COMPARASION OF METHODS FOR PRODUCING *MONASCUS PURPUREUS* IN SUBMERGED FERMENTATION

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ABSTRACT

Monascus purpureus is a fungus with great cultural and dietary importance for Asian peoples, but it also has great commercial potential due to the presence of compounds such as monacolins, gamma-amino butyric acid and dimerumic acid. In this work, a scope review was carried out with 11 articles, with the objective of grouping methodologies for the production of *M. purpureus* in submerged fermentation. In the literature, we can observe a variety of substrates and methodologies applied in the production of *M. purpureus*. However, this heterogeneity makes it difficult to define which would be the best parameters for the incubation of *M. purpureus* for biomass production. In addition, the values of the optimized parameters for RPM (180) and pH (5) are not applied in other exploratory works, which aim to test new substrates. Thus, we can state that the methodologies could be further optimized, consequently increasing the production of biomass..

Keywords: *Monascus purpureus*. Submerged fermentation. Mini-Review. Fungus.

1 INTRODUCTION

Monascus is a genus of small, filamentous, saprophytic fungi, classified under Eumycophyta, Ascomycotina, Plectomycetes, Eurotiales and Monascaceae,[4]. *Monascus purpureus* is widely known for its use in Asian cuisine, where it is used in a variety of dishes.[1] However, the most common consumption is through red mold rice, where M. purpureus growth in the rice resulting in its distinctive red color.[2] This color is caused by reddish pigments (rubropunctamine; monascorubramine) as well yellow pigments (monascin; ankaflavin) and orange (rubropunctatin; monascorubrin). *Monascus purpureus* also is related to medicinal properties such as monacolins (a group of anti-hypercholesterolemic agent and a red pigment), gamma-amino butyric acid (a kind of hypotensive agent), dimerumic acid (a natural antioxidant).[5] Thus, the present work aims to perform a mini review, using the search tool "Google Scholar", on submerged fermentation with *M. purpureus*, focusing on biomass production.

2 MATERIAL & METHODS

The keywords used were "Monascus purpureus", "Submerged fermentation" and "biomass". The research was carried out between March and May, in the year 2024, totaling 496 articles. The articles were selected based on the abstract and if they presented data on quantification of fungal biomass. In addition, the research was carried out only in English and gave preference to articles published in renowned journals such as Science direct; Emerad Insight and articles present in the Springer database. Thus, 11 articles were selected, in which 5 were used to compare the culture conditions of fermentation, shown in table 1 and 6 articles to embody the literature.

3 RESULTS & DISCUSSION

Table 1 Culture condition to *Monascus purpureus*'s growth

Strain	Substrate	Culture conditions	Biomassa yield (g/g)	Max biomass production (g/L)	Reference
M630	Hydrolyzed Rice Straw - 50 ml	Inoculum: $1.0 \, x \, 10^7$ spores/ml pH: N.A RPM: 150 Temp: 30°C Inc.time: 10 d	0,36	N.A	[2]
ATCC 16365	Loquat kernels – 60 g/L	Inoculum: $2 \, x \, 10^6$ spores/ml pH: 6.0 RPM: 200 Temp: 30°C Inc.time: 9 d	N.A	6	[6]
HBSD 08	Glucose Sucrose Lactose Maltose Glycerol Xylose (All 60 g/L)	Inoculum: 4% v/v (Seed medium) pH: N.A RPM: 140 Temp: 28°C Inc.time: 10 d	N.A	15 4 0 16 23 17	[7]
sjs-6	Corn starch – 60 g/L	Inoculum: 12% (v/v) pH: 5.0 RPM: 180 Temp: 30°C Inc.time: 8 d	N.A	33.7	[8]
AS3.531	Dried rice – 10 g	Inoculum: N.A pH: 5,5 RPM: 120 Temp: 3°C Inc.time: 7 d	0.012	N.A	[9]

According to Table 1, *M. purpureus* can grow on a variety of substrates, such as agroforestry waste like discarded fruit parts, such as loquat kernel powder[6] and rice straw,[2] it is also possible to use simple sugars such as glucose, sucrose, lactose, maltose, glycerol and xylose,[7] or more complex carbon structures such as corn starch [8] and rice.[9] It was found that loquat kernel powder could generate up to 6 g/L of biomass and 33 g/L for cornstarch.[8] Furthermore, it was possible to verify that rice straw and ground rice had very different yields, 0.36 g/g and 0.012 g/g respectively.[2] Furthermore, when using simple sugars it was possible to obtain 15 g/L (glucose), 4 g/L (sucrose), 0 g/L (lactose), 16 g/L (maltose), 23 g/L (glycerol) and 17 g/L (xylose). However, it was possible to observe that in most of the simple carbon sources (n=5), the highest biomass concentration was not at the end of the 10-day incubation. We can associate this oscillation in the amount of biomass to the autolysis of the cells.[7]

