A biotechnological approach to fungal biomass production: cultivation of *Fomitopsis pinicola* using plant-derived waste materials

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Abstract. The utilization of plant-derived waste materials as a nutrient source for the cultivation of basidiomycetes has attracted increasing interest within the scientific community due to its sustainability and biotechnological potential. Bulgaria, a major exporter of wheat and globally renowned for its high-quality rose and lavender oils, generates substantial volumes of agricultural by-products that remain underexploited. This study investigates the potential of such plant-based residues as alternative culture media for the *in vitro* cultivation of *Fomitopsis pinicola*, and to model the growth kinetics of a newly isolated strain from Bulgaria. Nine different media were evaluated using logistic and reversed autocatalytic growth models. Among the tested substrates, wheat straw with wheat bran (WSWB) supported the most efficient mycelial growth (μ_{max} = 0.841 ± 0.001d^{-1}). Comparable growth potential was also observed on media containing steam-distilled lavender straw and wheat bran (SDLSWB). Kinetic modeling revealed that the optimal cultivation temperature for *F. pinicola* on WSWB medium is 28°C, while pH 6.0 was determined to be the most favorable for the growth of the strain. These results highlight the suitability of plant-based residues as sustainable substrates for fungal biomass production and lay the groundwork for the development of eco-friendly biotechnological processes involving basidiomycete fungi.

1 Introduction

Fungal biomass has received significant attention in the field of biotechnology due to its potential as a source of proteins, enzymes, polysaccharides, secondary metabolites, and other bioactive compounds, as well as its role in the valorization of agricultural and plant-derived wastes. Filamentous and wood-decay fungi, particularly basidiomycetes, possess a unique ability to break down lignocellulosic material making them useful biological agents for green waste management approaches and the production of high-value compounds [1]. The concept of circularity in economies has its roots far back in history, although the term “circular economy” was introduced recently. Versions of the idea such as resource reuse and closed material loops appear in industrial ecology and industrial symbiosis. It has since been further developed by Walter Stahel's “product life extension” and the late-20th-century cradle-to-cradle approach [2, 3]. The adoption of the circular economy as a policy commitment and research focus has gathered strength since the 1990s, when a series of EU legislative packages and world sustainability initiatives renewed emphasis redefining waste as a valuable resource rather than a burden [2, 4].

Among the many daunting challenges under the classical „take-make-dispose“ approach are the large volumes of plant-derived by-products and lignocellulosic residues from agriculture and forestry. These materials are often underexploited or incinerated contributing to environmental pollution and carbon emissions. Converting these wastes into useful materials or commodities is therefore central the creation of a sustainable circular bioeconomy [4].

Basidiomycete fungi are particularly promising for sustainable biotechnological applications. As wood-decaying (xylotrophic) organisms, they possess a variety of enzymes such as lignin-modifying enzymes, cellulases, and hemicellulases that enable efficient degradation of complex lignocellulose polymers [1]. Their fundamental ecological role in the degradation of dead wood and forest litter highlights their intrinsic capability for organic material recycling and thus renders them the best possible candidate for the valorization of plant-derived waste [5].

Recent experimental research increasingly demonstrates the potential of basidiomycetes to convert industrial and agricultural by-products into valuable products. Examples include the use of brewery wastewater by species such as *Ganoderma lipsiense* and *Pleurotus ostreatus* to produce β-glucans while simultaneously reducing pollutant loads – an approach that couples biomass production and wastewater remediation [6]. Broader reviews highlight their capacities for xenobiotic degradation, wastewater processing, and conversion of wood- and agriculture-derived residuals into fungal biomass, enzymes, and bioactive metabolites [7].

Fomitopsis pinicola (Schwartz: Fr.) Karst., commonly known as the red-belted conk, is a perennial

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polypore widely occurring in boreal and temperate forests of the Northern Hemisphere. It colonizes both coniferous and deciduous species and functions primarily as a brown-rot agent causing significant wood decay and serving an important role in carbon cycling within forest ecosystems [8-10]. Its activity in decomposing lignocellulosic substrates reflect its enzymic competence in the breakdown of cellulose and hemicellulose by carboxylation modifications of the macromolecule structure of cellulose and hemicellulose, making it an efficient natural recycler of plant biomass [11].

