Evaluation of the Protein Production of Fungal Strains in Alternative Media

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The growing demand for sustainable protein sources in the context of global food security has fueled the development of innovative alternatives to animal meat, and in this research, the use of the fermentation process as a technological solution. In this context, mycoprotein produced by filamentous fungi, such as *Fusarium venenatum*, can be subjected to submerged fermentation to evaluate protein biomass production. Mycoprotein from filamentous fungi stands out as a promising alternative due to its nutritional value, sensory characteristics similar to those of meat, and lower environmental impact. It is necessary to identify the most efficient culture media to optimize biomass production and protein synthesis. Thus, the objective of this study is to evaluate the potential of *Fusarium venenatum* under certain medium compositions and fermentation conditions that can significantly favor mycelial growth and the final protein content of the biomass. Methods were established to evaluate spore growth, demonstrating greater efficacy of the DRBC culture medium. Fermentation tests were performed using liquid SD and FM medium, incorporating winemaking residues. This use of byproducts as an alternative medium contributes to greater mycoprotein sustainability. The parameters for the experiments were 180 rpm and 28°C, with quantification of dry biomass and protein. The results obtained demonstrated that adapted media generated greater biomass, while without adaptation, higher protein levels were observed. Significant growth was observed in the Adapted 2 (3%) medium, with 0.949g of dry biomass. The FM protein results were 35.6% in 24 hours. These findings reinforce the biotechnological potential of this microorganism as a promising source of alternative protein, contributing to technological innovation in the food sector.

Keywords: Mycoprotein. *Fusarium venenatum*. Submerged Fermentation. Alternative Protein.

The rapid growth of the world's population is driving a demand for food, especially protein, requiring an increase of up to 73% in global food production. However, the current food system, based on the high consumption of meat of animal origin, contributes to an increase in greenhouse gas emissions, excessive consumption of water and land, and loss of biodiversity [1,2].

In this scenario, filamentous fungi have emerged as a promising source for protein production due to their rich amino acid composition, natural binding capacity and fibrous texture. It is a valuable source of protein, minerals, vitamins and antioxidants and has a low fat content [2].

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These fungi have been studied in bioprocesses such as submerged fermentation, which allows the production of biomass and metabolites under controlled conditions, with less environmental impact and reduced costs, which generates great interest for industrial applications [1,3].

The sustainability of mycoprotein production can be improved by using agro-industrial waste as cultivation substrate. In the wine industry, byproducts of alcoholic and malolactic fermentations, rich in organic compounds, carbohydrates and nutrients, and a variety of macro- and micronutrients, present potential as alternative sources for filamentous fungi [4,5].

This study aims to prospect and characterize microorganisms with the potential to generate inputs for the alternative protein industry, taking as a starting point the kinetic characterization of microbial growth, including the evaluation of biomass and protein metabolism of *Fusarium venenatum* CML 3311, to offer biotechnological solutions for a more sustainable food system.

Materials and Methods

Submerged Fermentation

The strain used for this work was *Fusarium venenatum* CML 3311, obtained from the Institute of Health Technology (ITS), a laboratory located at SENAI CIMATEC University. Different types of culture media were used: Dichloran Rose Bengal Chloramphenicol Agar (DRBC Agar), Sabouraud Dextrose Broth (SD), GY medium [6], Fermentation medium (FM) [7], and the adaptation of GY and FM media enriched with malolactic and alcoholic fermentation residues from the wine industry.

The process began with the activation of the freeze-dried strain and subsequent testing stages for reactivation, purity and viability analysis. Starting with a fresh pre-culture, it was replenished on DRBC agar medium and incubated at 28°C [8] for a period of more than 10 days, depending on spore growth. Subsequently, the spore solution was prepared by adding 10 mL of sterile distilled water to the entire petri dish, adapted from Tong and colleagues [7], gently scraping the surface with a bacteriological loop, filtering the solution with gauze and the aid of a sterile beaker, removing a 10 uL aliquot and counting the spores in the Neubauer chamber, repeating the process until the macroconidia count is above 2,000 and adjusting the concentration to $5x10^6$ conidia [7]. With the concentration adjusted, the parameters were 28°C, 180 rpm and a maximum time of 72 hours.

