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Full Length Research Paper

Evaluation of cassava powder as material for production of calcium gluconate by *Penicillium citrinum* SCG-112

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The feasibility of using cassava powder as the main material for production of calcium gluconate by *Penicillium citrinum* SCG-112 was evaluated in this study. The effect of incubation temperature, initial pH of the medium and inoculum size on production of calcium gluconate was investigated. The maximum yield of calcium gluconate (155 g/L) was obtained after 36 h incubation. The result was both technically competitive and economically attractive.

Key words: Calcium gluconate, cassava, *Penicillium citrinum*.

INTRODUCTION

Calcium gluconate ($C_{12}H_{22}CaO_{14}\cdot H_2O$) finds extensive applications in the pharmaceutical and food industry. Research is ongoing to increase the production of this salt to meet its commercial demand (Bayraktar and Mehmetoglu, 2001). Most studies on calcium gluconate production have focused on the use of pure or easily fermentable substrates such as glucose or sucrose (Mariam et al., 2010; Liang et al., 2010). Due to the high costs of these pure materials, the process is less economic for industrial applications. The production cost of calcium gluconate might be significantly reduced if cheap raw materials could be used, such as starchy and cellulosic materials. Cassava is one of the most efficient crops in terms of carbohydrate production. It is a tropical perennial plant that grows on poor or depleted soils in which the yields of other crops are very low (Peters, 2007). Cassava is very rich in starch. Starch content of cassava root and dry cassava powder reached about 30 and 70%, respectively (Shen et al., 2009). Therefore, cassava has been successfully used as the main material for production of ethanol, lactic acid and sugar etc. (Shanavas et al., 2011; Kostinek et al., 2007; Abdul et al., 2005; Gaouar et al., 1998). Production of calcium gluconate from cassava is a promising strategy, for it will decrease the production cost. However, till now there is still no report regarding adoption of cassava as the substrate for production of calcium gluconate.

In the present study, the starch in cassava was enzymatically hydrolyzed (liquefaction with α-amylase and saccharification with glucoamylase) into glucose, which acted as the main material for production of calcium gluconate by a high-producing calcium gluconate strain, *Penicillium citrinum* SCG-112.

MATERIALS AND METHODS

Strain

P. citrinum SCG-112, a newly isolated calcium gluconate producer, was used in this study for its high yield of calcium gluconate. It was maintained on slants of potato dextrose agar and subcultured every month. The conidial suspension with the spores concentration of 10^8/ml was prepared form 3-4 day old slant of the strain.

Medium and fermentation

Cassava powder with the starch content of about 70% was purchased from local market in China. All other material and

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Table 1. Effect of temperature on production of calcium gluconate from cassava by *Penicillium citrinum* SCG-112.

Temperature (°C)	Maximum yield of calcium gluconate (g/L)	Time consumed to reach the maximum yield (h)
30	100±1.2	64
31	105±1.3	64
32	106±2.0	60
33	108±0.8	60
34	108±0.6	56
35	106±1.5	48
36	108±1.1	44
37	110±0.7	40
38	91±3.4	40
39	82±2.0	36
40	65±1.2	36

chemicals were also commercially available. Cassava powder was mixed with water to prepared cassava slurry with the concentration of 300 g/L. Thermo-stable α-amylase was added into the slurry according to 15 U per g cassava powder. Then the slurry was liquefied by heating it to 110°C and keeping at 110 °C for 5 min, then cooling it to 90°C and keep at 90°C for 2 h. Th e liquefied cassava was centrifugated and squeezed to remove cassava residues. The pH of obtained clarifying solution was adjusted to 4.6-4.8, added gulucoamylase at the ratio of 200 U per g cassava powder. After saccharification at 60°C for 24 h, the s tarch in cassava powder was converted to glucose thoroughly. The final glucose solution diluted to the concentration of 150 g/L with water was used for preparing medium for production of calcium gluconate. The basal medium containing (g/L): glucose 150, $(NH_4)_2SO_4$ 0.5, K_2HPO_4 0.05, $CaCO_3$ 42, natural pH (about pH 5.0). 50 ml medium in 500-ml flasks were autoclaved at 121° C for 15 min. One flask was inoculated with 1 ml spore suspension (10^8 spores/ml) and incubated on a rotary shake with the speed of 250 rpm. When the glucose in the medium reached less than 1 g/L, fermentation was terminated. During the process the sample was withdrawn at regular intervals to determine calcium gluconate yield and glucose concentration. The optimal levels of incubation temperature, initial pH of the medium, inoculum size were determined by varying them in the basal medium.

