 2018; Peer review completed: June 28, 2018; Revised version received and accepted: July 10, 2018; Published: July 19, 2018.  
DOI: 10.15376/biores.13.3.6722-6735