

Angkak Production in A Multi-Stage Fixed Bed Fermentation by Using Corn Cob as a Agriculture Residual Substrate

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Abstract

Monascus pigment (Angkak) is a natural red colorant which is usually made by culturing *Monascus spp.* on cooked rice. Changing from rice to corncob is to utilize a residue from agricultural waste. In Thailand, corn is produced many tons a year to be feed in animal farms. This research's objective is to improve a multi-stage fixed-bed fermenter for *Monascus* pigment production on corn cob. The period of solid-state fermentation was three weeks, and the experiment was divided in two parts. In the first part of experiment, corn cob was used as raw materials in a single fixed-bed fermenter with different height of column. The samples were taken and analyzed every week. The result was shown that the maximum pigment and glucosamine was produced at the third weeks. In the first week, the product yield was low and then increased clearly in the second and the third week. The 10 cm. height column was detected the pigments and glucosamine content higher than the 20 and 30 cm height. The metabolic heat of corn cob angkak in the 30 cm height column was higher than the 10 and 20 cm height column when it was monitored by using thermocouples and a datalogger. In the second experiment, the air flow rate was supplied to produce angkak in a three stages of 10 cm. height column vary from 0 to 8.6 L/min in each a fermenter (0, 1.5 ± 0.2 , 5.2 ± 0.2 , and 8.6 ± 0.2 L/min). The results were showed that the moisture content was decreased when the rate of air flow increased, and it affected to decrease the pigment and glucosamine production. The air supply was slightly affected to the different temperature in columns. Therefore, the maximum yield of pigment and glucosamine was shown in the unaerated multi-stage fixed bed fermenter.

Keywords: *Monascus spp.*, *Monascus* pigment, Multi-stage fixed-bed fermenter,

Introduction

Monascus pigment or angkak pigment is a product of rice fermentation by using *Monascus* spp. (red mould). It is a natural red colourant and has been used along with tradition in China and East Asian countries more than 600 years for producing wines and fermented food products (Ma et al., 2000). *Monascus* spp. produced three coloured pigments (yellow, orange and red), and have been used as a natural pigment in fish (surimi and other marine products), Chinese cheese, Chinese red wine, tomato ketchup and meat products (sausages and hams) including non-food as a dyestuff for textiles and leather (Carvalho et al., 2005; Dufossé et al., 2005; Erdooğrul & Azirak, 2004; Panagou et al., 2003; Rosenblitt et al., 2000; Wang & Lin, 2007). The biggest *Monascus* pigment production is in China. In 2010, *Monascus* pigment production in China was 10,000 tons and it was valued at \$150 million. *Monascus* pigment products from China were supplied to Japan, the Republic of Korea, the United States, Mexico and other countries (Luo, 2012).

At present, there is a trend to produce *Monascus* pigment changing from rice as a raw material to other agricultural products such as corn, oat, wheat, cassava, palm kernel, groundnut, adlay, tamarind seed and jackfruit seed. There are many agricultural waste products in Thailand can use to decompose with microbial. Many researches are reported that agro-industrial wastes such as rice bran, wheat bran, coconut oil cake, sesame oil cake, plam kerna el cake, and groundnut oilcake have been screened to select the best substrate for pigment production (Babitha et al., 2007; Velmurugan et al., 2011). Corn cob is a new material select form the central core of maize (*Zea mays* spp. L.) seed head or ear and used as a lingo-cellulosic material which contains amounts of polysaccharides (such as cellulose and hemicellulose) (Harmsen et al., 2010). It is to be an ideal alternative substrate which promote fungal growth and also increase *Monascus* pigment yield. However, the production of *Monascus* pigment on corn cob has been studied very little.

Traditionally, red yeast rice has been produced on a commercial scale in southern China, the Phillippines, and Indonesia by solid state fermentation (SSF), mainly in tray bioreactors (Rosenblitt et al., 2000). Due to the fact that the pigment production of the solid material was slowly, and the transport processes in the bed fermentation are limited to a layer of a few cm in the tray bioreactors. It also took much longer and has higher labour costs. However, product concentrations obtained in SSF are much higher than in submerged fermentation, potentially leading to reduced costs of product recovery and lower water and energy use. Therefore, SSF is an effective alternative for submerged fermentation (Dominguez-Espinosa & Webb, 2003; Rosenblitt et al., 2000). Temperature, pH, humidity and oxygen concentration are important factors to influence the growth of microorganisms and pigmentation (Carvalho et al., 2006). The objective of this work is varying different air flow rate to improve the SSF production process on corn cob in a multi-stage fixed bed fermentation.