Analytical methods

Calcium gluconate present in the supernatant sample was determined by disodium ethylene diamine tetra acetic acid (dEDTA) titration. The glucose in the medium was measured by SBA-80C biosensor analyzer (Institute of Biology, Shandong Academy of Sciences, China), which could provide quick measurements of glucose based on technology of the immobilized oxidases. All the experiments were run parallel in a set of triplicates. All values given are means of three determinations ± standard deviation.

RESULTS AND DISCUSSION

Effect of incubation temperature on production of calcium gluconate from cassava by *P. citrinum* SCG-112

As shown in Table 1, 37°C proved to be the best temperature for calcium gluconate in the present study. Incubation at lower temperature resulted in longer time to reach the maximum yield, though the maximum yield was near to the yield at 37°C. Meantime, temperature hi gher 37°C is not conducive to the production of calcium gluconate, neither. The possible reason for the observation is that higher temperature affected the fungus harmfully, and then decreased the calcium gluconate production.

Effect of initial pH on production of calcium gluconate from cassava by *P. citrinum* SCG-112

Table 2 indicated that the optimum pH of calcium gluconate production from cassava by *P. citrinum* was pH 6.5. pH less or more than 6.5 both decreased calcium gluconate synthesis. The possible reason may be that at pH 6.5, the strain grown best and its mycelia produced maximal enzyme glucose oxidase, then brought highest yield of calcium gluconate. These results are in agree-ment with the result reported previously by Sheu et al. (2002), Munk and Hanus (2005) and Mariam et al. (2010).

Effect of inoculum size on production of calcium gluconate from cassava by *P. citrinum* SCG-112

The inoculum size also plays a significant role in the fermentation process. As shown in Table 3, maximum yield was obtained when the inoculum size was 2 ml spore suspension (with the count of 10^8/ ml) per flask. A lower level of inoculum size may not be sufficient for initiating growth and enzyme synthesis. An increase in inoculum size ensures a rapid proliferation of biomass and enzyme synthesis. After a certain limit, production could decrease because of depletion of nutrients due to the enhanced biomass, which would result in a decrease

Table 2. Effect of initial pH on production of calcium gluconate from cassava by *Penicillium citrinum* SCG-112.

Initial pH	Maximum yield of calcium gluconate (g/L)	Time consumed to reach the maximum yield (h)
4.0	60±0.3	48
4.5	86±1.5	44
5.0	94±1.1	44
5.5	110±3.1	40
6.0	120±2.0	40
6.5	133±1.3	40
7.0	121±1.2	40
7.5	112±0.9	44
8.0	105±1.0	44

Table 3. Effect of inoculum size on production of calcium gluconate from cassava by *Penicillium citrinum* SCG-112.

Inoculum size (10^8 spores/mL)	Maximum yield of calcium gluconate (g/L)	Time consumed to reach the maximum yield (h)
0.5	126±0.4	60
1.0	133±0.7	40
1.5	141±1.9	40
2.0	155±2.3	36
2.5	132±1.4	36
3.0	128±1.0	32
3.5	113±0.3	32
4.0	102±1.3	32
4.5	100±0.2	32
5.0	96±0.9	32

in metabolic activity (Kashyap et al., 2002). A balance between the proliferating biomass and available substrate material would yield maximum enzyme.

Conclusion

Based on optimization, the fermentation conditions for production of calcium gluconate from cassava by *P. citrinum* SCG-112 and the maximum yield of calcium gluconate (155 g/L) was obtained after 36 h. This result is significantly competitive compared with the recent relevant report, in which the maximum yield was 110.35 g/L after 72 h incubation. The other advantage of our study is: we used a kind of relatively cheaper material (cassava) while other researchers adopted expensive pure chemicals (such as glucose). Therefore, the study not only brings technical advantage, but also it is economically attractive.

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