Appl Biochem Biotechnol (2010) 160:343–349
DOI 10.1007/s12010-008-8300-6

Kinetic Analysis and pH-Shift Control Strategy for Propionic Acid Production with *Propionibacterium Freudenreichii* CCTCC M207015

Xiaohai Feng · Hong Xu · Jun Yao · Sha Li · Hongyang Zhu · Pingkai Ouyang

Received: 5 March 2008 / Accepted: 10 June 2008 /
Published online: 15 July 2008

Abstract The production of propionic acid by *Propionibacterium freudenreichii* CCTCC M207015 was investigated in a 7.5-l stirred-tank fermentor. Batch fermentations by *P. freudenreichii* CCTCC M207015 at various pH values ranging from 5.5 to 7.0 were studied. Based on the analysis of the time course of specific cell growth rate (μ_x) and specific propionic acid formation rate (μ_p), a two-stage pH-shift control strategy was proposed. At first 48 h, pH was controlled at 6.5 to obtain the maximal μ_x, subsequently pH 6.0 was used to maintain high μ_p to enhance the production of propionic acid. By applying this pH-shift control strategy in propionic acid fermentation, the maximal propionic acid and glucose conversion efficiency had a significant improvement and reached 19.21 g/l and 48.03%, respectively, compared with those of constant pH operation (14.58 g/l and 36.45%). Fed-batch fermentation with pH-shift control strategy was also applied to produce propionic acid; the maximal propionic acid yield and glucose conversion efficiency reached 25.23 g/l and 47.76%, respectively.

Keywords *Propionibacterium freudenreichii* CCTCC M207015 · Propionic acid · Kinetic analysis · pH-Shift Control Strategy · Fed-batch fermentation

Introduction

Propionic acid had numerous applications in various fields of food and chemical industries [1, 2]. Propionic acid and its salts were widely used as preservatives in food and grains due to its antimicrobial activity. It plays an important role as an intermediate in the production of chemical products. Currently, propionic acid was mainly synthesized from petroleum feedstock [3, 4]. The biosynthesis of propionic acid was independent of petroleum-based resources and expected to be a promising candidate [5, 6]. However, there were still some existed problems in propionic acid fermentation processes, such as low propionic acid

X. Feng · H. Xu (✉) · J. Yao · S. Li · H. Zhu · P. Ouyang
State Key Laboratory of Materials-Oriented Chemical Engineering, College of Life Science and Pharmacy, Nanjing University of Technology, Nanjing 210009, People's Republic of China
e-mail: xuh@njut.edu.cn

productivity, low conversion efficiency, and by-product formation (e.g., acetic acid and succinic acid) [2, 3, 7].

pH is one of the most important environmental factors in propionic acid production. It was reported that most of propionic acid productions were conducted at a neutral pH [8–11]. However, pH was also concerned about the cell growth, substrate consuming, and byproduct formation. To achieve the maximal propionic acid production with an efficient conversion of substrate and decrease the byproduct formation, an optimization of propionic acid production on pH condition was needed to be further studied.

In this work, the effect of pH on propionic acid production from *P. freudenreichii* CCTCC M207015 was systematically studied. The formation of byproducts during propionic acid production was also investigated. A two stage pH-shift control strategy was proposed to enhance the production of propionic acid, based on which a fed-batch culture was carried out with a pH-shift control strategy, and a more efficient production was observed.

Materials and Methods

Microorganism and Media

P. freudenreichii CCTCC M207015, a propionic acid production strain, was originally isolated from cheese in our laboratory and used in this work [12]. The seed medium was composed of glucose 20 g/l, peptone 5 g/l, yeast extract 5 g/l, and NaCl 5 g/l; pH was adjusted to 6.9. The fermentation medium was containing glucose 40 g/l, peptone 5 g/l, yeast extract 10 g/l, NaCl 3 g/l, $(NH_4)_2SO_4$ 5 g/l, and KH_2PO_4 5 g/l.

Fermentation Conditions

The culture was transferred from deep agar into a flask containing 100 ml of fresh seed medium and incubated at 35 °C for 24 h. Seed culture (80 ml) was then transferred to 1,000 ml flask containing 500 ml of seed medium and incubated at 35 °C for 48 h. Anaerobiosis was established by sparging the medium of flask with nitrogen gas for 10 min. The seed culture (450 ml) was used to inoculate 4 l of the bioreactor culture medium. Batch fermentation was performed in a 7.5-l bioreactor (BioFlo 110, New Brunswick Scientific, USA) with a working volume of 4.5 l. The cultures were performed at 35 °C with a stirring of 100 rpm, and 0.1 l/min of nitrogen gas was applied to ensure the fermentations were under anaerobic conditions. In all cases, pH at 5.5–7.0 was automatically controlled with 6 M NaOH. For pH-shift control experiment, pH was controlled at 6.5 at first 48 h, then the pH was naturally decreased to 6.0 and controlled constant. Fed-batch fermentation with pH-shift control strategy for propionic acid production was also carried out when the residual glucose in the medium was below 5 g/l.