Beyond its ecological importance, *F. pinicola* has attracted considerable research interest due to its production of bioactive compounds. Both its fruiting bodies and cultured mycelium contain polysaccharides, triterpenoids, and phenolic compounds that exhibit verified antioxidant, antimicrobial, anti-inflammatory, and anticancer properties [8, 12]. These metabolites have been explored for their potential use in the pharmaceutical, nutraceutical, and functional food industries. Furthermore, research on the submerged fermentation of *F. pinicola* has underscored its ability to generate mycelial biomass and exopolysaccharides under optimized conditions, with carbon and nitrogen sources, pH levels, and mineral supplementation recognized as key factors influencing yield [13, 14].

Despite the promising potential, systematic investigations on low-cost, plant-derived substrates for *F. pinicola* cultivation remain limited. Bulgaria is a major producer and exporter of wheat and wheat-based products and is globally recognized for its high-quality rose and lavender essential oils. In addition, the country's extensive forest resources support sustainable timber production. A common characteristic of these sectors is the generation of substantial quantities of plant-based by-products. The present study explores the feasibility of repurposing these agricultural and forestry residues as complex substrates for the cultivation of *F. pinicola*, and aims to characterize and model the growth kinetics of a newly isolated Bulgarian strain.

2 2 Materials and methods

2.1 Fungal strain

Basidiomycetous strain *Fomitopsis pinicola* was obtained from the fungal collection of the Department of Microbiology and Biotechnology, University of Food Technology, Plovdiv, Bulgaria. Specimen was initially isolated from decayed pine wood from Sredna Gora, Bulgaria [15]. The strain was cultivated at 28°C on Mushroom Complete Medium (MCM), composed of (g/L): glucose – 20.0, KH_2PO_4 – 0.5, K_2HPO_4 – 1.0, $MgSO_4$ – 0.5, peptone – 2.0, yeast extract – 2.0, agar – 2.0, at pH 5.5 – 6.0. Fully developed culture was stored at 4°C and subcultured every 60 days.

2.2 Media composition

Nine different culture media were formulated for the experimental assays and divided into two main groups according to the type of plant-derived substrates incorporated. The first group included mono-substrate media that used single lignocellulosic residue: wheat bran (WB), hexane-extracted rose flowers (HERF), wheat straw (WS), steam-distilled lavender straw (SDLS), or pine sawdust (PS). Each medium contained 40 g/L of the respective biowaste and 20 g/L of agar. The second group included dual-substrate media combining two plant-waste materials in equal proportions (1:1, w/w). The used combinations were as follows: steam-distilled lavender straw with wheat bran (SDLSWB), wheat straw with wheat bran (WSWB), hexane-extracted rose flowers with wheat bran (HERFWB), and pine sawdust with wheat bran (PSWB). Each medium contained constituent at 20 g/L of WB and 20 g/L of the respective biowaste, alongside with 20 g/L of agar.

2.3 Cultivation and modeling of the process kinetics

Inoculation was performed by transferring agar disks (d=10 mm) of a fully grown culture. Surface cultivation was conducted under static conditions in thermostat at 25 ± 1 °C for 14 days. The mycelium's radial growth was assessed daily, and the respective mycelium diameter and density were recorded.

The obtained data was subsequently used for modeling the growth kinetics by applying the logistic curve model (Equation 1) and the reverse autocatalytic growth model (Equation 2):

$$\frac{dD}{d\tau} = \mu_{max}\left(1 - \frac{D}{D_m}\right) \quad (1)$$

$$\frac{dD}{d\tau} = k_1 S_0' D - \frac{k_1}{D_m} D^2 \Rightarrow \quad (2)$$

$$\Rightarrow D = \frac{D_0 \times S_0' \times \frac{K}{1+K}}{D_0 + \left(S_0' \cdot \frac{K}{1+K} - D_0\right) e^{-k_1 S_0' \tau}}$$

where parameters of interest included: μ_{max} (specific growth rate, d^{-1}), D_0 (initial diameter of the mycelium, mm), D (current diameter of the mycelium, mm), D_m (maximal diameter of the mycelium, mm), k_1 (rate constant for biomass formation, d^{-1}), and S_0' (initial substrate quantity in cell units described with the diameter of the mycelia, mm). The applied models were solved by the Runge–Kutta methods of 4th order, and the identification of their parameters was achieved by minimization of the difference in the square between the experimental and model data by applying Excel's Solver function [16, 17].