Two methodos were applied to quantify the biomass: one with the original media and samples taken every 24 hours, and the other with adapted GY and FM media, using concentrations of 0.7; 2 and 3% of wine fermentation residues, replacing the yeast extract in the original FM formula [7], with samples taken only after 72 hours. The other stages included filtering the biomass using autoclavable systems, a Whatman membrane and a vacuum pump [9], weighing the wet biomass, collecting aliquots for pH measurement, and drying the biomass in a forced-air oven at 40°C for 24 hours [10].

Protein Quantification

To quantify the proteins using the Kjeldahl method, the tests were carried out in the Physical-Chemical Food Laboratory (SENAI CIMATEC University). The procedure was according to Lutz's method [11] with the conversion factor being kept at 6.25 for calculating total biomass proteins. Another methodology was evaluated to quantify the protein content. In the method carried out using the Bradford protocol, reagent and a BSA standard curve prepared at the SENAI CIMATEC Biotechnology Laboratory were used, using a methodological standard prepared at the laboratory and following the methodology of Oliveira [12], which was read on a spectrophotometer at an absorbance of 595nm and with the calculations referring to the standard curve of the laboratory in which the test was carried out.

Results and Discussion

Medium of Culture

To enable the pure and isolated growth of *Fusarium venenatum* CML 3311, the DRBC Agar medium(DichloranRoseBengalChloramphenicol) was determined to be the most viable. With this medium, it was possible to visualize the reproductive structures of macroconidia according to those described by Lazarotto [13].

The design of the liquid media was also determined, with the classic Saboraud Dextrose Broth (SD) medium, the enriched Fermentation medium (FM) medium and, finally, adaptations using malolactic and alcoholic fermentation residues from this culture medium, which had already been validated as viable in previous fermentations.

The first results described the establishment of the ideal culture media for the characteristics that favored the DRBC Agar medium to demonstrate greater efficiency in the sporulation of *F. venenatum* which is attributed to its

formulation, which modulates the fungal metabolism by combining controlled oxidative stress (rose bengal) with the availability of metal cofactors *F. venenatum* sporulation is attributed to its formulation, which modulates fungal metabolism, combining controlled oxidative stress (rose bengal) and the availability of metallic cofactors, inducing the macroconidia required for the concentration adjustment phase of this work, visually compared with Lazarotto's findings [13] to determine the target cells of macroconidia reproductive structures. Taking into account the media that were modified with the adaptation of yeast extract, its rich and complex composition that provides peptides, aminoacids, vitamins and some essential carbohydrates for microbial growth [12], justifying that this approach can meet the demand for cheaper cultivation media and reduce the impacts associated with the disposal of this waste, thus being a relevant alternative [4].

Submerged Fermentation

Based on validation at a temperature of 28°C, carried out in other studies, the ideal speed to stimulate biomass production was determined, with 180 rpm being the best agitation in this temperature range. The fermentation processes were started with four different media, according to the method of Tong and colleagues [14].

Four growth curves were constructed (Figures 1 to 4), with quantification of dry biomass (Tables 1 and 2) and evaluation of post-fermentation pH variation (Tables 3 and 4), within the established submerg parameters and evaluated for a maximum of 72 medium hours.

Both curves in Figures 1 and 2 show exponential growth between 24 and 48 hours and a deceleration phase at 72 hours, according to Table 1.

The curves (Figures 3 and 4) showed higher biomass production with 3% residue. Both showed similar growth to the FM medium with 0.7%, showing that the higher the concentration, the higher the level of biomass generation, as shown in Table 2.

Figure 1. Growth curve of *F. venenatum* in submerged fermentation at 28°C, 180 RPM with FM medium without adaptation.

