

The effects of forced aeration and initial moisture level on red pigment and biomass production by *Monascus ruber* in packed bed solid state fermentation

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Abstract—Colorants produced by various *Monascus* sp. are well established in Asiatic foods. Industrial production of *Monascus* pigments involves expensive submerged culture fermentation. A less expensive alternative is the use of solid state fermentation. This study reports on color production by *Monascus ruber* ICMP 15220 in packed bed solid state culture. The fungus was grown in packed beds (0.05 m in diameter and 0.17 m in depth of substrate) of moistened rice. The bed of rice was continuously aerated by sparging with humidified air (95–97% relative humidity). The effects of different aeration rates (0.05–2.0 L min⁻¹) and initial moisture level (45, 57.5, 70% (w/w)) in the substrate, on color production are reported. The packed bed solid state fermentations were carried out at a nominal temperature of 30 °C. A forced aeration rate of higher than 0.5 L min⁻¹ reduced the production of the pigments and biomass as a consequence of water loss from the bed. Highest levels of pigments were obtained at forced aeration rates of between 0.05 and 0.2 L min⁻¹. Under these conditions, the pigment content reached ≥ 98 AU per g dry matter in the bed. An initial moisture level of 45% (i.e. g water per 100 g wet substrate) resulted in very low production of pigments and biomass with final moisture content of 29.6%. An initial moisture level of 70% gave the highest pigment production in the fermented dry solids and also the highest pigment productivity, which were 1,415 AU per g dry matter and 101 AU per g dry matter per day, respectively. The results suggest that forced aeration rate and initial moisture level in the substrate have a strong influence on pigment production in solid state fermentation of *M. ruber*.

Index Terms—Aeration rate, food colorants, *Monascus ruber*, solid state fermentation.

I. INTRODUCTION

Species of the fungus *Monascus* are used to produce intense red color in some Asiatic foods. Commercial production of *Monascus* pigments as food colorants relies on

Manuscript received December 19, 2009. This work was supported in part by the Ministry of Higher Education Malaysia.

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a relatively expensive submerged fermentation. A less expensive alternative is the use of solid state fermentation (SSF) but this is difficult to scale up. Solid state fermentations involve a moistened solid substrate in the absence or near absence of free water [9], [11], [12]. Microbial species used in SSF are generally filamentous fungi because they can grow in a relatively dry environment and can spread over and penetrate the solid substrate without needing excessive agitation. Fungi are obligate aerobes and require a continuous supply of oxygen to grow. Oxidation of the substrate by the fungus produces heat that must be removed to prevent temperature rise to a level that could kill the fungus. Removal of heat in the absence of flowing water can be a significant challenge [4], [15]. Potentially, beds of solid substrate can be aerated and cooled using a forced flow of moistened air [8], [10], [14]. This work reports on the effects of forced aeration rate and initial moisture level of the substrate on the production of biomass and pigments by *M. ruber* grown in packed beds of rice.

II. MATERIAL AND METHODS

A. Microorganism

Monascus ruber ICMP 15220 isolated from a palm kernel in Auckland, New Zealand, was used. The fungus was maintained on potato dextrose agar (PDA) plates kept at 4 °C and subcultured once every three weeks.

B. Inoculum preparation

Potato dextrose agar (PDA) slants inoculated with the fungus were incubated for 7 days at 30 °C to achieve full sporulation. Sterile distilled water (10 ml) was added to a slant and the spores were scraped into the water. This spore suspension was used as the inoculum. The suspension contained approximately 10⁷ spores per mL.

C. Substrate preparation and inoculation

Polished long grain rice (Sun Rice, premium white long grain rice, product of Australia) was purchased locally. 110 g of rice was placed in a 500 ml beaker and distilled water was added to achieve the required initial moisture level of 45, 57.5 and 70% w/w (g water per 100 g wet substrate). In all cases, the water contained 11 mL of 0.128 M ZnSO₄ solution. Rice was soaked for 1 h at 30 °C. The rice was then autoclaved at 121 °C for 15 min, cooled to room temperature

and inoculated with 11 mL of spore inoculum (10^6 spores per g dry substrate). The inoculated substrate was placed in glass packed bed columns (0.05 m in diameter, 0.22 m empty height) that had been autoclaved ($121\text{ }^\circ\text{C}$ for 25 min) prior to use. The packed bed bioreactors were incubated at $30\text{ }^\circ\text{C}$ for the specified number of days. Rice beds with constant initial moisture level of 45% were aerated at various constant rates of 0.05, 0.2, 0.5, 1.0 and 2.0 L min^{-1} using humidified air (relative humidity of 95–97%). Various initial moisture levels of 45, 57.5 and 70% were aerated using constant aeration rates of 0.2 L min^{-1} .