2.4 Determination of temperature optimum

The growth of *F. pinicola* was examined by incubating culture at seven different temperatures (19°C, 22°C, 25°C, 28°C, 31°C, 34°C, and 37°C). Surface cultivation was carried out for 14 days under static conditions. Radial extension of mycelia and colony morphology were monitored and recorded at regular intervals throughout the incubation period.

2.5 Determination of pH optimum

To determine the optimal pH for the growth of *F. pinicola*, culture media were adjusted to six pH levels (4.5, 5.0, 5.5, 6.0, 6.5, and 7.0). Surface cultivation was conducted for 14 days under static conditions. The strain was incubated at 28°C for 14 days, and mycelial growth was evaluated by measuring the diameter of the resulting colonies.

2.6 Statistical analysis and kinetic modeling

All cultivations were performed with five replicates (n=5). The results obtained are presented as the arithmetic mean of the five replicates, with the standard deviation (SD) indicated as a measure of the variability. The statistical significance was determined by the analysis of variance (ANOVA) and Tukey HSD tests; the value of $p < 0.05$ indicated a statistical difference [18].

3 Results and Discussion

3.1 Cultivation on different complex culture media and kinetic modeling of the process

The valorization of waste from the food and agricultural industries has become an increasingly important focus. In this context, the reproductive capacity of the *F. pinicola* strain was evaluated under solid-state cultivation on a series of complex nutrient media (HERF, WB, SDLS, WS, PS, HERFWB, SDLSWB, WSWB, and PSWB). This fungal strain is the first documented representative of this species in Bulgaria and was previously reported [15]. The mycelial diameter was measured over a 14-day cultivation period on each medium, and the experimental results are summarized in Figure 1.

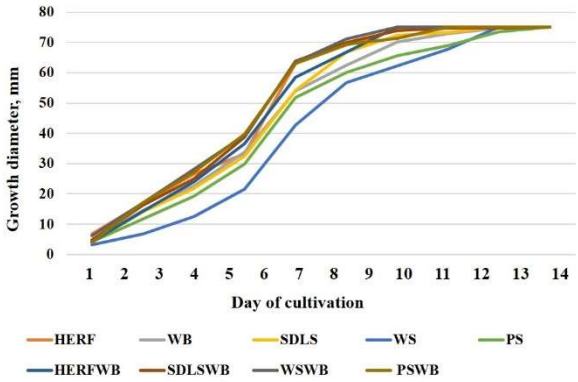

Fig. 1. Growth dynamics of *F. pinicola* on different culture media.

The figure clearly shows that the mycelial mass reaches its maximum diameter of 75 mm by day 12 of cultivation and remains unchanged thereafter. This pattern is consistent across all complex nutrient media, indicating that the optimal cultivation period for the process is between 12 and 14 days. It is also evident that the mixed media – HERFWB, SDLSWB, WSWB, and PSWB support higher and comparable growth rates. This is likely due to their richer nutrient composition compared to the single-component media, which may lack certain growth factors essential for *F. pinicola* reproduction. The lowest growth rate was observed on WS medium, while HERF, WB, SDLS, and PS show intermediate growth performance.

To further elucidate the effect of complex medium composition on strain growth, modeling of the process kinetics was performed. For this purpose, the models of a logistic curve and reversible autocatalytic growth were applied, both of which incorporate parameters with clearly defined biological significance. Parameter identification was conducted, and the corresponding model constants were determined (Table 1).

Table 1. Kinetic parameters of the *F. pinicola* cultivation models on different comlpex culture media.

