

Production of *Monascus* Pigment in low cost fermentation

T. Raja Rajeswari, V. Ponnusami and K.R. Sugumaran*

School of Chemical and Biotechnology, SASTRA University, Thanjavur – 613402,
Tamilnadu, India.

*Corres.author: sugumaran@biotech.sastra.edu

Abstract: Colorants are considered to be important in food industries. Edible pigments of *Monascus* sp have an established history of use in certain Asian foods. *Monascus* sp cultivated on starch rich substance. In this study, both solid and submerged fermentation were investigated and it was observed that maximum pigment was obtained in solid state (0.59AU/gds) than in submerged fermentation (0.34AU/gds). Corn and sugarcane bagasse were observed to be the best substrate for monascus pigment. The *Monascus* pigment production by varying the initial pH of fermentation broth was determined, initial pH 3.6 for corn and 5.6 for sugarcane bagasse. The initial pH on pigment production was substrate related.

Key words: *Monascus* pigment, initial pH, low cost fermentation.

Introduction

Requirements of colorants are increasing day by day in human lifestyle. People always have argued on two types of colorants (natural and synthetic). Preference of natural colorants has been high concern because by studying the harmful effect of synthetic colorants. Pigments are derived from plant, animals and microorganism. *Monascus* pigments have been traditionally used in food application.

Monascus is a filamentous fungus belonging to the genus *Monascus*, family monascaceae and class ascomyceta. This has the power to synthesis secondary metabolites as the bio pigments with connected polyketide structure. *Monascus* sp produces six primary pigments, the colours of which are yellow (anka flavin, monascine), orange (rubropunctatin, monascorubrine) and red (rubropuntamine, monascorubramine) pigment¹. The color produced by *Monascus* sp depends on some environmental factors during the fermentation process. *Monascus* pigments are structurally related, for example red pigment is derivative of orange pigment. *Monascus* acquire on starch sample substance, both in solid and submerged fermentation gives pigment production. Liquid or semi-solid medium can be used for submerged fermentation for desirable products. Fermentation conditions such as aeration, agitation and pH should be controlled during submerged fermentation. Agro industrial residues and crop such as coconut oil cake, corn cob, rice bran, jack fruit seed, groundnut oil cake, wheat bran were used as substrate this resulting in lower price fermentation and high pigment output.²

Monascus pigment was used in food and therapeutic application³. Recent analyses account that *Monascus* metabolites has a lessened blood level of cholesterol⁴, antioxidant activity, antimicrobial activity, antimalarial and antifungal⁵. *Monascus* metabolites lessened insulin and blood levels in a group of type II diabetes⁶.

2. Materials and Methods

2.1 Culture collection

Monascus purpureus (MTCC 369) purchased from IMTECH Chandigarh, India was grown on PDA plates and slants at 4 °C and sub cultured 30 days once.

2.2 Fermentation

Substrates were purchased from Thanjavur local market, cleaned and sundried for 3 days and cut it into small pieces and these pieces were used for studies.

Five percentage (v/v) of fully sporulated (7-8 days old) PDA plate culture was suspended in sterile distilled water and this suspension was used as inoculums. Optimization studies was directed in 250 ml erlenmeyer flask containing production media⁷. The above media was autoclaved, inoculated & incubated at 30 °C for 7 days.

2.3 Extraction and estimation of pigments

Pigment appraisal was done as described by (Babitha, Soccol, & Pandey, 2006)⁸ in which pigment from the fermented substrate with *monascus purpureus* was extracted using 90 % ethanol. The extraction was done by shaking at 30 °C for 3 hrs. The insoluble debris was filtered and the absorbance of the supernatant was found out spectrophotometrically for quantitating the pigments. Optical density was determined at representing wavelength and expressed as value per gram of dry fermented substrate (gdfs). Calculated the pigment yield as OD units.

3. Result and Discussion

3.1 Selecting suitable method and screening of substrates for the preparation of pigment

Fig 1 and 2 shows that solid-state yielded greater amount of pigment^{9,8} compared to submerged fermentation. This phenomenon had been attributed to the pigment because of low solubility in the fermentation medium and pigment accumulation within the mycelium in submerged culture¹⁰. In contrast, in solid-state fermentation, the *Monascus* sp penetrated into solid substrate and pigment released onto the surface. Solid state not only issued nutrient, but also acted as anchorage for cells, this may have bestowed to the celebrated eminent productivity. Solid state environment is similar to environment of fungi which was normally excited. (Hamano & Kilikian, 2006)¹¹ obtained high pigment production from corn steep liquor. (Daroit, Silveira, Hertz, & Brandelli, 2007)¹² obtained pigment concentration from grape waste. (Velmurugan et al., 2011)⁷ reported that pigment yield from potato powder. (Sharmila et al., 2013)¹³ reported pigment production from corn cob. (El-batal & Al-habib, 2012)¹⁴ reported that palm fruit extract showed good yield from *Monascus purpureus*. Tropical agro industrial residues and crops are tested. Among the substrate tested, corn and sugarcane bagasse showed better yield in solid-state fermentation compared to submerge. Corn and sugarcane bagasse was taken for further studies.