D. Biomass estimation

The amount of fungal biomass in the bed of fermenting substrate was determined by measuring the *N*-acetyl glucosamine released as a consequence of acid hydrolysis of chitin present in the cell wall of the fungus [13]. Fermented solids were freeze dried at $-80\text{ }^\circ\text{C}$ (24 h) and milled at room temperature to a powder using a grinder.

0.5 g of ground solids were mixed with concentrated sulfuric acid (2 mL, 60% sulfuric acid) and the mixture was then kept at $25\text{ }^\circ\text{C}$ for 24 h. This mixture was then diluted with distilled water to produce a 1 N solution of sulfuric acid and autoclaved at $121\text{ }^\circ\text{C}$ for 1 h. The cooled mixture was neutralized with 1 N NaOH to pH 7 and the final volume was made up to 60 mL with distilled water.

The resulting solution of 1 mL was filtered through a membrane filter $0.45\text{ }\mu\text{m}$, mixed with 1 mL of acetyl acetone reagent (2% v/v of acetyl acetone in 1 N Na_2CO_3) and incubated in a boiling water bath for 20 min. After cooling, ethanol (6 mL) was added followed by 1 mL of Ehrlich reagent (2.67% w/v of *p*-dimethylaminobenzaldehyde in 1:1 mixture of ethanol and concentrated HCl). The resulting solution was incubated at $65\text{ }^\circ\text{C}$ for 10 min. The solution was then cooled to room temperature and its optical density was read at 530 nm against the reagent blank by using a spectrophotometer [13].

Glucosamine measurements of dried solids were made during the course of the fermentation. Measurements from a submerged culture fermentation that involved only dissolved components in the medium were used to convert the glucosamine measurements to the concentration of dry biomass. Based on data of the submerged culture, 340.59 mg of glucosamine corresponded to 1 g dry fungal biomass.

E. Pigment extraction and analysis

1 g of dried ground fermented solids was extracted with 10 mL of 95% ethanol in a shake flask (180 rpm for 2 h). The extract was then centrifuged at 7,540-g for 20 min to remove suspended solids. The supernatant was measured by a spectrophotometer using 95% ethanol as blank [5], [7], [2]. Pigment concentration was measured using a double beam spectrophotometer (Shimadzu, UV 1601) at 500 nm for the red pigment. The results were expressed as absorbance units (AU) per gram of dried solids, correcting for any dilution [6].

F. Moisture content determination

The moisture content of fermented solids (1 g) was determined by heating the solids in a pre-dried and weighed pan in a hot air oven at $105\text{ }^\circ\text{C}$ for 24 h. The weight loss was measured after the sample had been cooled to room temperature in a desiccator [5], [16].

G. Water activity (a_w) determination

The water activity of fermented solids was determined at room temperature using a Aqualab water activity meter (Decagon Devices, Model CX-2, Pullman, Washington, USA). The equipment was calibrated with saturated salt solutions in the a_w range of interest (Decagon Devices, Model CX-2 Manual). Each measurement was in duplicate. Under these conditions the accuracy of the measurement was $\pm 0.003 a_w$.

III. RESULTS AND DISCUSSION

A. Biomass and pigment production at different aeration rates

Figure 1 shows the effect of five different forced aeration rates on pigment and biomass production at day 6 of a batch packed bed solid state fermentation. Although a peak biomass production occurred at an aeration rate of 0.05 L min^{-1} , pigment production rate peaked at a higher aeration rate of 0.2 L min^{-1} . Higher aeration rates adversely affected both the production of pigment and the biomass. An aeration rate of 0.05 L min^{-1} appeared to be clearly sufficient for providing the fungus with oxygen and removing carbon dioxide [3]. Aeration rates of higher than 0.2 L min^{-1} may have improved oxygen supply but also tended to rapidly reduce the moisture content of the bed (Fig 2) so much so as to reduce biomass and pigment production. Good production of pigment and biomass appeared to require the substrate moisture content to be maintained at between 65 and 70% (Fig 2).

Thus greatest pigment production occurred at 0.02 L min^{-1} , corresponding to a bed moisture content of 62%. Increasing rates of aeration appeared to aid heat removal as indicated by the temperature of the bed on day 6 (Fig 2). The high temperature values on day 6 at aeration rates of 0.05 L min^{-1} and 0.2 L min^{-1} suggest a high rate of metabolism and a possible relatively low rate of heat removal.