Medium	Logistic curve model			Reversible autocatalytic growth			
	μ_{max}, d^{-1}	δ, $mm.d^{-1}$	R^2	k_1, d^{-1}	S_0', mm	K/1+K	R^2
HERF	$0.720 \pm 0.007^{b,c,d}$	$0.0104 \pm 0.0001^{b,c,d}$	0.9939	$0.0076 \pm 0.0002^{b,c}$	95 ± 3^{a}	0.8138 ± 0.0224^{c}	0.9956
WB	$0.677 \pm 0.059^{c,d}$	$0.0090 \pm 0.0001^{c,d}$	0.9955	$0.0080 \pm 0.0005^{b,c}$	85 ± 2^{b}	$0.8901 \pm 0.0323^{a,b}$	0.9966
SDLS	$0.704 \pm 0.059^{c,d}$	$0.0092 \pm 0.0004^{b,c,d}$	0.9969	$0.0080 \pm 0.0002^{b,c}$	88 ± 5^{b}	0.8666 ± 0.0496^{b}	0.9969
WS	0.645 ± 0.009^{d}	0.0084 ± 0.0001^{d}	0.9983	0.0072 ± 0.0002^{c}	89 ± 5^{b}	0.8600 ± 0.0377^{b}	0.9983
PS	0.627 ± 0.005^{d}	0.0084 ± 0.0001^{d}	0.9710	$0.0075 \pm 0.0003^{b,c}$	93 ± 5^{a}	0.7991 ± 0.0977^{c}	0.9962
HERFWB	$0.748 \pm 0.005^{b,c}$	$0.0098 \pm 0.0004^{a,b,c,d}$	0.9969	0.0084 ± 0.0001^{b}	89 ± 3^{b}	0.8589 ± 0.0517^{b}	0.9969
SDLSWB	$0.807 \pm 0.019^{a,b}$	$0.0107 \pm 0.0003^{a,b}$	0.9985	0.0105 ± 0.0003^{a}	77 ± 4^{c}	0.9875 ± 0.0514^{a}	0.9985
WSWB	$0{,}841 \pm 0{,}001^{a}$	0.0110 ± 0.0001^{a}	0,9885	0.0104 ± 0.0003^{a}	78 ± 4^{c}	0.9752 ± 0.0529^{a}	0.9985
PSWB	$0.772 \pm 0.001^{a,b,c}$	$0.0104 \pm 0.0001^{a,b,c}$	0,9939	0.0100 ± 0.0002^{a}	80 ± 2^{c}	0.9356 ± 0.0341^{a}	0.9971

Data are expressed as mean ± SD (n = 3); a, b, c, d – different letters in the rows indicate significantly different values (Tukey HSD tests, $p < 0.05$).

Although research on the cultivation of *F. pinicola* using various plant-derived waste substrates remains limited, the findings of this study align with observations reported by other authors. For example, Li et al. explored the valorization of wheat bran through solid-state fermentation with *Inonotus obliquus* and reported significant increases in soluble dietary fiber, total phenolic content, and antioxidant activity, underscoring the beneficial effects of wheat bran on fungal growth and metabolite production [19]. Similarly, Charpentier-Alfaro et al. demonstrated that the addition of cereal substrates such as millet or wheat markedly enhanced the growth rates of different fungal isolates, including *Gloeophyllum carnosum*, *Trametes versicolor*, and *Pleurotus ostreatus*. In these cases, wheat and millet grains served as nitrogen sources, promoting faster fungal growth [20].

A similar trend was observed for the biomass formation rate constant (k_1), which was 0.0104 ± 0.0003 and 0.0105 ± 0.0003 d^{-1} for the two media. This finding is further supported by the increase in the substrate utilization coefficient (K/ (1 + K)), which reached 0.9752 ± 0.0529 and 0.9875 ± 0.0514, respectively. These values exceed those obtained for *F. pinicola* cultivated on the single-component media WB, SDLS, and WS, where the K/ (1 + K) parameter was 0.8901 ± 0.0323, 0.8666 ± 0.0496, and 0.8600 ± 0.0377, respectively.

The next medium that supported relatively rapid biomass formation was PSWB, for which the biomass formation rate constant (k_1) reached 0.0100 ± 0.0002 d^{-1}. The substrate utilization coefficient was 0.9356 ± 0.0341, which is again close to unity, indicating that the incorporation of wheat bran alongside the other components supplies additional substrates essential for fungal growth.