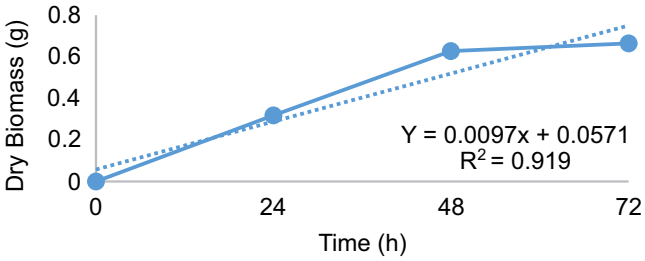

Figure 2. Growth curve of *F. venenatum* in submerged fermentation at 28°C, 180 RPM with SD medium.

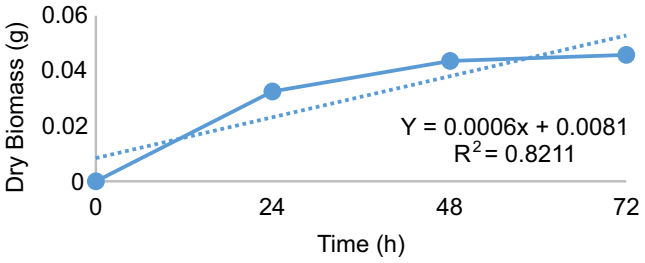

Figure 3. Growth curve of *F. venenatum* in submerged fermentation at 28°C, 180 RPM with FM medium adapted with malolactic residue.

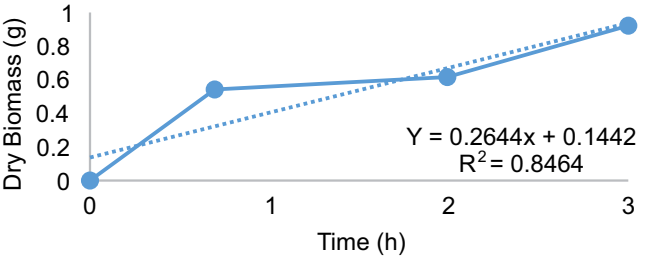

Figure 4. Growth curve of *F. venenatum* in submerged fermentation at 28°C, 180 RPM with FM medium adapted with alcoholic residue.

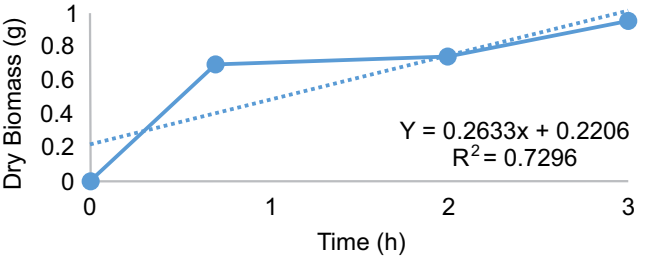

Table 1. Dry biomass (g) of *F. venenatum* under conditions of 180 rpm agitation at 28°C with media without adaptation.

Time	FM	SD
24h	0.319	0.032
48h	0.633	0.043
72h	0.669	0.045

Table 2. Dry biomass (g) of *F. venenatum* under conditions of 180 rpm agitation at 28°C with FM medium adapted.

Residue Concentration	FM malolatic	SD alcoholic
0.7%	0.541	0.697
2%	0.621	0.737
3%	0.923	0.949

In the FM medium (without adaptation), there was a continuous increase in biomass over 72 hours, with exponential growth of 98.43% up to 48 hours and a subsequent slowdown, with an increase of 5.69% (Table 1). As reported by Tong and colleagues [7] using *F. venenatum* strain TB01 grown in a bioreactor with FM medium, 5.80 g/L of biomass was reached after 72 hours and Ahmad and colleagues [5] with *F. venenatum* ATCC 20334, which produced 5.46 g/L of biomass in 72 hours in a complex medium with glucose as a carbon source. When the values from this study are converted into g/L, there is a positive potential, since 72 hours would be 6.69 g/L.

In the SD medium, growth was substantially lower, with 34.3% in 48h and an increase of only 4.65% in 72h, possibly due to the absence of specific mineral salts present in the FM medium, which may have prevented more vigorous mycelial growth. Compared to the literature, Pradeep and colleagues [9] presented a maximum biomass production of 4.47 0.12 g/L for *Fusarium moniliforme* KUMBF1201 in Potato Dextrose Broth (PDB) medium, which is also a less enriched medium, but obtained a significant result. The magnitude of the final biomass obtained by Pradeep and colleagues [9] (which would be 0.447 g in 100 mL) is approximately ten times greater than the 0.045 g/100mL produced in the SD medium, suggesting that the Fusarium strain used requires nutrients not provided by the SD medium.

