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Improvement of L-Lactic Acid Production under Glucose Feedback Controlled Culture by *Lactobacillus rhamnosus*

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Abstract In this study, a glucose feedback controller had been designed. Under the controller, the glucose concentration in L-lactic acid culture could be automatically controlled at 15±1 g/L. Furthermore, L-lactic acid production by *Lactobacillus rhamnosus* LA-04-1 in fed-batch culture was significantly increased to 170 g/L L-lactic acid using the controller compared with 130 g/L L-lactic acid in pulse fed-batch and 135 g/L in L-lactic acid constant feed rate fed-batch cultures. The results showed that the controller was effective at glucose control in L-lactic acid culture. The control strategy could also be used in other fermentation processes.

Keywords L-Lactic acid · Feedback · Controller · Glucose · *Lactobacillus rhamnosus*

Abbreviations

GC	Glucose concentration
HPLC	High-performance liquid chromatograp
GL	The amount of glucose consumption
AM	The amount of ammonia consumption

Introduction

Lactic acid and its derivatives are widely used in food, pharmaceutical, leather, and textile industries [1]. Recently, more attention has been paid to its great potential in the

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manufacture of environmentally friendly biodegradable plastics, which could substitute for synthetic plastics derived from petroleum [2]. Approximately 90% lactic acid was produced by lactic acid bacteria culture in recent years [3]. Fed-batch culture is an effective strategy for lactic acid and many other culture products [4]. Hirata et al. reported that supplying feed medium could enhance the production of lactic acid [5], and glucose control method was the key factor to obtain higher lactic acid production in fed-batch culture. Our previous studies showed that the performance of constant glucose concentration (GC) in broth was better than that of inconstant GC in broth [6] since the varied GC in culture broth could decrease the bacteria activity [3]. Therefore, it is difficult to control the GC at the suitable concentration under a simple feeding strategy.

An online GC controller was used by Hirata et al. to control GC in culture [3]. Compared to the high-performance liquid chromatography (HPLC) system, the experimental errors of the GC controller were within 5% in offline. However, the values measured by the GC controller were lower than those by the HPLC system online. The authors implied that the error might be due to the dialysis membrane polluted by bacteria and protein in the culture broth. Therefore, further investigation is needed on how to control GC effectively.

In this paper, a glucose controller had been developed to control GC in culture broth, and the performance under the glucose-controlled fed-batch culture was investigated to improve L-lactic acid production.

Materials and Methods

Bacterial Strain and Media

The *Lactobacillus rhamnosus* LA-04-1 (screened by this research group and maintained at the Key Laboratory of Bioprocess of Beijing, Beijing University of Chemical Technology, People's Republic of China) was used in all experiments. It was maintained on semisynthetic medium consisting of (in grams per liter): 20 glucose, 5 yeast extract (YE), 10 soya peptone, 10 beef extract, 5 NaCl, 10 sodium acetate, 2 triammonium citrate, 0.2 $MgSO_4 \cdot 7H_2O$, 0.05 $MnSO_4 \cdot 7H_2O$, and 15 agar. The stock cultures were maintained at 4 °C. The medium for cell growth or inoculum preparation contained the following (in grams per liter): 40 glucose, 20 YE, salts (0.01 NaCl, 0.5 sodium acetate, 0.2 triammonium citrate, 0.2 KH_2PO_4, 0.2 $MgSO_4 \cdot 7H_2O$, and 0.05 $MnSO_4 \cdot 7H_2O$), and 16 $CaCO_3$.

Cultivation Conditions

Seeds were cultivated at 42 °C and 150 rpm in 250-ml flasks containing 100 ml of seed medium. L-Lactic acid was neutralized by an equivalent amount of $CaCO_3$.

Batch culture was performed in a 5-L fermentor (Zhenjiang, China) with an initial broth volume of 2 L equipped with temperature, agitation, and pH controllers. The temperature was maintained at 42 °C and the rotation speed was 150 rpm. The pH was controlled at 6.25±0.05 by addition of ammonia.

In fed-batch culture, initial GC was 125 g/L. The pH was controlled at 6.25 by the addition of 33% (*w*/*w*) calcium hydroxide solution (before L-lactic acid concentration reached 85 g/L) and ammonia (after L-lactic acid concentration reached 85 g/L); 770 g/L glucose was fed once GC was lower than 15 g/L.