The initial cultivation of *M. purpureus* can be done on potato dextrose agar (PDA) between 30°C and 28°C, with a growth time of between 7 and 10 days of incubation. [2 6 7 8 9] After this growth period, the methodology used to resuspend spores is through the use of distilled water [2 8 9] or physiological water (0.9% NaCl) together with a surfactant (0.2 mL/L Tween 80)[6]. Pinar N (*et al.*, 2020)[6], when suspending spores, filtered the suspension using three layers of muslin in order to eliminate unsuspended hyphae and conidia

As a standardization in the amount of inoculum, concentrations ranging from 1.0 x 10^7,[2] 2,5 x 10^7 [8] e 2.0 x 10^6, [6] spores per ml to be inoculated into the medium for incubation are used. However, it is also possible to use the seeding medium itself as inoculum, as is the case in the work of Zeng H (*et al.*, 2019)[7], Lv J (*et al.*, 2017) [8] and Zhang L (*et al.*, 2013).[9] In which 4% (v/v)[7] or 12% (v/v) [8] of the volume of the seeding medium is used as inoculum. This approach of using the seeding medium as inoculum may have as its principle, in addition to fungal growth, the transfer of extracellular enzymes important for the metabolism of carbon sources, such as α-amylase, β-amylase, and glucoamylase.[10] This step, although fundamental, there are indications that in inoculum volumes in the range of 2% to 5% (v/v) the result is similar and better, however, in volumes of 1% and 6% (v/v) there is a reduction in biomass. [11]

Therefore, as an alternative to incubating spores in media intended for incubation, it is possible to use seeding media that may contain glucose, yeast extract, sodium nitrate, iron sulfate, magnesium sulfate, ammonium sulfate, monopotassium phosphate, peptone, cornstarch and yeast malt extract. [2 7]

However, not all articles use seeding media (n = 1) as a step in the development of the experiment.[6] Furthermore, a strategy to optimize the growth of *M. purpureus* is to use a seeding medium with a composition similar to that of the incubation medium. In this way, it is possible to pre-adapt the fungus to the incubation medium, therefore reducing the lag phase of growth, thus allowing a probable increase in growth and consequently greater biomass and secondary metabolites in less time.[10] However, this strategy

was only used in one study. [8] Another strategy used was to inoculate a sample of *M. purpureus*, subsequently neutralized, in the incubation medium to produce an amount of reduced sugars (8% w/w).[9]

The composition of incubation media may have several complementary components. In order to supplement quantities of nitrogen and mineral salts, yeast extracts, malt extracts, peptone, dipotassium and monopotassium phosphate, calcium chloride, magnesium sulfate, iron sulfate, zinc sulfate, manganese sulfate, ammonium sulfate, ammonium nitrate, glucose, sodium nitrate, [2 9 7 8]. However, it is also possible to find studies that use media composed of only one component, serving as the sole source of carbon, nitrogen and mineral salts.[6]

The incubation temperature of *M. purpureus* varies little between studies, some using 32°C [9] ,30°C [2 6 8] and others 28°C.[7] The incubation time to reach the highest biomass rate of *M. purpureus* varied from 10, [2 7] 9,[6] , 7 [9] and 8 days.[8]

The agitation of the culture system varies greatly between studies, and can range from 120 to 200 rpm.[6] The pH does not seem to be a factor of concern in the incubations, since no checks are carried out at pH. [7] When they are carried out, only the initial pH is determined (6 pH),[6] and subsequently, no changes or control of this parameter are made, only one study optimized its initial pH to 5.5.[9] Both RPM and pH appear to be parameters that are not necessarily optimized to allow for greater biomass production in the studies, since a more acidic pH tends to form freely dispersed small mycelial pellets, in which a pH value of 5.0 demonstrated the best characteristics for a more suitable morphology, that is, shorter, thicker and multi-branched hyphae. Besides that, RPM also influences biomass production, allowing for mixing of the medium, oxygen transfer and also the morphology of the fungus, in which higher speeds cause great hyphae fragmentation or possible death and low speeds poor growths. Thus, 180 RPM proved to be the best speed parameter, given its superiority in biomass production.[8]

4 CONCLUSION

Based on the review, it is possible to note that the cultivation in submerged fermentation of *M.purpureus* is possible, however, it is necessary to optimize the cultivation parameters. As documented in the literature, there are a variety of substrates and methodologies applied in the production of *M. purpureus*. However, this heterogeneity makes it difficult to define which would be the best parameters for the incubation of *M. purpureus* for biomass production. In addition, the values of the optimized parameters for RPM (180) and pH (5) are not applied in other exploratory works, which aim to test new substrates. Thus, we can state that the methodologies could be further optimized, consequently increasing the production of biomass.

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