Material and Method

Microorganism

Monascus ruber (strain TISTR3006, obtained from Thailand Institute of Scientific and Technological Research, Thailand) was used to produce *Monascus* pigment. Strain preservation and spore formation were performed on Potato Dextrose Agar (PDA) (Merck, Germany). Spore suspensions were prepared with 10^5 CFU/mL as seed cultures.

Substrate and solid-state fermentation

Corn cobs were obtained from local agricultural fields in Chiang Mai, Thailand. Cobs were washed thoroughly, dried with hot air at 60°C for a day and ground with a hammer mill (armfield FT2, UK) to around 3 mm. particle size. Corn cob was boiled in boiling water for 15 minute and then transfer to a plastic bag. After that, the material was sterilised at 121°C for 15 minutes in an autoclave (Daihan labtech, LAC-5100SD, Korea), cooled to room temperature. The initial moisture content was controlled at 87% in plastic bags. The 5 mL of spore suspension was added and mixed in the bag. Then, the material was incubated at 30°C for a day and transferred in a bioreactor to ferment at room temperature ($\sim 30 \pm 2^{\circ}\text{C}$) until three weeks. The heat production during fermentation was monitored by using thermocouples and a datalogger. *Monascus* products was dried, grounded and taken as a sample for analysis.

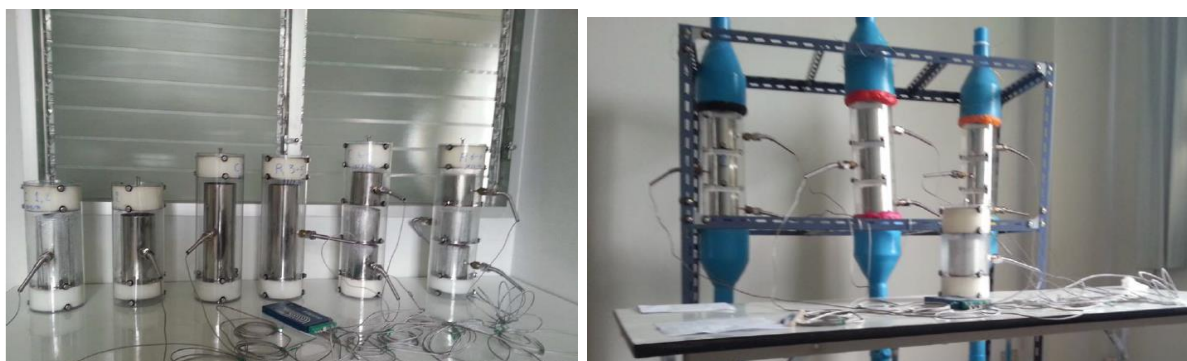


Figure 1 Experimental setups for *Monascus* pigment production in solid state culture

Biomass concentration

Fungal biomass was also estimated the N-acetyl glucosamine is found in the cell wall of fungi and it also estimate fungal biomass by determining its concentration in the sample (Carvaiho et al., 2006; Babitha et al., 2007). The glucosamine concentration is therefore considered to be representative of the fungal cell concentration in foods. Firstly, a 0.1 g from a pigment powder sample was subjected to acid hydrolysis by dissolving it in 5 mL of 5 M HCL for 16-18 hours until the solution was clear. The acid hydrolysis sample was then mixed with 1 mL acetylacetone reagent and incubated in a bath of boiling water for 20 minutes. After cooling, 1.5 mL ethanol was added followed by the addition of 1 mL of Ehrlich reagent. After

that, the optical density was measured by using a UV-visible spectrophotometer (Algilant Cary 60 cary, Malasia) at 530 nm against a reagent blank. N-acetyl glucosamine obtained from Sigma-Aldrich was used as the standard (Babitha et al., 2007).

Pigment concentration

Fermented corn cob was dried in the oven at 55°C overnight until the weight was constant. 0.5 g pigment powder (angkak powder) was dissolved with 5 mL of 70% (v/v) ethyl alcohol and shaken for eight hours on a reciprocal shaker (Daihan Labtech LSI-3016R, Korea) at 150 rpm. Supernatants were obtained by centrifugation (Nuve NF200 bench top, Turkey). Optical densities of yellow, orange and red pigments were estimated using a UV-visible spectrophotometer (Algilant Cary 60 cary, Malasia) at 400, 470 and 500 nm respectively (modified from Kim et al., 2002; Pattangul et al., 2008; Yongsmith et al., 2000).