With respect to the maximum specific growth rate, the PSWB, HERF, and SDLS media occupy an intermediate position, yielding relatively high values of 0.772 ± 0.001, 0.720 ± 0.007, and 0.704 ± 0.059 d^{-1}, respectively. The corresponding biomass formation rate constants (k_1) for HERF and SDLS are similar – 0.0076 ± 0.0002 and 0.0080 ± 0.0002 d^{-1}. These complex media also exhibit substrate utilization coefficients of 0.8138 ± 0.0224 and 0.8666 ± 0.0496.

The lowest maximum specific growth rates were observed for the WB, WS, and PS media, at 0.677 ± 0.059, 0.645 ± 0.009, and 0.627 ± 0.005 d^{-1}, respectively. Their k_1 values are comparable, measuring 0.0080 ± 0.0005, 0.0072 ± 0.0002, and 0.0075 ± 0.0003 d^{-1}. Substrate utilization coefficients for these media (0.8901 ± 0.0323, 0.8600 ± 0.0377, and 0.7991 ± 0.0977, respectively) are lower than those observed for the two-component mixtures.

The initial substrate content expressed in cell units was highest in the HERF and PS media, at 95 ± 3 and 93 ± 5 mm, respectively. However, their lower K/ (1 + K) values indicate rapid depletion of key nutrients, leading to growth cessation despite the presence of residual, unutilized substrates. Similar observations apply to the WB, SDLS, WS, and HERF+WB media, which exhibit S_0' values of 85 ± 2, 88 ± 5, 89 ± 5, and 89 ± 3 mm, each exceeding the experimentally determined maximum mycelial diameter of 75 mm.

In contrast, the S_0' values for the SDLSWB, WSWB, and PSWB media (77 ± 4, 78 ± 4, and 80 ± 2 mm) are close to the maximum diameter measured at the end of cultivation. This suggests that these media provide a more balanced nutrient composition and are utilized more completely. This conclusion is further supported by their high K/ (1 + K) coefficients.

The growth inhibition coefficient δ ranged from 0.0110 ± 0.0001 to 0.0084 ± 0.0001 $mm \cdot d^{-1}$, indicating that the complex nutrient media do not contain inhibitory components. The slower growth of the strain on certain media is therefore attributable to the composition of the substrates rather than the presence of growth inhibitors.

A statistical comparison of the maximum specific growth rates on the different culture media was performed using a modified Tukey HSD method from the Real Statistics software package for MS Excel 2016. The analysis revealed statistically significant differences in the maximum specific growth rate of the strain on the WSWB, SDLSWB, and PSWB media compared with the WB, SDLS, WS, and PS media. A weak but statistically significant difference was detected for the HERF and PSWB media, as their μ_{max} values fell within overlapping statistical groups. A significant difference was also observed between the PS and WS media and all other complex media, although these two did not differ significantly from each other. Overall, the analyses indicate that the strain exhibits the highest maximum specific growth rate on the WSWB and SDLSWB media, which therefore represent the most suitable substrates for cultivation. Their average μ_{max} values form a distinct statistical group with the highest values.

For the biomass formation rate constant (k_1), the SDLSWB, WSWB, and PSWB media clustered within the group exhibiting the highest k_1 values, with no statistically significant differences among them. However, these media differed significantly from all other nutrient media. No significant differences in k_1 were observed among the PS, WB, HERF, and SDLS media, whose values overlapped with those obtained for the WS medium, indicating only a weak statistical distinction among these substrates.

Collectively, the statistical evaluation of parameters derived from the reverse autocatalytic growth model confirms that WSWB and SDLSWB are the most suitable complex media for the development of the strain. Among them, WSWB supported the highest maximum growth rate of *F. pinicola*, making it the most favorable substrate overall. Accordingly, WSWB was selected for subsequent experiments to determine the temperature and pH optimum for the strain's development.

3.2 Determination of temperature optimum

The WSWB nutrient medium was also used to assess the effect of temperature on the growth dynamics of *F. pinicola*. This analysis allowed the determination of both the temperature optimum and the activation energy

associated with fungal growth. The primary parameter used to evaluate growth was the mycelial colony expansion rate. Figure 2 illustrates the changes in mycelial diameter during cultivation on WSWB medium across a temperature range of 19 ºC to 31 ºC.

