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Kinetic study of growth and biopigments production using xylose and ethanol as substrates to *Monascus ruber* cultivation

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ABSTRACT

The increasing demand for sustainable products is driving research towards alternatives with economic, environmental, and social potential. Monascus biopigments emerge as alternative to synthetic colorants because, in addition to providing color, they offer health benefits due to their characteristics as antioxidant, antimicrobial, and anticancer properties. The aim of this study was to analyze the effect of using xylose and ethanol as substrates for biopigments production, performing kinetic studies of cell growth, substrate consumption, and production of yellow, orange, and red biopigments. The fungus *Monascus ruber* was cultivated in a medium based on commercial xylose (40 g/L), ethanol (10 g/L), and yeast extract (4 g/L) in Erlenmeyer flasks (30°C at 150 rpm). After 20 days of cultivation, a cell growth of 5.23 g/L, a residual concentration of xylose and ethanol of 32.08 g/L and 0.473 g/L, respectively, and a production of 16.24 UA, 12.06 UA, and 11.53 UA of red, orange, and yellow biopigments, respectively, were obtained. Kinetic studies showed fast cell growth between days 6 and 8, with a specific growth rate of 0.784 day^{-1}, and specific production rates of 1.35, 0.479, and 0.628 day^{-1} for red, orange, and yellow biopigments, respectively. The maximum specific consumption rates of xylose and ethanol were 0.981 day^{-1} (on the 10th day) and 5.43 day^{-1} (initial days of fermentation), respectively. Based on the presented data, it was possible to observe xylose and ethanol consumption, with maximum pigment production in 17 days of fermentation. The results will be basis for new studies involving sequential production of ethanol and biopigments and aiming at optimizing this process in bioreactors.

Keywords: Pentoses. Azaphilone. Microbial colorants. Sustainability. Dyes.

1 INTRODUCTION

Dyes and pigments are compounds with essential characteristics for various industrial sectors, such as the food industry, where they are used as color additives and antioxidants[1]. However, most of such products used in the industry are obtained by synthetic route, and some of them can be harmful to human health, with allergenic and, depending on specific dosages, carcinogenic properties, even though the involved mechanisms are not yet well understood[2].

The use of alternatives of biological origin emerges as interesting to the market, as they not only mitigate the risks for consumers but also bring health and environmental benefits due to their biodegradability[3]. Among the various types of natural biopigments, those produced by fungi of the *Monascus* genus stand out. Monascus biopigments have significant market value, mainly due to their versatility in industrial applications. This is due to their high stability to light, pH, and temperature, solubility in polar solvents like water and alcohol, and proven biological properties such as antioxidant, antimicrobial, and anticancer properties, which contribute to the added value of the product[3].

Aiming to favor a larger use of Monascus biopigments, studies about the use of alternative cheap raw materials are important. Among them, xylose from hydrolysates of hemicellulosic fraction of lignocellulosic biomass can be interesting, as the biopigments could be produced from abundant agricultural by-products, as sugarcane bagasse, and could be included in a biorefinery context[5]. Besides the raw material, evaluation of influential variables and new process alternatives must be carried out in research works aiming at an optimized biopigment production. Recent studies, for example, indicated ethanol addition to the medium could be beneficial to the process, increasing biopigment production. Those benefits may be due to act of ethanol's potential as a carbon source to promote mycelial growth and biopigments production, affecting the metabolic pathway of Monascus pigments[6].

In this way, kinetic studies are fundamental to increase the knowledge about the influent variables in the process and to serve as basis for design of bioreactors adequate for industrial production. Therefore, this study aimed to perform a kinetic study of cell growth, substrate consumption, and production of biopigments (yellow, orange, and red) by *Monascus ruber* cultivated in a medium based on commercial xylose and ethanol.

2 MATERIAL & METHODS

The cultivation to obtain the kinetic profile of growth, substrate consumption (xylose and ethanol), and biopigment production (yellow, orange, and red) was conducted in 50 mL Erlenmeyer flasks, with 20 mL of pre-sterilized liquid culture medium (121°C for 15 minutes). The culture medium was formulated with xylose (40 g/L), yeast extract (4 g/L), and ethanol (10 g/L), with an initial pH adjusted to 6.0 and mineral supplementation[4].

As inoculum, 1 agar-mycelium disc (8 mm in diameter) per flask was used, and the cultivation was conducted over a period of 20 days at 30°C, in the dark, and under agitation (150 rpm). For periodic sampling, a sacrificial flask strategy was used, in which each analyzed point corresponded to a flask removed from incubation and fully analyzed. In each sample (1 flask per sample), cell growth, xylose concentration, ethanol concentration, and the production of yellow, orange, and red biopigments were analyzed. With the experimental data, the fermentation parameters were determined: cells and products yields ($Y_{X/S}$, $Y_{P/S}$), and volumetric productivities (Q_X).