At these low aeration rates, the high temperature in the bed (Fig 2) did not adversely affect biomass growth and pigment production (Fig 1). A relatively good production of pigment throughout the bed at an aeration rate of $\leq 0.2\text{ L min}^{-1}$ is clearly seen in Figure 3.

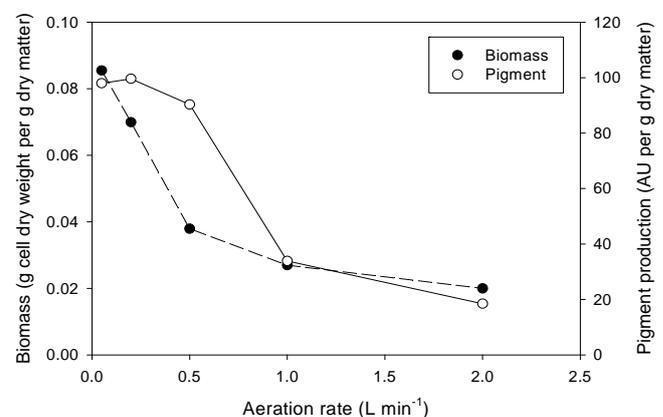


Fig. 1: Pigment and biomass production (day 6) at different aeration rates

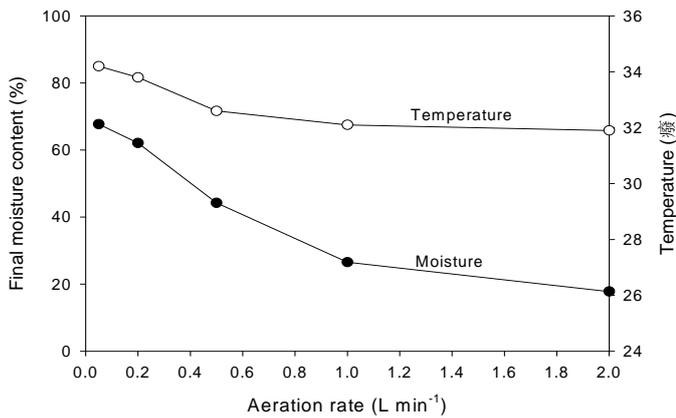


Fig. 2: Temperature and final moisture content (day 6) at different forced aeration rates. The temperature was measured in the center of the bed at a height of 6 cm from the bottom of bed.

B. Biomass and pigment production at different initial moisture level

As shown in Figs 4 and 5, production of biomass and pigments was strongly affected by the initial moisture level. An initial moisture level of 70% was clearly the best for maximizing the production of biomass and pigments at a constant aeration rate of 0.2 L min⁻¹. There was a slight increase of final moisture content and water activity during the fermentation. Pigment production reached 1,415.7 AU per g dry matter.

Lower moisture levels adversely affected fungal growth along with pigment production. Decreasing the initial moisture level to 57.5% resulted in a fairly constant moisture content of the bed with slight reduction of the water activity (Figs 6 and 7), but pigment production was reduced to 734.1 AU per g dry matter.

An initial moisture level of 45% resulted in a decrease in moisture content and water activity of the bed over the 18 day fermentation. Figures 6 and 7 show that at the end of fermentation on day 18, the moisture content and water activity of the bed were only 29.6% and 0.80, respectively. Thus least pigment production (170.7 AU per g dry matter) occurred at 45% initial moisture level.

The peak production of pigment and biomass was attained by day 14 at an initial moisture level of 70%. Continuing the fermentation to day 18 did not substantially enhance the production of biomass or pigment. Data in Figs 4 and 5 were obtained at a fixed aeration rate value of 0.2 L min⁻¹ that had proved optimal in earlier studies (Fig 1). All fermentations exhibited a lag phase of about 6 days. Growth was rapid from day 6 to day 10. At initial moisture level of 70% the maximum productivity of the pigment was 101 AU per g dry matter per day and product formation per unit biomass, q_p was found to be 90.7 AU per g cell dry weight per hour.

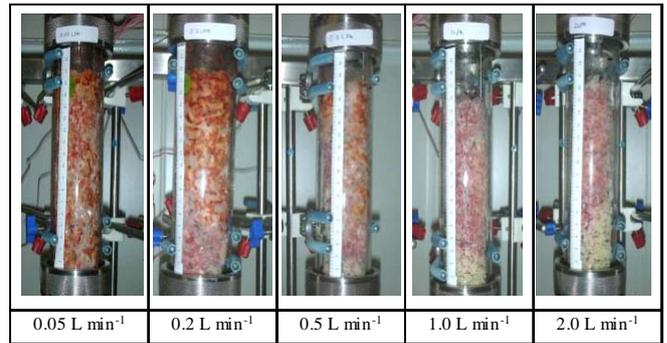


Fig. 3: Red color development (day 6) in the packed beds at different forced aeration rates (the initial moisture level was 45%).