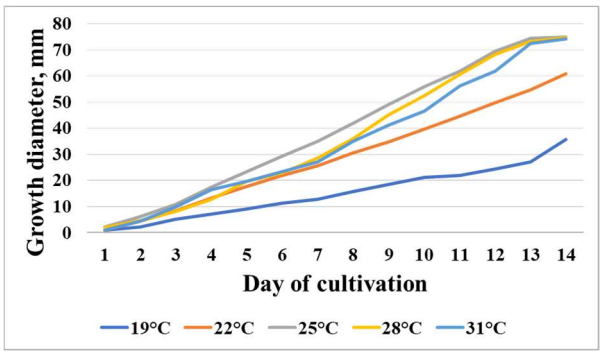

Fig. 2. Temperature-dependent growth dynamics of *F. pinicola* on WSWB medium.

The results demonstrate that temperature has a pronounced effect on the radial expansion of the mycelium, with the highest growth rates observed at 25ºC and 28ºC. At these temperatures, the colony diameter reached approximately 75 mm by the 13th and 14th day of cultivation, respectively. At 22ºC, the final diameter was about 61 mm, while at 31ºC it approached 73 mm by day 14. The lowest growth intensity occurred at 19ºC, with the mycelium attaining only 35 mm after two weeks of incubation. Overall, these findings clearly show that *F. pinicola* exhibits significantly enhanced growth at 25ºC and 28ºC compared to the other tested conditions (Figure 2).

Since the growth intensity on WSWB medium was similar at 25ºC and 28ºC, the maximum specific growth rate (μ_{max}) was determined at both temperatures to identify the optimal growth temperature (Figure 3).

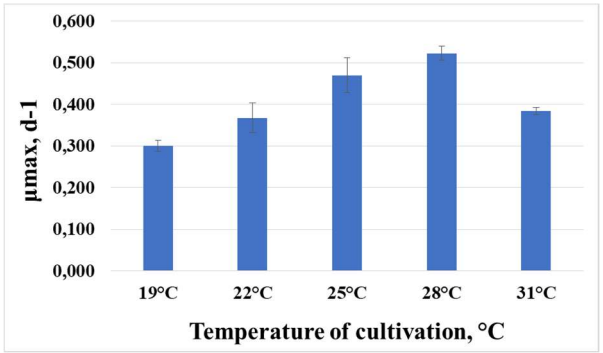

Fig. 3. Temperature dependence of the maximum specific growth rate (μ_{max}) of *F. pinicola* cultivated on WSWB medium.

The maximum specific growth rates (μ_{max}) of *F. pinicola* on WSWB medium were 0.301 d^{-1} at 19ºC, 0.368 d^{-1} at 22ºC, 0.470 d^{-1} at 25ºC, 0.523 d^{-1} at 28ºC, and 0.385 d^{-1} at 31ºC (Figure 3). The increase in μ_{max} up to 28ºC reflects enhanced metabolic activity and enzymatic efficiency within this temperature range. Beyond 28ºC, the observed decline in μ_{max} indicates partial inhibition of key enzymatic systems or thermal stress affecting protein stability and membrane integrity. Figure 3 clearly demonstrates that μ_{max} rises steadily with increasing temperature up to 28ºC, reaching a maximum of 0.523 ± 0.017 d^{-1}, which defines the optimal cultivation temperature for the strain. Further temperature elevation results in a reduction of μ_{max} to 0.385 ± 0.008 d^{-1} at 31ºC, suggesting partial thermal inhibition of metabolic activity. Previous studies have reported variations in the optimal growth temperature of *F. pinicola* depending on the strain. For example, Du et al. observed a slightly higher optimal temperature of 31 °C [21], whereas Choi et al. identified 25 °C as optimal for both mycelial growth and exopolysaccharide production [22]. These findings collectively indicate that the cultivation conditions of *F. pinicola* are strain-dependent.