Analytical Methods

Cell growth was estimated by measuring the absorbance of cell suspensions at 660 nm wavelength in a spectrophotometer (Unico 2800, China). To determine the dry cell weight (DCW), cells were harvested by centrifugation at 10,000 ×*g* for 10 min, washed twice with distilled water, and dried at 80 °C for 24 h. Glucose was analyzed by a biosensor equipped with glucose oxidase electrode (Institute of Biology, Shandong Academy of Sciences SBA-

40C, China) [13]. Propionic, acetic, and succinic acids were determined by the high-press liquid chromatography (Agilent 1200, USA) with an Aminex HPX-87H column (300× 7.8 mm, Bio-Rad) by an ultraviolet detector. A 0.04 M H_2SO_4 solution was used as mobile phase with a flow rate of 0.6 ml/min at 60 °C.

Results and Discussion

Time Course of Propionic Acid Fermentation at Different pH Values

The effects of pH values at the range of 5.5–7.0 on propionic acid fermentations by *P. freudenreichii* CCTCC M207015 were investigated. The experimental results indicated that the pH played a vital role in propionic acid production and cell growth. As was shown in Fig. 1, the propionic acid yield and the maximum DCW obtained at pH 5.5 were only 10.27 and 6.91 g/l, respectively. Similarly, low level of the propionic acid yield (9.22 g/l) and the maximum DCW (7.88 g/l) were observed at pH 7.0. Either lower pH (5.5) or high pH (7.0) was not beneficial for the propionic acid production and cell growth. pH 6.0 was the optimum pH condition for the production of propionic acid and the maximum production of propionic acid reached 14.58 g/l. However, it was more suitable for the cell growth at pH 6.5 and 8.26 g/l of DCW was obtained. It was indicated that the optimum pH for cell growth was not suitable to the propionic acid production [14, 15]. According to the above

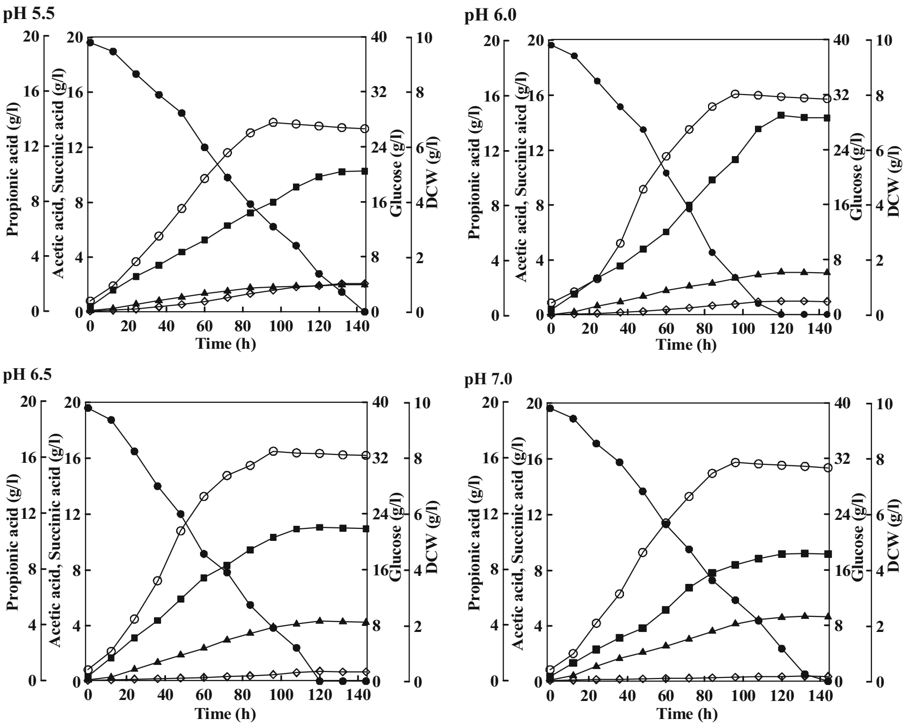

Fig. 1 Time course of propionic acid production at different pH values (pH 5.5–7.0). The symbols were used: propionic acid (*filled square*), acetic acid (*filled triangle*), succinic acid (*unfilled square*), glucose (*filled circle*), DCW(*unfilled circle*)

results, it was necessary to investigate the role of pH for cell growth and propionic acid production by *P. freudenreichii* CCTCC M207015.