Design of the Glucose Feedback Controller

As shown in Fig. 1, a glucose feedback controller (#1, the main board in Fig. 2) has been designed to control GC automatically. The controller could measure pH by collected information from a pH meter (#2) with a transducer. Furthermore, pH in the culture broth could be maintained at 6.25±0.05 by opening a peristaltic pump (#7) to add ammonia (#3) into the broth, and the amount of ammonia addition was calculated by a related software "BUCTFMCS" (which was developed by ourselves using Labview 8.0) through computing the times of the peristaltic pump opening (the time and speed of the pump was kept constant).

In addition, the software could also calculate the amount of glucose solution that should be added into the broth to keep a desirable GC according to $Y_{GL/AM}$ (where GL and AM are the amount of glucose and ammonia consumption during the batch culture). Then, the controller activated the peristaltic pump (#6) periodically in order to add glucose solution (#4) into the fermentor.

Glucose Feedback Controlled Fed-Batch Culture

The pH was maintained constant at 6.25±0.05, and the controller activated the peristaltic pump (#1; adding ammonia) when pH fell below 6.20, then 5 mol/L ammonia was fed. The controller recorded the amount of ammonia consumption at each 5-min interval and computed the amount of glucose solution needed. Then, the peristaltic pump (#2) was activated by the controller to add glucose solution. One milliliter broth was withdrawn at regular intervals.

Analytical Methods

One milliliter culture broth was centrifuged at 8,000 rpm for 5 min, and the supernatant liquor was used for determination. Glucose and L-lactic acid were measured using a SBA-40C biosensor analyzer (Institute of Biology, Shandong Province Academy of Sciences, People's Republic of China) [2].

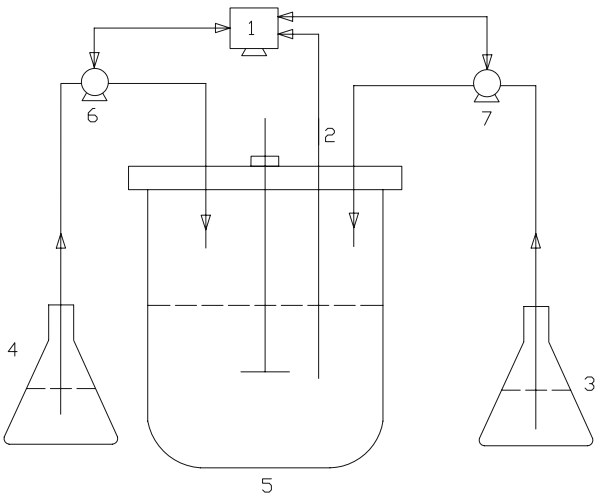

Fig. 1 Glucose control system: *1* glucose controller, *2* pH meter, *3* ammonia, *4* glucose solution, *5* fermenter, *6* glucose pump, *7* ammonia pump

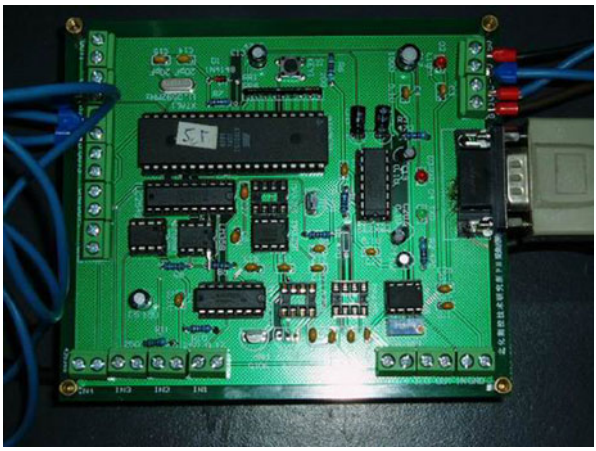

Fig. 2 The main board of the glucose feedback controller

Results and Discussion

Batch Culture

The batch culture of L-lactic acid by *L. rhamnosus* was shown in Fig. 3. The initial GC was 130 g/L, and the L-lactic acid production was 90 g/L. Under this condition, glucose consumed and ammonia added to neutralize L-lactic acid accumulated were calculated from plots of GL versus AM (where GL and AM are the amount of glucose and ammonia consumption during the batch culture). $Y_{GL/AM}$ was estimated to be 1.4261 (R^2=0.9958), and the good linear relationship indicated that GC in culture broth could be controlled by coupling glucose solution addition with ammonia addition to neutralize the L-lactic acid. The relationship between the amount of glucose and ammonia consumption was used to control the GC.