Polynomial models are commonly used to describe the relationship between μ_{max} and temperature, similar to approaches applied for characterizing the effect of pH [23]. In this study, a fourth-degree polynomial provided the most accurate representation of the experimental data, expressed as follows:

$$\mu_{max} = -2.62187 + 0.711638 \times T - 0.0620216 \times T^2 + 0.00228601 \times T^3 - 0.0000298354 \times T^4$$

The μ_{max} values predicted by the polynomial model for the different temperature conditions were compared with the experimentally obtained values, as shown in Figure 4.

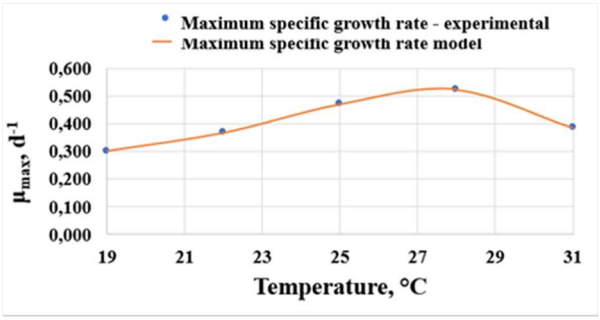

Fig. 4. Comparison of the maximum specific growth rate (μ_{max}) of *F. pinicola* on WSWB medium, obtained from the polynomial model with the experimentally determined value.

The graphical comparison demonstrates a strong correlation between the modeled and observed data, confirming that the polynomial model accurately captures the temperature dependence of μ_{max} within the studied range. The minimal deviations between the predicted and experimental values indicate that the fourth-degree polynomial reliably reflects the biological response of *F. pinicola* to temperature variation and can be effectively applied for predictive modeling and optimization of cultivation conditions.

In addition to polynomial regression, the Ratkowsky model is a well-established approach frequently used to describe the effect of temperature on microbial growth kinetics [24]. This empirical model provides a mechanistic interpretation by relating the square root of the specific growth rate to temperature. Its simplicity and biological relevance make the Ratkowsky model particularly suitable for characterizing microbial and fungal growth across a wide range of environmental conditions.

$$\mu_{max} = b^2 \times (T - T_{min})^2 \times [1 - e^{(c \times \{T - T_{max}\})}]^2$$

The Ratkowsky model offers the advantage of estimating the theoretical minimum and maximum growth temperatures. Temperature in the model can be expressed in either degrees Celsius or absolute units (Kelvin). Applying this model to the growth data revealed a strong correlation between the experimental measurements and theoretical predictions. The results, along with the comparison between experimental data and model predictions, are presented in Figure 5, which demonstrates excellent agreement between observations and model outputs.

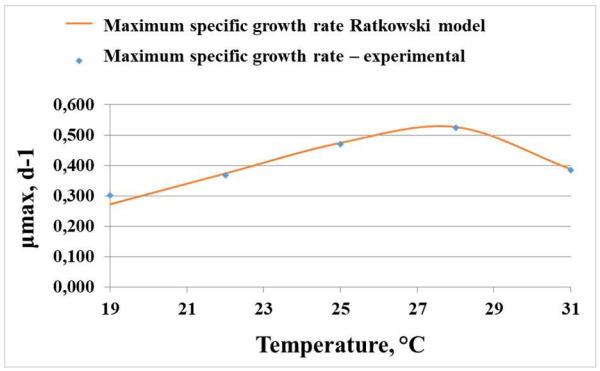

Fig. 5. Comparison of the maximum specific growth rate (μ_{max}) of *F. pinicola* on WSWB medium, predicted by the Ratkowsky model with the experimentally determined value.

According to the Ratkowsky model, the theoretical minimum growth temperature (T_{min}) for *F. pinicola* is 2.3ºC, and the maximum (T_{max}) is 34.1 ºC. The regression coefficients b and c were determined as 0.0313 and 0.378, respectively. The model achieved a high coefficient of determination (R^2 = 0.9878) and a low identification error (ε = 0.04), confirming its accuracy in describing the experimental data. These results indicate that the Ratkowsky model provides a reliable framework for interpreting the temperature dependence of the maximum specific growth rate of *F. pinicola* cultivated on the complex WSWB medium.