In FM modified with malolactic residue, there was a 70.6% increase in biomass, and in alcoholic FM, a 36.1% increase between the lowest and highest concentrations (Table 2). The experiment carried out by Tong and colleagues [14] showed a production of 9.53 g/L (0.953 g/100mL) of *F. venenatum* mycoprotein in 72 hours, with the pH dropping from 6 to 4 after 48 hours of fermentation, comparable to the production of 0.923g and 0.949g of biomass at the 3% concentration in this study. When comparing these results, the addition of winemaking waste to FM resulted in higher biomass production, with gains proportional to the increase in concentration.

The variation of pH (Table 3) indicates that an increase in biomass in media with an initial pH 6 causes a reduction in this value, which is typical of fermentative processes, due to carbon consumption and the production of organic acids. With regard to the pH of growth in SD medium (Table 4), the stabilization at around 5.11 to 5.24 contrasts with the sharp drop of 3.03 on average in FM medium, reflecting greater metabolic activity and biomass production. On the other hand, the lower biomass production in the SD medium implies less intense metabolic activity [9].

Protein Quantification

The protein production analysis of the biomass generated provided an estimate of biomass production and the synthesis of metabolites of interest.

The quantification of total proteins was evidenced in the Kjeldahl analysis, for the determination of the total nitrogen content in a sample, which is correlated with the total nitrogen content of protein [10].The other method performed by Bradford, is a colorimetric technique [12]. The time-varying protein analyses show a pattern of protein reduction (Tables 5-7).

Table 3. pH results of fermentation under conditions of 180 rpm agitation at 28°C with media without FM and SD adaptation.

Time	FM	SD
24h	3.33	5.24
48h	2.80	5.17
72h	2.74	5.11

Table 4. pH results of fermentation under conditions of 180 rpm agitation at 28°C with media FM adaptation.

Residue Concentration	FM malolatic	FM alcoholic
0.7%	3.25%	3.06%
2%	3.30%	2.98%
3%	3.07%	2.76%

Table 5. Protein analysis using the Kjeldahl method under conditions of 180 rpm at 28°C with FM medium without adaptation.

Time	Protein
24h	35.6%
48h	30.0%
72h	28.4%

Table 6. Protein analysis using the Kjeldahl method under conditions of 180 rpm at 28°C for 72 hours with adapted FM media.

Residue Concentration	FM malolatic	FM alcoholic
0.7%	15.5%	20.7%
2%	19.7%	21.5%
3%	20.3%	24.0%

Table 7. Protein analysis using Bradford reagent under conditions of 180 rpm at 28°C with SD medium.

Time	Protein
24h	1151µg/mL
48h	1068µg/mL
72h	746µg/mL

The analyses showed a reduction of the protein over time, characteristic associated with fungal metabolism in submerged cultures. The drop in pH and the slowdown in growth indicate the progressive consumption of nutrients and the reaching of a plateau in the experimental conditions, where the lower pH generates stress and reduces the production of protein in the biomass Srivastava and colleagues [3]. On the other hand, increasing the concentration of the residue resulted in a higher protein content.

Increasing the percentage of residue stimulates protein production, with better performance in alcoholic residue. However, the method did not prove to be effective, as at 72 hours the values were below those of the non-adapted medium.

According to a report by Tong and colleagues [14], using a wild strain (WT) of *F. venenatum* TB01, results of 39.4% were found at 48h, which remained stable at 72h, close to that found in this work at 24h for FM (35.6%). This difference in protein percentages may be due to other additional factors in this article, such as the strict control of aeration mentioned above.

Thomas and colleagues [10], on the other hand, obtained 49.99% protein in 48h, with a different carbon source and greater agitation, suggesting that these parameters should be evaluated in the future to optimize the protein percentage, since this result is also higher than the aforementioned article which also evaluated under 28°C and 180 rpm.

