ISSN:2521-0858 (Print)
ISSN:2521-0866(Online)

Contents List available at RAZI Publishing

Science Heritage Journal / Galeri Warisan Sains (GWS)

Journal Homepage: http://www.razipublishing.com/journals/galeri-warisan-sains-gws

https://doi.org/10.26480/gws.01.2017.13.15

Red Pigment Production By Monascus Purpureus In Stirred-Drum Bioreactor

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ARTICLE DETAILS

Article history:

Received 27 September 2016
Accepted 13 December 2016
Available online 10 January 2017

Keywords:

Monascus purpureus, oil palm fronds, red pigments, stirred drum bioreactor, solid state fermentation.

ABSTRACT

Several studies has been conducted to economically cultivate the Monascus sp. However, the potential of using stirred drum bioreactor in solid state fermentation (SSF) for Monascus sp. cultivation has been relatively understudied. Oil palm frond (OPF) petiole has been used as a potential substrate due to its nutritional contents and to add more value to local agricultural waste. This study reports the production of red pigment by Monascus purpureus FTC 5357 in a 2.3 L bench top - stirred-drum bioreactor. The fungus was grown on moistened OPF substrate (60 % (w/w)) supplemented with 2% (w/w) of soy meal peptone. The effects of different aeration rates (0.3-1.0 vvm of humidified air), agitation programme (4-8 cycles per day), and substrate load capacity (25-40 % (v/v) of total drum capacity) on red pigment production are reported. Aeration rate showed a positively correlated interaction to red pigment production in which the highest red pigment were produced using1.0 vvm (6.09 AU/g dry solid), and non-aerated culture showed the lowest red pigment production (0.81 AU/g dry solid). The agitation programme was also showing the positive trend of interaction, in which 8 cycles per day showed the highest red pigment production (4.34 AU/g dry solid) and 4 cycles per day agitation showed the lowest red pigment production. The red pigment production was peaked at 30% (v/v) drum loading capacity (5.61 AU/g dry solid) and the lowest at 25% (v/v) (0.89 AU/g dry solid), whereas 40% (v/v) substrate capacity was incapable of being mixed due to low power output of agitating motor. Results suggested that OPF was a potent source of substrate for the cultivating Monascus purpureus using SSF and all 3 factors (aeration, substrate load capacity and agitation programme) were significantly influenced the red pigment production.

1. INTRODUCTION

Generally, most of the manufactured food will be introduced to colorant to enhance the attractiveness of the product. These added colors usually will increase the product consumption due to manipulation of normal human behavioral responses [1] thus significantly increase the demand of colorants production. Nowadays, the concern of utilization of organic or natural based product is gaining positive acceptance among the consumers. Natural colorants are in great demand especially for use in foods and beverages as its market was valued at $465 million USD in 2007, a raised of 4.6% from the year 2004 [2].

One of the microorganism with high potential for large scale natural pigment production is Monascus sp., due to its ability to produce an intense red pigment as well as other beneficiary metabolic by-products (e.g. lovastatin, antioxidants) [3][4][5]. In this regard, the use of bioreactors are advantageous because of its potential to process significantly larger scale of substrate with the aid of parameters control system. As for solid state fermentation (SSF) bioreactors, these respective systems are loosely associated with flowing water during the fermenting process.

In typical stirred-drum bioreactor, the bioreactor body remains stationary, equipped with impellers mounted on a shaft running along the central axis of the bioreactor rotating within the drum. These impellers are usually classified in accords to the blades diameter relative to the vessel, and for close-clearance agitation, impellers usually having proximity to the tank inner wall for efficient bulk blending capability [6].

Despite the high pigment yields in SSF compared to submerged cultivation [7], however, the mechanical aspects such as the bioreactor design for Monascus sp. fermentation are vastly unexplored. Hence, this study has been focused on the SSF of Monascus purpureus using OPF as a potential source of substrate in stirred-drum bioreactor, for the purpose of red pigment production.