The experimental data obtained from the cultivation were further adjusted using the software Microsoft Excel, employing polynomial functions, with the derivates calculated with aid of the software Scilab 2024.0.0. Based on the rates and biomass concentration, the values of specific cell growth rates (μX), substrate consumption rates (μS), and product formation rates (μP) were obtained.

3 RESULTS & DISCUSSION

Figure 1 presents the cell growth profile (experimental data) and the μ_X values over a 20-day cultivation period.

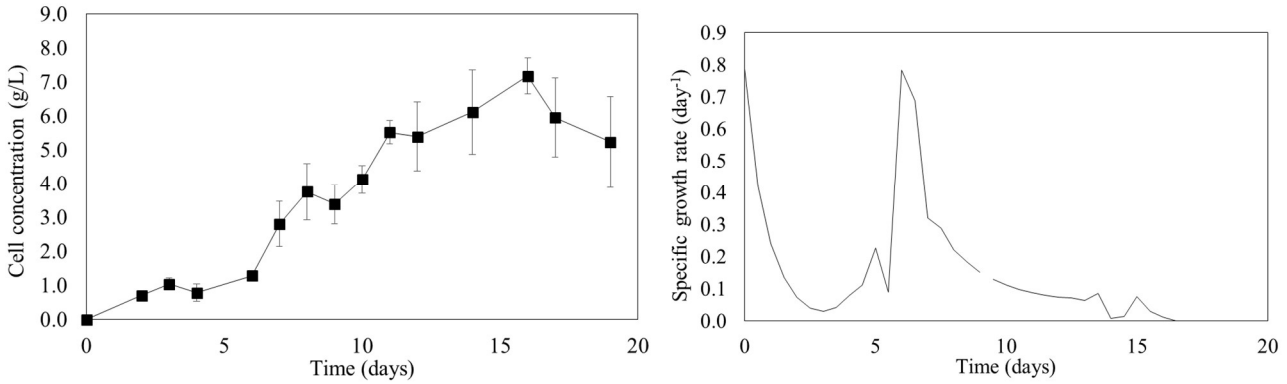

Figure 1. Cell growth (dry biomass) (a) and specific growth rate values (μX) (b) obtained in the cultivation of the fungus *M. ruber* during 20 days

During the cultivation, based on Figure 1, it was noted that the cell concentration reached its maximum growth on day 16, with a concentration of 7.17 g/L. However, the maximum cell growth rate per cell unit occurred between days 6 and 8, with a value of 0.784 day^{-1}.

In terms of biopigment production, maximum production was observed on the seventeenth day, with 19.45, 14.83, and 13.97 UA for red, orange, and yellow, respectively. Figure 2 summarizes the results of biopigment production and the specific product formation rates.

Based on Figure 2, the specific biopigment production rates (μ_P) reached maximum values between days 9 and 10 for the orange and red biopigments, with 1.35 day^{-1} and 0.479 day^{-1}, respectively. The yellow biopigment peaked in production rate between days 5 and 6, with a μP of 0.628 day^{-1}. Additionally, a second region of maximum specific biopigment production rates was observed between days 14 and 16, with 0.142, 0.187, and 0.202 day^{-1} for red, orange, and yellow biopigments, respectively. The behavior of μ_P curves for the three products still need to be clarified; however, the most likely hypothesis relates to the microorganism's biochemical pathway for producing biopigments using different substrate sources (xylose and ethanol) in the various phases of fermentation.