Effect of pH on the Specific Cell Growth Rate (μ_x) and Specific Propionic Acid Formation Rate (μ_p)

Specific cell growth rate (μ_x) and specific propionic acid formation rate (μ_p) were applied to describe the propionic acid fermentation process. As was shown in Figs. 2, the profiles of μ_x and μ_p had similar tendencies and the maximum of μ_x and μ_p were appeared at about 12 h. Compared with pH 6.0, μ_x was higher at pH 6.5 at the beginning of the propionic acid fermentation (about 48 h); it was showed that pH 6.5 was better for cell growth at the beginning of the propionic acid fermentation (Fig. 2a). But after 48 h, pH 6.0 was beneficial for propionic acid formation with high valve of μ_p; it was obviously that pH 6.0 was appropriate for propionic acid production after 48 h (Fig. 2b).

Based on the analysis of μ_x and μ_p, a two-stage pH-shift control strategy was proposed as following: pH 6.5 was controlled to reach the optimal biomass during 0–48 h, and pH was shifted to 6.0 after 48 h to maintain the high μ_p for the fermentation of propionic acid with *P. freudenreichii* CCTCC M207015.

Two-Stage pH-Shift Control Strategy for Propionic Acid Fermentation

Time course of pH-shift control strategy for propionic acid fermentation was shown in Fig. 3. pH 6.5 was controlled at first 48 h to obtain maximal μ_x, then the pH was naturally decreased to 6.0 and controlled constant. After pH was shifted to 6.0 at 48 h, μ_p continued keeping at high values to produce more propionic acid. Finally, the maximum yield of propionic acid and DCW reached 19.21 and 8.38 g/l, respectively. The results were both higher than that of constant pH process (14.58 g/l of propionic acid at pH 6.0 and 8.26 g/l of DCW at pH 6.5). Especially, the final glucose conversion efficiency with pH-shift control strategy was enhanced from 36.45% to 48.03% (theoretical glucose conversion rate to propionic acid was 54.8%) [4].

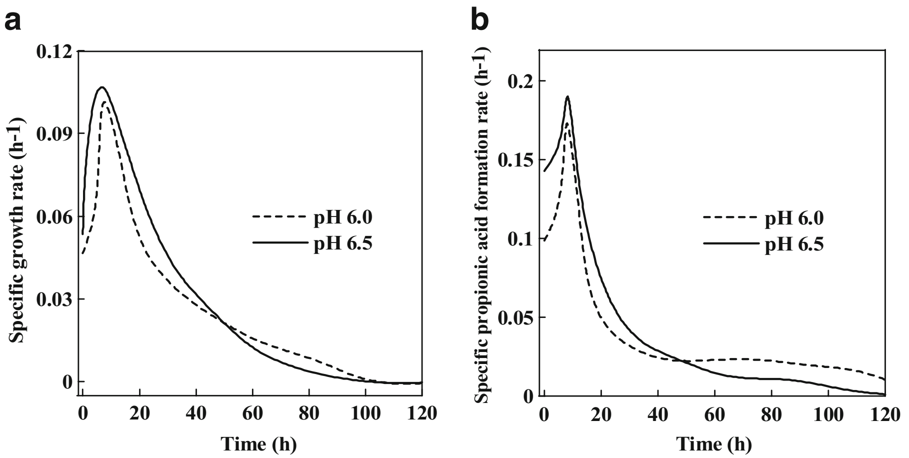

Fig. 2 Comparison of specific growth rate (μ_x) and specific propionic acid formation rate (μ_p) between pH 6.0 and pH 6.5. **a** Comparison of specific growth rate (μ_x) between pH 6.0 and pH 6.5. **b** Comparison of specific propionic acid formation rate (μ_p) between pH 6.0 and pH 6.5