2. EXPERIMENTAL

In this study, a specifically fabricated bioreactor from the Faculty of Chemical and Natural Resources Engineering (FKKSA) laboratory, UMP, was used (Figure 1). This bioreactor consists of (1) 2.3 L stainless steel drum mounted with a self-modified impeller and a direct current-geared motor (model: SPG30-30K, Cytron Technologies, Malaysia) with a metal heating-jacket at the bottom of the drum, (2) a control panel for agitation and temperature control, and (3) a commercial outlet air pump (ADA, China).

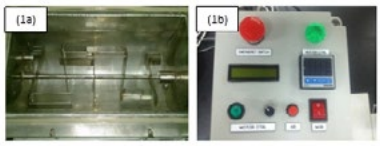

Figure 1 Major component of the bioreactor.

(1a) Fermenting drum that consists of a metal body, a double-bladed impeller (fabricated), a DC-geared motor, and a transparent tempered glass for monitoring the fermenting process. (1b) Control panel for agitation and temperature control of the bioreactor.

Monascus purpureus strain FTC 5357 stock culture was maintained on Potato Dextrose Agar (PDA) and incubated in the dark at 30oC for 7-8 days, which was then preserved at 4oC. Sub-culturing was done once a month for adequate propagation of the strain [8].

Fully sporulated agar slant culture (after 6-8 days incubation) was prepared prior to inoculum preparation. Sterile distilled water was added to the slant culture, followed by gentle scrapping on the slant surface to harvest the spore. The spore concentration was measured and adjusted to approximately 105 spores/mL.

The fresh oil palm fronds (OPF) were obtained from a local palm oil plantation in Felda Bukit Goh, Kuantan, Pahang. The fresh OPF were cut into smaller pieces (approximately 3-4 cm in length), and thoroughly washed using diluted detergent. Later, the OPF were rinsed with tap water several times. The clean OPF were then dried at 60o C for 2 to 4 days. Next, the dried OPF were chipped using a large scale wood chipper before being pulverized into smaller particles (<1.0 mm) using a commercial grinder (Retsch ZM-200). The OPF particles were autoclaved with distilled water in 1:18 ratio (w/v) at 121oC for 15 minutes [9]. The pre-treated OPF were filtered and washed with distilled water, before being oven dried at 60o C.

Experiments were conducted in a 2.3 L stirred drum bioreactor. An empty bioreactor drum and the treated OPF having an initial moisture content of 60% (w/w) was supplemented with 2% (w/w) of peptone, were separately autoclaved. After being cooled to room temperature, the substrates were inoculated with 105 spores/mL of Monascus purpureus FTC 5357, evenly mixed and aseptically transferred to the bioreactor. The cultures were cultivated for 16 days in the dark at room temperature. Red pigments

production was determined using a UV-VIS spectrophotometer (Hitachi U-1800).

Series of experiments had been conducted to study the effect of aeration rate, agitation and loading capacity of the drum to red pigment production by Monascus purpureus strain FTC 5357 in a stirred drum bioreactor.All of conducted experiment were subjected to initial moisture content of 60% (w/w) of substrate supplemented with peptone (2%, w/w), 0.5 vvm of humidified air, loading capacity of 30% (v/v) and were agitated for 6 rotations/ day in the dark at room temperature in 8 days, unless otherwise mentioned.

As for factor of aeration studies, the treated OPF were aerated at three aeration rates of 1, 0.5 and 0.3 vvm of humidified air. For programme of agitation studies, OPF wereagitated at different frequency of 4, 6 or 8 rotations/day. The time duration between each rotation was evenly distributed. Lastly, for effect of the drum loading capacity studies, treated OPF were loaded to the drum at 35%, 30%, or 25% (v/v) of drum capacity, separately.

After fermentation, the fermented OPF-solvent solution was allowed to settle for 15 min followed by filtration through a Whatman No.1 filter paper. Unfermented substrate was used as a blank. Analysis of pigment concentration was done using a UV-VIS spectrophotometer (Hitachi U-1800) by measuring absorbance at 500 nm. The yield was expressed as absorbance units (AU) per gram of dried solids [10][11] [12]. Glucose from the fermented OPF was extracted by a simple contact method previously outlined by Hong et al.,(2012) with slight modifications [13]. The fermented OPF were suspended in deionized water in a ratio of 1:10 (w/v), and then incubated for 90 min at 30o C in a rotary shaker set at 150 rpm. The suspension was then centrifuged at 5000 rpm for 30 min, 4 °C, and then filtered through a Whatman No. 1 filter paper. The glucose measurements of the sample were estimated using standard dinitrosalicylic (DNS) colorimetric method [14].