Moisture content

The moisture content of cooked corn cob and fermented corn cob (angkak) was measured by drying a sample in an oven (Daihan Labtech LSI-3016R, Korea) at 105°C for 24 hours. The weight loss was measured after the sample had been cooled to room temperature in a desiccator. The moisture content of material was calculated at the constant weight. The results (n=3) are expressed as a percentage (AOAC, 1995, Johns & Stuart, 1991; Maritotti et al., 2006).

$$\% \text{Moisture} = \frac{(\text{Original weight of sample} - \text{Dried weight of sample}) \times 100}{\text{Original weight of sample}}$$

Results and discussion

Biomass and pigment production on corn cob at different column heights

Figure 2 shows the effect of three different height columns at 10, 20 and 30 cm. on pigment and biomass production on corn cob for three weeks in a fixed bed fermentation. Moisture content of the bed tended to reduce when fermentation period increased. Pigment value and glucosamine content changed with time. The rate of pigment and glucosamine production increased in the first week and dropped in the second and third week.

The pigment value and glucosamine content were detected slightly different in each height of column. The pigment values and glucosamine content in the 10 cm. of height column is higher than the 20 and 30 cm height. In the 10 cm. height column, the yellow, orange and red pigment concentrations were 7.32, 7.49 and 11.09 units per gram respectively and 42.13

mg per gram of glucosamine content. Temperature in 10, 20 and 30 cm. height column maintain around 32°C. The temperature in 30. cm height column was slightly higher than 10 and 20 cm. height column.