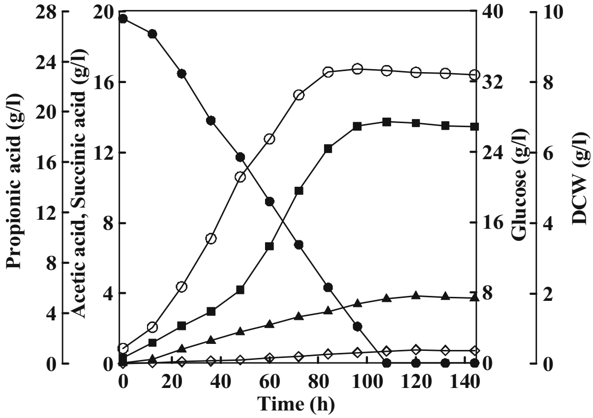

Fig. 3 Two-stage propionic acid fermentation with pH shifted from 6.5 to 6.0 at 48 h. The symbols were used: propionic acid (*filled square*), acetic acid (*filled triangle*), succinic acid (*unfilled square*), glucose (*filled circle*), DCW(*unfilled circle*)

Fed-Batch Fermentation of Propionic Acid Fermentation with pH-Shift Control Strategy

As was shown in Figs. 1 and 3, it was observed that the propionic acid yield could not increase when the glucose was exhausted. A fed-batch culture by feeding of glucose with pH-shift control strategy was carried out. As was shown in Fig. 4, glucose was added to fermentation culture when the substrate was below 5 g/l. After 168 h fermentation, the propionic acid yield and glucose conversion efficiency reached 25.23 g/l and 47.76%, respectively, at the end of the fermentation.

The Analysis of Parameters at Different Culture Conditions

Table 1 listed the results of constant pH experiments, pH-shift control strategy, and fed-batch fermentation with pH-shift control strategy, and the parameters were analyzed. It was observed that pH-shift control strategy could not only considerably improve the production of propionic acid but also increase the propionic acid productivity. The production of propionic acid was increased by 31.75% (from 14.58 to 19.21 g/l), com-

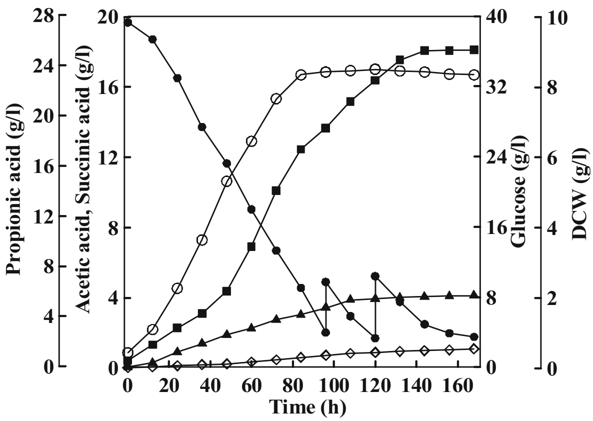

Fig. 4 Fed-batch fermentation of propionic acid fermentation with pH-shift control strategy. The symbols were used: propionic acid (*filled square*), acetic acid (*filled triangle*), succinic acid (*unfilled square*), glucose (*filled circle*), DCW(*unfilled circle*)

Table 1 The analysis of parameters at different fermentation culture conditions.

Parameters	pH 5.5	pH 6.0	pH 6.5	pH 7.0	pH-shift control strategy	Fed-batch fermentation with pH-shift control strategy
DCW (g/l)	6.91	8.05	8.26	7.88	8.38	8.51
Propionic acid (g/l)	10.27	14.58	11.05	9.22	19.21	25.23
Acetic acid (g/l)	1.93	3.11	4.25	4.63	3.84	4.12
Succinic acid (g/l)	2.00	1.01	0.67	0.31	0.80	1.09
Conversion efficiency (%)	25.68	36.45	27.63	23.05	48.03	47.76
Propionic acid productivity (g/l/h)	0.071	0.122	0.092	0.064	0.178	0.150
Propionic acid/acetic acid (mol/mol)	4.31	3.80	2.11	1.61	4.06	4.96
Glucose exhausted time (h)	144	120	120	144	108	–

pared with the constant process of pH 6.0. At the same time, the productivity of propionic acid was enhanced from 0.122 (pH 6.0) to 0.178 g/l/h with this strategy. The results showed that the two-step pH-shift control strategy remarkably improved the fermentation of propionic acid. Moreover, the pH-shift control strategy was also beneficial for the cell growth, and 8.38 g/l of DCW was obtained at the end of the fermentation, compared with the constant process of pH 6.0 (8.05 g/l). The fed-batch fermentation with pH-shift control strategy could further enhance the production of propionic acid. Compared with the solely pH-shift control strategy fermentation, the yield was increased by 31.34% with high glucose conversion rate to propionic acid (47.76%).