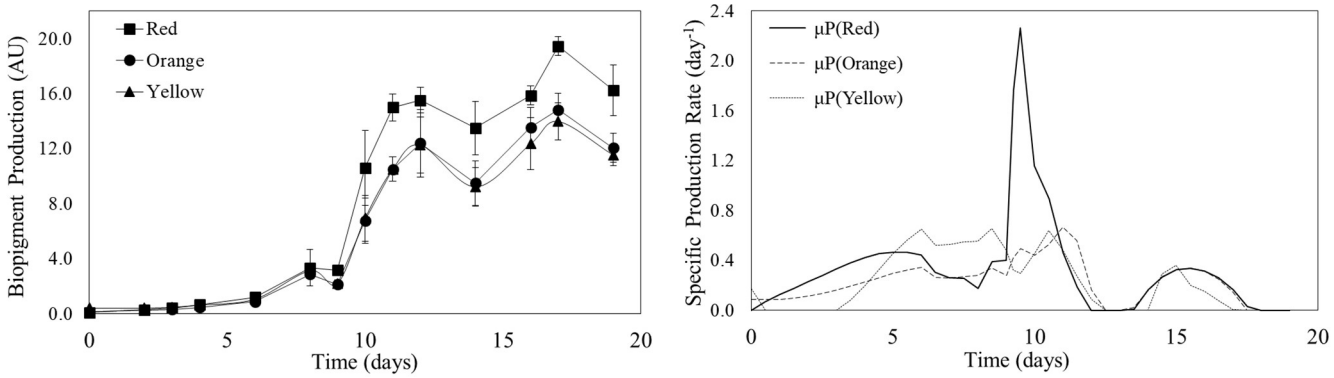

Figure 2. Biopigment production in Absorbance Units (U.A) x time (days) for red, orange, and yellow. Kinetic production profile and specific production rate μP (day^{-1}).

Finally, substrate consumption was also analyzed. Figure 3 presents the kinetic profile of substrate concentration and the specific consumption rate observed. During the initial days of fermentation, a high decrease in ethanol concentration was observed, with a maximum specific consumption rate of 5.43 day^{-1}. Regarding xylose, there was a decrease in this substrate's concentration starting from day 8, with a maximum specific consumption rate of 0.981 day^{-1} on day 10, remaining a concentration of 32.08 g/L at the end of the process.

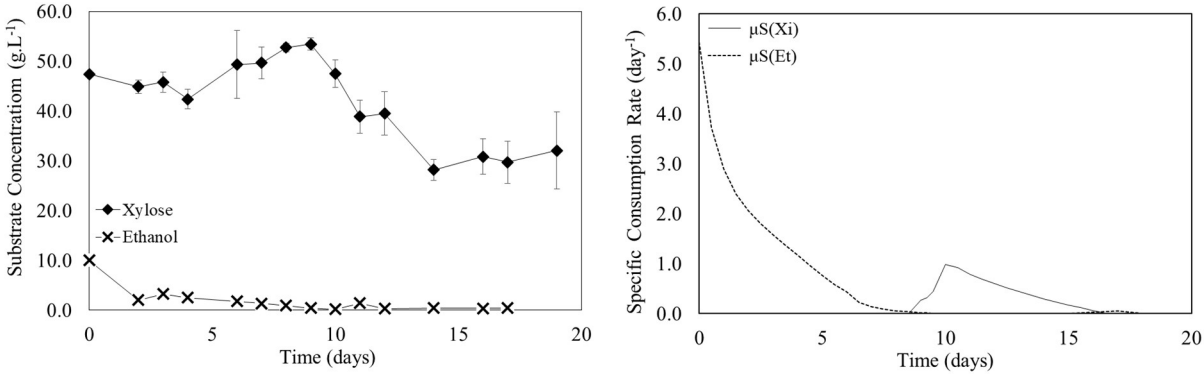

Figure 3. Kinetic profile of substrate concentration (g/L) over time (days) and the specific consumption rate μS (day^-1) over time (days) for the experiment.

The low consumption of xylose can be explained by the microorganism's preference for using other carbon sources. An alternative to overcome this issue could involve a preliminary culture to adapt the microorganism to a medium containing the pentose, employing biomass immobilization techniques to facilitate the transfer of the adapted cells to a new culture medium.

Finally, fermentation parameters were calculated. Table 1 shows the yield values $Y_{(X/S)}$ and $Y_{(P/S)}$ for ethanol and xylose substrates.

Table 1. $Y_{X/S}$ and $Y_{P/S}$ values obtained for biomass and for the substrates ethanol and xylose

Substracts	$Y_{(X/S)}$	$Y_{(PRed/S)}$	$Y_{(POrange/S)}$	$Y_{(PYellow/S)}$
Ethanol	0.529	1.68	1.24	1.16
Xylose	0.287	0.913	0.674	0.628

Also, a Q_X value of 0.269 g/L.day was obtained, as well as Q_P values of of 0.856, 0.632, 0.589 AU/L.day for the red, orange, and yellow biopigments, respectively, with a Q_s of 0.937 and 0.508 g/L.day for xylose and ethanol, respectively.

4 CONCLUSION

In the kinetic study of fermentation, cell growth reached maximum specific production rate around day 6, with 0.784 day^{-1}, with a maximum cell concentration of 7.17 g/L observed in 16 days of process. Regarding biopigments production, there were maximum production values of 19.45, 14.83, 13.97 AU (red, orange, and yellow, respectively) on day 17 and two regions of maximum specific production rates, probably associated to the two used substrates. Additionally, there was a preference of the microorganism for ethanol consumption, with higher consumption of this alcohol at the beginning of the cultivation, with a Yx/s value (0.53) almost twice that one observed for xylose.

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