Glucose Feedback Controlled Fed-Batch Culture

The constant GC by manual operation in fed-batch L-lactic acid production was 152.5 g/L, which was higher than that of the inconstant GC by pulse fed-batch (130 g/L) and constant

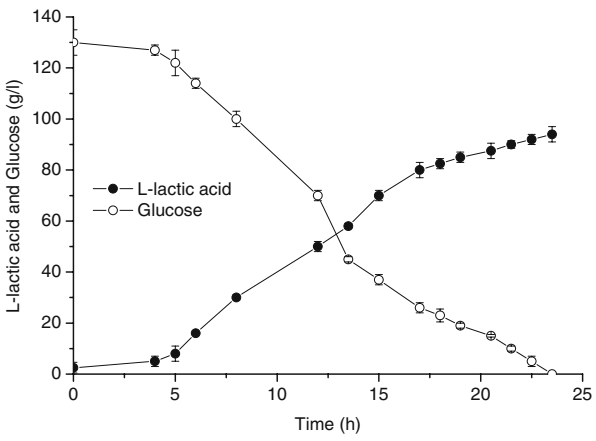

Fig. 3 L-Lactic acid production by *L. rhamnosus* in batch culture. The culture was performed in a 5-L fermentor at 42°C and 150 rpm. The initial glucose was 130 g/L and the pH was maintained at 6.25±0.05 by automatic addition of ammonia

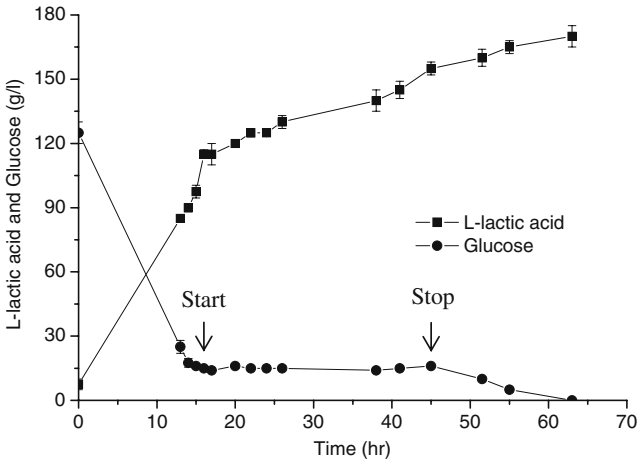

Fig. 4 Fed-batch culture of *L. rhamnosus* with glucose feedback controller. The fed-batch culture was performed in a 5-L fermentor at 42 °C and 150 rpm. The initial glucose was 125 g/L and the pH was maintained at 6.25±0.05 by automatic addition of 33% (*w*/*w*) calcium hydroxide solution (before L-lactic acid concentration reached 85 g/L) and ammonia (after L-lactic acid concentration reached 85 g/L). The *arrow* indicates the start and stop of feeding 770 g/L glucose

feed rate fed-batch (135 g/L) cultures [7]. Therefore, it was a suitable strategy for L-lactic acid culture.

Glucose was added to the culture broth by the glucose feedback controller (Fig. 1) once GC was lower than 15 g/L in order to eliminate glucose inhibition [4]. The concentrations of glucose and ammonia were 770 g/L and 5 mol/L, respectively. As shown in Fig. 4, the initial GC was 130 g/L, and glucose feeding began at 14 h and ended at 45 h. Residual GC ranged from 14 to 16 g/L during the feeding. Apparently, the glucose feedback controller achieved the goal of keeping the GC constant during the culture. L-Lactic acid production increased quickly during the feeding and reached 170 g/L at 63 h.

The effect of different residual GC on the production of L-lactic acid by *L. rhamnosus* was investigated [6]. When residual GC were higher than 40 g/L, L-lactic acid biosynthesis was inhibited and its production decreased. Therefore, L-lactic acid production could be significantly enhanced by the glucose feedback controller in fed-batch culture, which was 1.89 times higher than that of batch culture.

Conclusions

In this paper, a glucose feedback controller had been designed. Under the controller, the GC in L-lactic acid culture could be automatically controlled at constant. Furthermore, L-lactic acid production by *L. rhamnosus* LA-04-1 in fed-batch culture was significantly increased to 170 g/L L-lactic acid using the controller compared with pulse fed-batch and constant feed rate fed-batch cultures. The results showed that the controller was effective at glucose control in L-lactic acid culture.

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