FERMENTATION TECHNIQUE AND PH CONTROL STRATEGY FOR IMPROVEMENT OF KOJIC ACID PRODUCTION BY *ASPERGILLUS FLAVUS* USING SAGO STARCH AS CARBON SOURCE

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Introduction

High production of kojic acid using gelatinised sago starch as substrate was achieved in shake flask culture of *A. flavus* (Rosfarizan et al. 1998). However, kojic acid production by this fungus in pilot scale fermenter (50 L) using gelatinised sago starch as substrate was drastically reduced due to non-optimal aeration conditions. High aeration rate (>70-80% saturation) was required during growth phase to produce mycelium that contained high enzyme activities responsible for the conversion of glucose to kojic acid during the production phase (Ariff et al. 1996). In this report, the fermentation technique which can be used to enhance kojic acid production by *Aspergillus flavus* Link 44-1 using gelatinised sago starch in 8 l fermenter is proposed.

Materials and Methods

The fungus, *Aspergillus flavus* Link 44-1, was used for kojic acid production. Gelatinized starch was prepared by heating starch slurry to slightly above 70°C, which is the gelatinisation temperature of sago starch (Haska et al. 1992). Prior to gelatinisation, the pH of the starch slurry was adjusted to 3 using concentrated HCl. All fermentation were performed using 8 l stirred tank fermenter (MBF-800 ME; Eyela Ltd., Tokyo) equipped with temperature, pH and dissolved oxygen controllers. Two fed-batch fermentations with different starch feeding modes were carried out. Both fed-batch fermentations were initiated with 2 l initial batch culture using a medium containing 60 g/l gelatinised sago starch. In the first type of fed-batch fermentation. 1 l of 140 g/l gelatinised sago starch was added after 2 days of fermentation (i,e. gelatinised starch was only added once during the fermentation). In the second type of fed-batch fermentation, 200 ml of 140 g/l gelatinised sago starch was added intermittently at 2 days intervals up to 10 days fermentation (i,e. 200 ml gelatinised starch was added 5 times during the fermentation). The aeration control strategy for optimum kojic acid production was employed in all fermentations (Ariff et al. 1996).

Results and Discussion

Kojic acid reached a maximum concentration (4.51 g/l) after 10 days of batch fermentation. The yield of kojic acid based on starch consumed and overall productivity was 4.51% and 0.45 g/l/d, respectively. Reduced kojic acid production in batch fermentation using gelatinised sago starch as a carbon source may be due to non-optimal aeration conditions. The dissolved oxygen tension (DOT) level during batch fermentation drastically decreased to 0% of saturation after about 2 days fermentation and remained at 0% till the end of the fermentation. This observation indicated that the DOT control system applied was failed to control the DOT at the required value (i.e. 80% saturation during active growth phase). High medium viscosity and imperfect mixing during the initial stages of the fermentation led to low oxygen transfer rate and hence, reduced the DOT level.

In fed-batch fermentation of kojic acid, in which large volume of high concentration of starch was added at 2 days of fermentation, growth profile and maximum cell concentration attained were similar to batch fermentation. The DOT level was only dropped to 0% saturation after about 4 day, indicating that the required DOT level during growth phase was slightly improved compared to batch fermentation but still failed to maintain at high levels (70-80% saturation) during growth phase which is required for optimal kojic acid fermentation. Rapid kojic acid production was observed during non-growing phase and reached a maximum concentration of 16.43 g/l at the time glucose became depleted. Further improvement of kojic acid was achieved by feeding small volume of concentrated starch intermittently to the active fungal culture at two days intervals. In this fermentation run, DOT level was maintained at about 40-50% saturation during the growth phase and dropped to 0% saturation after 6 days. Very high glucose accumulation was obtained after 4 days and then drastically reduced due to consumption for kojic acid synthesis. Rapid kojic acid production was only started after day 4 and reached to a maximum concentration of 31.07 g/l after 15 days fermentation. Obviously, high levels of DOT during growth phase enhance the production of mycelia with a great ability in synthesising kojic acid in which the mycelia effectively converted glucose accumulated into kojic acid during the production phase. Fermentation without pH control (started with initial pH 3 and was not controlled during the fermentation) increased kojic acid production significantly compared to fermentation with single and two-phase pH control strategies.

Conclusions

Different levels of DOT also influenced the secretion of amylolytic enzymes especially α-amylase. High DOT levels during growth phase enhanced α-amylase production and resulted with high accumulation of glucose in the culture. Low glucose accumulation during growth phase in batch fermentation caused very low kojic acid production compared to fed-batch fermentation. The yield and overall productivity of kojic acid production by *A. flavus* using gelatinised sago starch were comparable to those obtained in fermentation of the same fungal strain using pure glucose as a carbon source. Kojic acid production obtained in this study was also comparable to that reported in the literature.

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