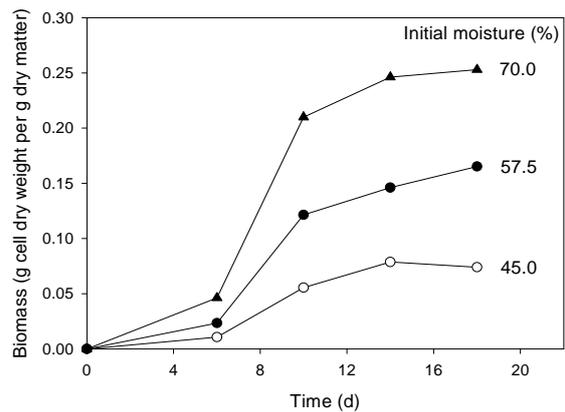


Fig. 4: Biomass production at different initial moisture level

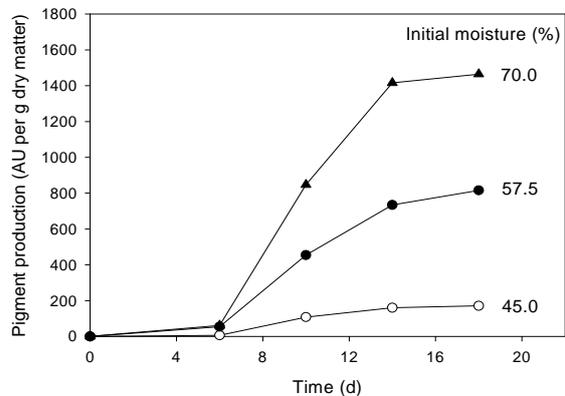


Fig. 5: Pigment production at different initial moisture level

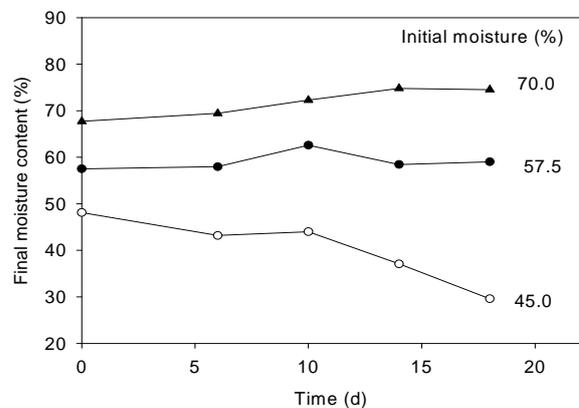


Fig. 6: Final moisture content at different initial moisture level

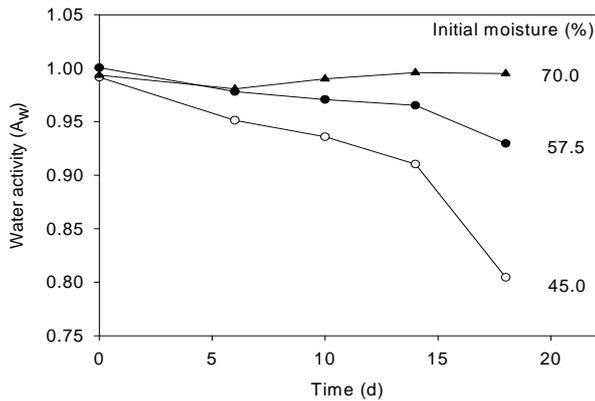


Fig. 7: Water activity at different initial moisture level

Initial moisture level of > 70% was not examined because according to Carvalho et al. (2007) at $\geq 80\%$ of moisture, the culture medium becomes too wet and free water is present. Under these conditions, aeration becomes less efficient, as tracking in the bed becomes a problem. Such conditions could no longer be considered to be solid state fermentation.

IV. CONCLUSION

Moisture content and water activity of the bed had a strong influence on biomass growth and pigment production. The optimal initial moisture level of 70% effectively stabilized the final moisture content at around the same value and prevented a decline in water activity of the bed during operation. This ensured a good biomass growth and biomass specific production of the pigment. The identified optimal initial moisture level did not flood the bed to adversely affect diffusion of oxygen. Aeration rate was another major influence on the productivity of this packed-bed solid state fermentation.

ACKNOWLEDGMENT

The authors acknowledge the financial support from Ministry of Higher Education Malaysia.

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