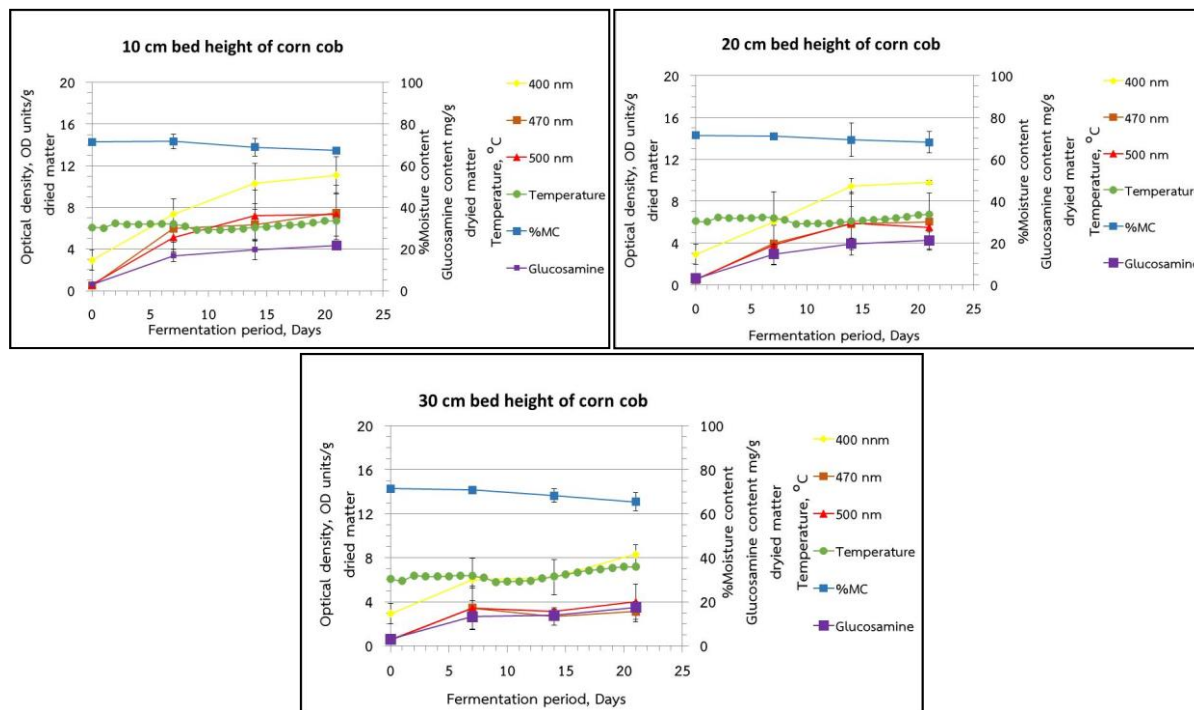


Figure 2 *Monascus* pigment production on corn cob in difference bed heights

Biomass and pigment production on corn cob at different air flow rate

Figure 3 shows the effect of four different aeration rates at 0, 1.5, 5.2 and 8.6 L min⁻¹ on pigment and biomass production on corn cob after three weeks. Obviously, the high air flow rate was decrease moisture content especially at the bottom bed of fermenter. Pigment and glucosamine production were also decreased when the air flow rate increase from 0 to 8.6 L min⁻¹. The best condition cob in a multi-stage fixed bed fermentation may be slightly air flow rate or no airflow. No air flow condition gave yellow, orange and red pigment concentrations of 3.69, 4.08 and 12.20 units per gram respectively and 31.48 mg per gram of glucosamine content. Farhan et al. (2010) reported that the greatest pigment production occurred at 0.2 L min⁻¹ in 0.05 m diameter packed bed column. Too high of aeration rates (higher than 2.0 L min⁻¹ in packed bed column) may improve oxygen supply but also tended to rapidly reduce the moisture content of the bed so much so as to reduce biomass and pigment production. However, the high air flow rate at 8.6 L min⁻¹ air flow in 0.10 m diameter multi-stage fixed bed fermenter, which had been still pigment value and glucosamine, is better than 2.0 L min⁻¹ in packed bed column. Temperature was not reported because it is not much change in different condition.

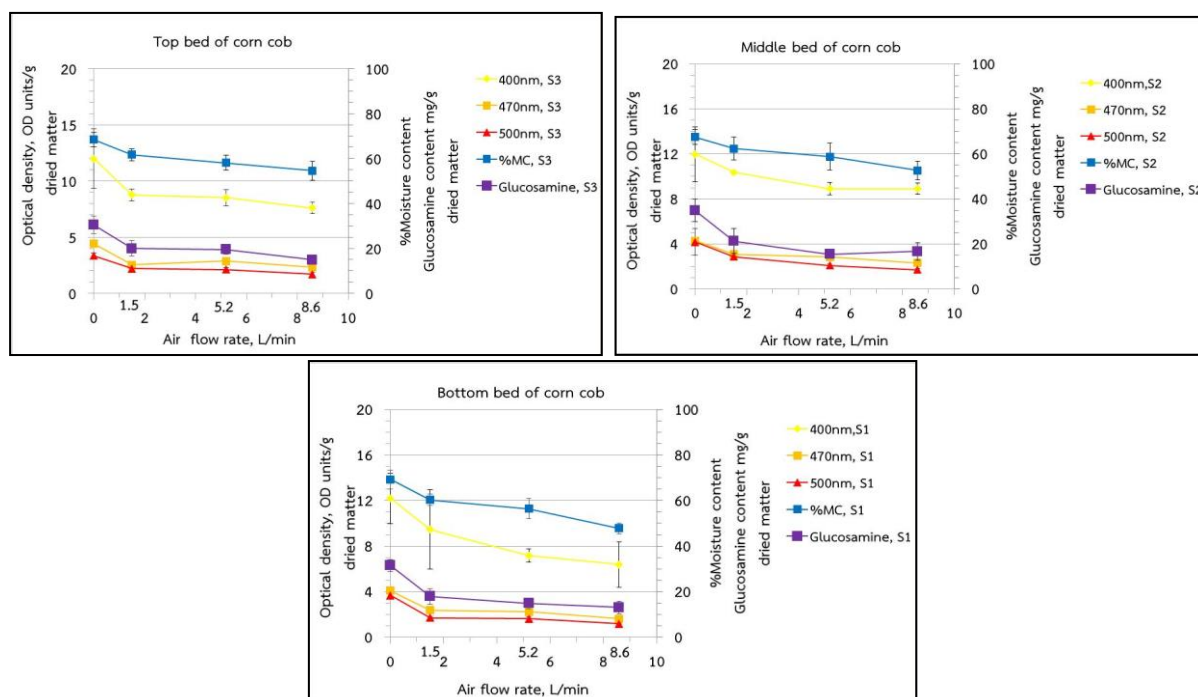


Figure 3 *Monascus* pigment production on corn cob in difference air flow rates

Conclusions

Moisture and oxygen content were the main factor strongly which influenced on biomass growth and pigment production. Too long bed column may not produce a pigment production at the bottom due to limitation of oxygen diffuse through the material bed. Too high air flow passes the bed column reduced the moisture content of fermenter and effect on metabolic heat temperature. It was also give negative effect on biomass growth and pigment production.

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Reference

- AOAC, (1995). Anon., (1995). *Official Methods of Analysis*, 16th ed. Gaithersburg, MD, USA: Association of Official Analytical Chemists.
- Babitha, S., Soccol, C.R., & Pandey, A., (2007). Solid state fermentation for the production of *Monascus* pigments from Jackfruit seed. *Bioresource Technology*, 98(8), 1554-1560.
- Carvalho, J.C.D., Oishi, B.O., Pandey, A., & Soccol, C.R. (2005). Biopigments from *Monascus*: strains selection, citrinin production and color stability. *Brazilian Archives of Biology and Technology*, 48(6), 885-894.