Propionic acid fermentation was a multiproduct symbiotic system. Acetic and succinic acids were accompanied with the propionic acid production in this work. It was reported that the acetic acid yield was not significant affected by the pH and the succinic acid yield increased with the pH increasing [14]. As was shown in Table 1, the acetic acid yield increased with pH increasing (from pH 5.5 to 7.0). In contrast, the succinic acid yield decreased with pH increasing. The experimental results mentioned above were different from the previous reports. The theoretical molecular ratio of propionic acid yield to acetic acid yield was 2:1 with glucose as substrate [4]. But in our work, the proportion of propionic acid to acetic acid decreased from 4.31 (pH 5.5) to 1.61 (pH 7.0). It was clear that pH-shift control strategy fermentation could considerably enhance the production of propionic acid, and the proportion of propionic acid to acetic acid was distinctly increased. The molecular ratio was enhanced from 3.80 with none controlled strategy (pH 6.0) to 4.06 with pH-shift control strategy fermentation or 4.96 with fed-batch and pH-shift control strategy.

Furthermore, the propionic acid production with pH-shift control strategy could also reduce the course of fermentation. The fermentation time was shortened from 120 to 108 h (40 g/l of glucose), which was advantageous of the production of propionic acid with this strategy.

Conclusion

In this paper, a two-stage pH-shift control strategy as a regulator was employed to improve the production of propionic acid by *P. freudenreichii* CCTCC M207015. Based on the kinetic analysis of μ_x and μ_p, an optimal pH-shift control strategy was developed as following: pH was controlled at 6.5 during 0–48 h, it was beneficial for the cell growth with maximize of μ_x, then it was shifted to 6.0 after 48 h to maintain the high value of μ_p for the fermentation of propionic acid. By applying this pH-shift control strategy, the propionic acid yield and productivity were increased by 31.75% and 45.90%, respectively, compared with the best results of constant pH process. Considering the formation of byproduct, the acetic acid, the main by-product of propionic acid production, increased with pH increasing (from pH 5.5 to 7.0), while the succinic acid were decreased. Fed-batch was also applied to propionic acid production; the maximal propionic acid yield and glucose conversion efficiency reached 25.23 g/l and 47.76%, respectively.

Acknowledgements This work was supported by the National Basic Research Program of China (973) (2007CB714304), the National High Technology Research and Development Program of China (2006AA03Z453), and the National Nature Science Foundation of China (20674038).

References

1. Kerjean, J. R., Condon, S., Lodi, R., Kakantzopoulos, G., Chamba, J. F., Suomalainen, T., et al. (2000). *Food Research International*, *33*, 281–287.
2. Woskow, S. A., & Glatz, B. A. (1991). *Applied and Environmental Microbiology*, *57*(10), 2821–2828.
3. Jin, Z., & Yang, S. T. (1998). *Biotechnology Progress*, *14*, 457–465.
4. Playne, M. J. (1985). Propionic and butyric acids. In M. Moo-Young (Ed.), *Comprehensive biotechnology* (pp. 731–755). New York: Pergamon.
5. Huang, Y. L., Wu, Z., Zhang, L., Cheung, C. M., & Yang, S. T. (2002). *Bioresource Technology*, *82*, 51–59.
6. Yang, S. T., Zhu, H., Li, Y., & Hong, G. (1994). *Biotechnology and Bioengineering*, *43*, 1124–1130.
7. Rickert, D. A., Glatz, C. E., & Glatz, B. A. (1998). *Enzyme and Microbial Technology*, *22*, 409–414.
8. Roberto, M. C., & Mayra, T. (2002). *Biotechnology Letters*, *24*, 427–431.
9. Himmi, E. H., Bories, A., Boussaid, A., & Hassani, L. (2000). *Applied Microbiology and Biotechnology*, *53*, 435–440.
10. Paik, H. D., & Glatz, B. A. (1994). *Applied Microbiology and Biotechnology*, *42*, 22–27.
11. Czaczy, K., Trojanowska, K., & Grajek, W. (1997). *Applied Microbiology and Biotechnology*, *48*, 630–635.
12. Xu, H., Hong, H. S., Li, S., Xiong, Q., Yang, L., & Ouyang, P. K. (2007, March 13) CN. Patent 2007100205798.
13. Xu, H., Jiang, M., Li, H., Lu, D., & Ouyang, P. K. (2005). *Process Biochemistry*, *40*, 519–523.
14. Hsu, S. T., & Yang, S. T. (1991). *Biotechnology and Bioengineering*, *38*, 571–578.
15. Obaya, M. C., Ramos, J., Villa, P., Valdes, E., & Eng, F. (1994). *Acta Biotechnologica*, *14*(1), 45–51.