The Bradford method is widely recognized for its sensitivity and speed for quantifying proteins in micrograms, based on protein-dye binding. In Oliveira's experiment [12] on lipase production by *Fusarium oxysporum* in submerged fermentation (180 rpm, 28 °C) and protein determination by the Bradford method, the values ranged from 0.11; 0.42 and 9.55 mg/mL, depending on the optimization of the medium. These results were higher than those obtained in this study at 24 hours (1.151 mg/mL) compared to 9.55 mg/mL, and lower than the lowest value found at 72 hours (0.726 mg/mL) compared to 0.11 mg/ml in the

article, showing that quantification depends on the medium used.

Conclusion

The project therefore, has broad biotechnological potential, with relevant results on biomass growth and protein quantification in different media. This work shows the potential for producing proteins from the fungal cultivation of the Fusarium strain, as well as demonstrating the potential for using industrial waste. However, limited time and resources indicate the need to optimize parameters in order to increase production over time, and this study could be part of more comprehensive research of interest to the food industry.

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References

1. Heidemann MS, Pereira IO, Maske BL, Massaki S, Manzoli MC, Valentini N, et al. Fermentação no Brasil: o potencial para a produção de proteínas alternativas. São Paulo: Tikibooks; The Good Food Institute Brasil; 2025.
2. Gonçalves LGF. Avaliação do perfil de diferentes grupos alimentares em relação ao consumo de micélios de fungos como proteína alternativa [trabalho de conclusão de curso]. 2023.
3. Srivastava S, Pathak N, Srivastava P. Identification of limiting factors for the optimum growth of *Fusarium oxysporum* in liquid medium. 2011. doi:10.4103/0971-6580.84262.
4. Karimi S, Mahboobi SN, Lundh T, Mahboubi A, Kiessling A, Taherzadeh MJ. Evaluation of filamentous fungal biomass cultivated on vinasse as an alternative nutrient source of fish feed: protein, lipid, and mineral composition. Fermentation. 2019. doi:10.3390/fermentation5040099.
5. Ahmad ML, Farooq S, Alhamoud Y, Li C, Zhang H. A review on mycoprotein: history, nutritional composition, production methods, and health benefits. Trends Food Sci Technol. 2022. doi:10.1016/j.tifs.2022.01.027.
6. Tong S, An K, Chen W, Zhau W, Sun Y, Wang Q, Li D. Evasion of Cas9 toxicity to develop an efficient genome editing system and its application to increase ethanol yield in *Fusarium venenatum* TB01. 2022. doi:10.1007/s00253-022-12178-5.
7. Tong S, An K, Zhou W, Chen W, Sun Y, Wang Q, Li D. Establishment of high-efficiency screening system for gene deletion in *Fusarium venenatum* TB01. J Fungi. 2022.
8. Leslie JF, Summerell BA, editors. The Fusarium laboratory manual. 2006.
9. Pradeep FS, Pradeep BV. Optimization of pigment and biomass production from *Fusarium moniliforme* under submerged fermentation conditions. Int J Pharm Pharm Sci. 2013.
10. Thomas AB, Shetane TD, Singha RG, Nanda RK, Poddar SS, Shirsat A. Employing central composite design for evaluation of biomass production by *Fusarium venenatum: in vivo* antioxidant and antihyperlipidemic properties. Appl Biochem Biotechnol. 2017.
11. Instituto Adolfo Lutz. Métodos físico-químicos para análise de alimentos. São Paulo: Instituto Adolfo Lutz; 2008.
12. Oliveira BH. Imobilização e caracterização parcial de lipase produzida por *Fusarium oxysporum* em fermentação submersa [dissertação]. 2012.
13. Lazarotto M. Identificação e caracterização de *Fusarium* spp. e *Pestalotiopsis* spp. associados a Carya illinoinensis no Rio Grande do Sul [tese]. 2013.
14. Tong S, Chen W, Hong R, Chai M, Sun Y, Wang Q, Li D. Efficient mycoprotein production with low CO_2 emissions through metabolic engineering and fermentation optimization of *Fusarium venenatum*. J Agric Food Chem. 2024.