- Carvalho, J.C.D., Pandey, A., Oishi, B.O., Brand, D., Rodriguez-Leon, J.A., & Soccol, C.R. (2006). Relation between growth, respirometric analysis and biopigments production from *Monascus* by solid-state fermentation. *Biochemical Engineering Journal*, 29(3), 262-269.
- Dominguez-Espinosa, R.M., & Webb, C. (2003). Submerged fermentation in wheat substrate for production of *Monascus* pigment. *World Journal of Microbiology and Biotechnology*, 19(3), 329-336.
- Dufossé, L., Galaup, P., Yaron, A., Arad, S.M., Blanc, P., Murthy, K.N.C., & Ravishankar, G.A. (2005). Microorganisms and microalgae as sources of pigments for food use: a scientific oddity or an industrial reality. *Trends in Food Science and Technology*, 16(9), 386-406.
- Erdooğrul, Ö., & Azirak, S. (2004). Review of the studies of the red yeast rice (*Monascus perpureus*). *Turkish Electronic Journal of Biotechnology*, 2, 37-49.
- Harmsen, P. F. H., Huijgen, W. J. J., Bermúdez López, L. M., & Bakker, R. R. C. (2010). *Literature review of physical and chemical pretreatment processes for lignocellulosic biomass*. Wageningen, Netherlands: Wageningen UR Food & Biobased Research.
- Johns, M. R., & Stuart, D.M. (1991). Production of pigments by *Monascus purpureus* in solid culture. *Journal of Industrial Microbiology*, 8(1), 23-28.
- Kim, H.J., Kim, J.H., Oh, H.J., & Shin, C.S. (2002). Morphology control of *Monascus* cells and scale-up of pigment fermentation. *Process Biochemistry*, 38(5), 649-655.
- Luo, T.T. (2013) Molecular biology into *Monascus* research opportunity. Retrieved from <http://www.scienanet.en/sbhtm/news/20112/12/266656.shtm>.
- Ma, J., Li, Y., Ye, Q., Li, J., Hua, Y., Ju, D., Zhang, D., Cooper, R., & Chang, M. (2000). Constituents of red yeast rice, a traditional Chinese food and medicine, *Journal of Agricultural Food Chemistry*, 48(11), 5220-5225.
- Mariotti, M., Alamprese, C. Pagani, M.A., & Lucisano, M. (2006). Effect of puffing on ultrastructure and physical characteristics of cereal grain and flours. *Journal of cereal science*, 43(1), 47-56.
- Panagou, E. Z., Skandamis, P. N., & Nychas, G.J.E. (2003). Modelling the combine effect of temperature, pH, and a_w on the growth rate of *Monascus ruber*, a heat-resistant fungus isolated from green table olives. *Journal of Applied Microbiology*, 94(1), 146-156.
- Pattangul, P., Pinthong, R., Phianmongkhol, A., & Tharatha, S. (2008). Mevinolin, citrinin and pigments of adlay angkak fermented by *Monascus* sp. *International Journal of Food Microbiology*, 126(1-2), 20-23.
- Rosenblitt, A., Agosin, E., Delgado, J., & Pérez-Correa, R. (2000). Solid substrate fermentation of *Monascus purpureus*: growth, carbon balance, and consistency analysis. *Biotechnology Progress*, 16(2), 152-162.

- Said, F.M., Chisti, Y., & Brooks, J. (2010). The effects of forced aeration and initial moisture level on red pigment and biomass production by *Monascus ruber* in packed bed solid state fermentation. *International Journal of Environmental Science and Development*, 1(1), 1-4.
- Velmurugan, P., Hur, H., Balachandar, V., kamala-Kannan, S., Lee, K.-J., Lee, S.M., Chae, J.-C., Shea, P. J. , & Oh, B. - T. (2011). *Monascus* pigment production by solid state fermentation with corn cob substrate. *Journal of Bioscience and Bioengineering*, 112(6), 590-594.
- Wang, T.H., & Lin, T.F. (2007). *Monascus* rice products. *Advances in Food and Nutrition Research*, 53, 123-159.
- Yongsmith, B., Kitprechavanich, V., Chitradon, L., Chairsisook, C., & Budda, N. (2000). Color mutants of *Monascus sp.* KB9 and their comparative glucosamylases on rice solid culture. *Journal of Molecular Catalysis B: Enzymatic*, 10(1-3), 263-272.