3. RESULTS AND DISCUSSION

The yield of red pigment was monitored and observed from day 0 until day 16 of incubation (Figure 2). The fermentation was done at room temperature, 60% (w/w) initial moisture content, 2% (w/w) of peptone in a stirred drum bioreactor with loading capacity of 30% (v/v) and agitated of 8 cycles per day.

According to Zahari et al., (2012), pressed juice from OPF petiole consisted of approximately 71% glucose; 27% sucrose and 2% fructose[15], supporting the similar finding was found on OPF in-situ enzymatic hydrolysis results of Hong et al., (2012) where the most common product of OPF was glucose (approximately 69.2 %) (Figure 2)[13].

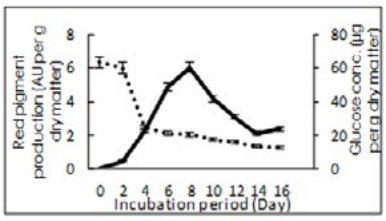

Figure 2 Red pigment production and glucose consumption of Monascus purpureus FTC 5357 in a stirred drum bioreactor. (¬__) indicates pigment profile,(¬....) indicates glucose profile.

Heat removal can be a notable constrain in maintaining the efficiency of SSF. In the present study, aeration with humidified air seems to be an adequate solution to overcome this limitation. The effect of aeration on red pigment production is shown in Figure 3. The non-aerated culture showed the lowest red pigment production (0.81 AU/g dry matter) (Figure 3). The cause of this phenomenon was due to an inadequate air flow to remove accumulated heat from fungal metabolisms especially from central and bottom of the substrate bed, thus increase the temperature of the vessel. Secondly, loss of gaseous and water required for metabolism of Monascus sp. due to evaporation. Oostra et al., (2000) [16] have stated that the low rate of liquid diffusion would strongly inhibit oxygen transfer at the particle level of fungal hyphae, thus notably affecting the overall cultivation performance. In addition, similar finding was found by Han and Mudgett (1992) where under anaerobic condition the fungal growth and pigment production were retarded [17].

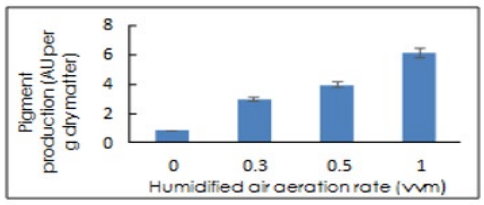

Figure 3 Effect of aeration rate on red pigment production.

The productions of red pigment by aerated cultures in this study were significantly higher compared to those of the non-aerated cultures, showing the increasing trend of red pigment production due to increased aeration. The culture with 1 vvm aeration indicated the highest red pigment production suggesting that an effective transformation of heat, water and oxygen, between the reactor's headspace and substrate particles. These results were contrary to those of packed bed bioreactor study [18], where the optimal value was obtained at 0.05 vvm might be due to the saturated air in the drum bioreactor not directly flowing through the substrate bed, thus, higher rate of airflow were needed to remove the accumulated heat in the substrate bed.

Successful fungal growth is strongly influenced by the heat transfer between the bed and headspace [19], which are dependent on effective mixing. Figure 4 shows the frequency of agitation per day was directly proportional to the rate of red pigment production. At 8 cycles per day the highest red pigment production was 4.34 AU/g dry solid while 4 cycles per day resulted in the lowest red pigment production at 1.915 AU/g dry solid.