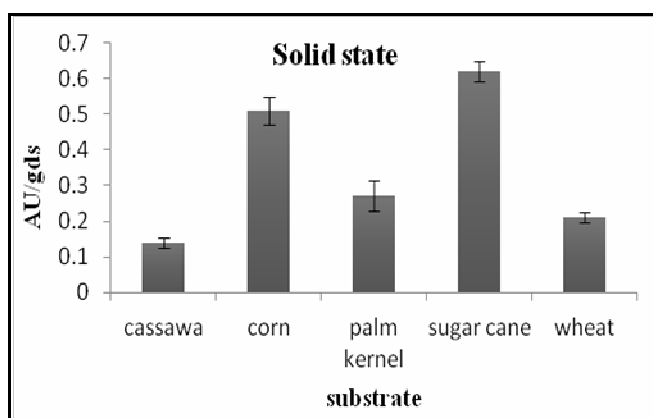


Fig 1 Effect of solid and substrate on pigment production

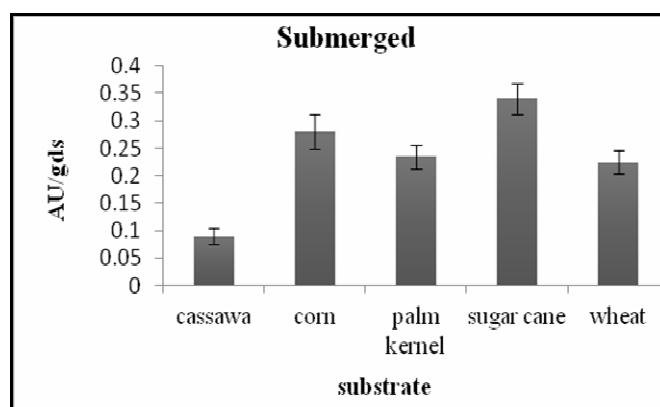


Fig 2 Effect of submerged and substrate on pigment production

3.2 Effect of pH on pigment production

Initial pH in fermentation medium influenced the pigment synthesis. Various pH ranges were subjected for pigment production in that, pH 5.6 for sugarcane bagasse and 3.6 for corn showed better yield respectively. From (Babitha, Soccol, & Pandey, 2007)¹⁵ report buffering nature of the substrate changes the initial pH which plays role in change in pigment production.

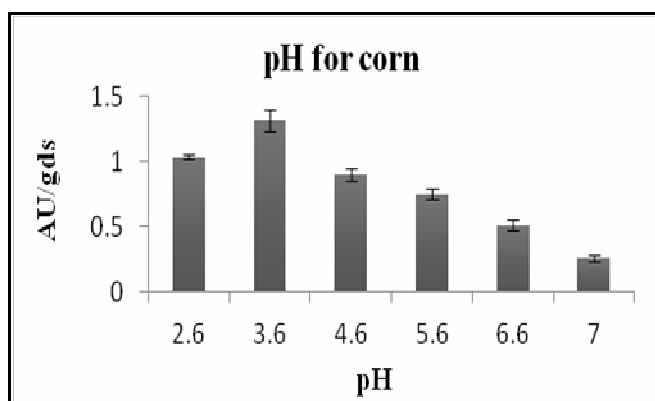


Fig 3 Effect of pH on corn

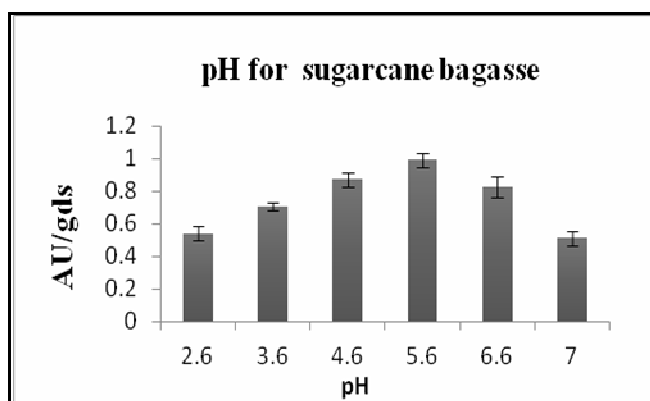


Fig 4 Effect of pH on sugarcane bagasse

3.3 Solubility of pigment

Solubility of pigment was tested using three different solvents such as water, methanol and chloroform. Condensed form of ethanol extract was completely soluble in water and partially soluble in methanol and chloroform. The chloroform suspension was yellow in colour, methanol suspension was orange in colour and water soluble was red in colour.

4. Conclusion

From this study it was concluded that agro industrial waste and residue could be an alternative option for monascus pigment production. The use of corn and sugarcane bagasse as substrate was cost effective and environmental friendly. Maximum production was obtained in solid-state fermentation having initial pH as 3.6 for corn and 5.6 for sugarcane bagasse.

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