Basic water requirement was being supplied by the saturated air within the headspace, suggesting a continuous mixing as a more relevant mode of agitation in order to achieve uniformity of water distribution in the substrate bed. Thus, it may increas the contact surfaces between the substrate and saturated air. However, excessive agitation can be detrimental toward process performance, causing damages to fungal hyphae due to shear and impact forces from the agitator [19]. Alternative intermittent agitation was adopted to minimize the effect of shear forces on fungal growth, without jeopardizing the importance of mixing.

Furthermore, the growth of fungal mass may not be necessarily uniform within the bed. Depletion of nutrient supply may occur to the older and central hyphae while the aerial hyphae at the surface may propagate better on the substrate to acquire new sources of nutrients and maintain their growth [20]. Thus, implementing the intermittent agitation will most probably optimise the dynamics of fungal growth inside the bioreactor. In this case, 8 cycles/day programme was the most suitable agitation program in which timing of the mixing met the favourable growth condition of Monascus sp. for red pigment production (Figure 4).

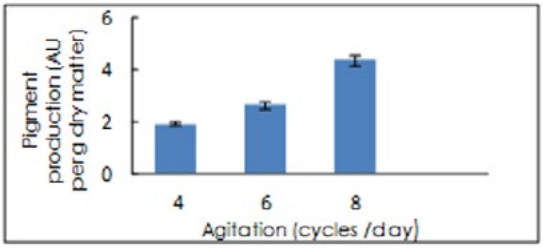

Figure 4 Effect of agitation rate on red pigment production.

To attain an optimal gaseous transfer and avoid excessive accumulation of generated heat in the substrate bed, especially during non-agitating (static) periods, it is crucial to limit the volume of the substrate bed. Otherwise the continuous mixing would most probably be necessary in order to aerate a relatively large volume of substrate, particularly in the central and at the bottom of the bed. Substrate capacity also strongly affects the fungal growth, as surface-mass ratio of the substrate was directly related to the surface area available for the growth to occur [21].

A method to determine the optimal loading capacity of a bioreactor has not been well established, especially in regard to this study. However, a few investigative approaches were available in the literature [22]. Considering the attempt to achieve optimal height/volume of substrate bed, fractional filling will allow an optimum utilisation of the drum volume. However, preferable the maximum working capacity for a reasonable mixing is about 40% of the total drum volume [23]. Using this experimental approach, the

optimized volume for each combination of substrate and microorganism should be determined.

In this study, the 40% (v/v) substrate capacity was not being agitated by the impeller, due to limitation of the DC-geared motor power output. Thus, 35% (v/v) of working substrate capacity was assumed as the maximum loading capacity of the drum. Experimental results in Figure 5, showed that 30% (v/v) of load were sufficient for red pigment production, by achieving a higher red pigment yield (5.605 AU/g dry solid) compared to the other parameters.The 25% (v/v) loading capacity produced the lowest red pigment production, suggesting that the sheer force of agitation (rpm was held constant for all experiments) was intolerable for the amount of substrate used (Figure 5). Also at 25% (v/v) loading capacity, fewer substrates will achieve greater shear force resulting in a higher mycelial disruption rate than mycelial formation rate, thus affecting the product formation. On the other hand, at a larger loading capacity of 35% (v/v), slightly lower red pigment production was obtained.

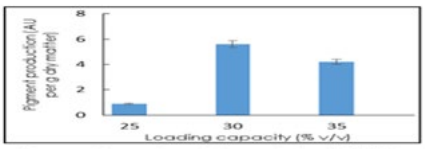

Figure 5 Effect of substrate working capacity on red pigment production. (4.213AU/g dry solid) (Figure 5) suggesting that an optimal condition for gaseous exchange and an optimal mixing of the substrate.

4. CONCLUSION

The present study demonstrated OPF was capable of being fermented by Monascus purpureus to produce a relatively significant amount of red pigment using SSF. Factors of aeration rate, substrate load capacity and agitation programme were significantly influenced the red pigment production in a stirred drum bioreactor suggesting a further need for optimization.

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Cite this article as: Mohamad Al Aamin Razali, Farhan M. Said. Red Pigment Production By Monascus Purpureus In Stirred-Drum Bioreactor. G. War. Sains 1(1) (2017): 13–15