3.3 Determination of pH optimum

A series of experimental studies was carried out to investigate the effect of pH on the growth of *F. pinicola* cultivated on WSWB medium. The dynamics of mycelial expansion at different pH values are presented in Figure 6.

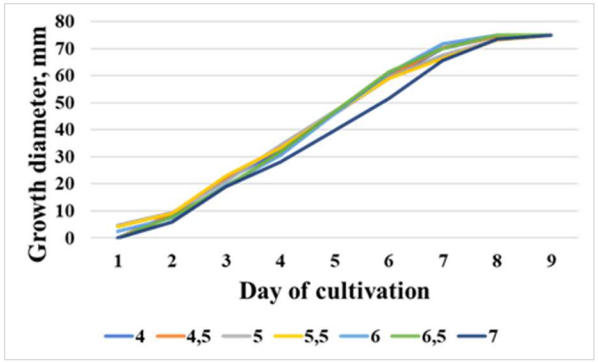

Fig. 6. pH-dependent growth dynamics of *F. pinicola* on WSWB medium.

The fungus exhibited markedly enhanced and relatively stable growth within the pH range of 6.0 to 6.5 compared to other tested conditions. Regardless of initial growth dynamics, the mycelial front typically reached a maximal diameter of approximately 75 mm by the 14th day of incubation. To further quantify the pH-dependent growth behavior of *F. pinicola* on WSWB medium, a logistic growth model was applied, enabling the determination of maximum specific growth rates (μ_{max}) under each experimental condition.

As shown in Figure 7, the highest specific growth rate (μ_{max} = 0.582 d^{-1}) was observed at pH 6.0, identifying this value as the optimal pH for *F. pinicola* proliferation on WSWB medium.

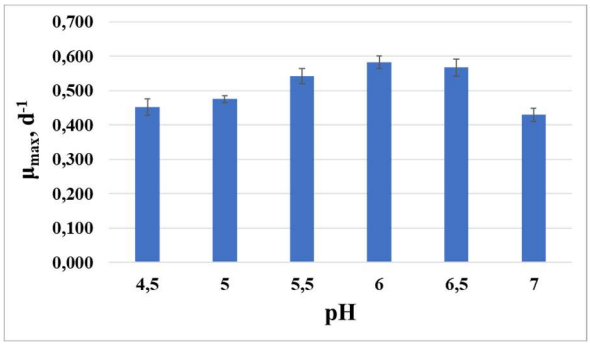

Fig. 7. pH dependence of the maximum specific growth rate (μ_{max}) of *F. pinicola* cultivated on WSWB medium.

The data indicate that increasing the medium pH leads to a progressive rise in μ_{max} up to pH 6.0, where it attains 0.582 ± 0.018 d^{-1}. At pH 6.5, a slight, statistically insignificant decrease to 0.568 ± 0.018 d^{-1} is observed, forming a pseudo-plateau. Further elevation of pH results in a pronounced decline in μ_{max}, reaching 0.430 ± 0.019 d^{-1} at pH 7.0. The results clearly demonstrate that the growth dynamics of *F. pinicola* are strongly influenced by the pH of the cultivation medium, with the highest maximum specific growth rate (μ_{max} = 0.582 d^{-1}) observed at pH 6.0. This finding is consistent with previous studies on *F. pinicola* and related polypore species, which report optimal mycelial growth and enzyme production under mildly acidic to near-neutral conditions. For example, Sun et al. identified pH 6.0 as optimal for maximal cellulase activity in *F. pinicola* cultures, with further increases in pH leading to a significant reduction in enzymatic yield [25]. Similarly, Krupodorova et al. reported substantial biomass accumulation and biosynthetic activity across a broad pH range (2.5 – 7.5), with maximum antioxidant potential and phenolic compound synthesis occurring near pH 6.0 [13]. These observations support the notion that the physiological processes governing hyphal growth and secondary metabolism are closely linked and optimized at comparable pH values.

To describe the relationship between μ_{max} and pH, polynomial models of second or higher degree are commonly employed [23]. Accordingly, for the investigated pH range (4.5 – 7.0), a fifth-degree polynomial model was derived, expressed as follows:

$$\mu_{max} = 155.060000 - 134.865133 \times pH + 46.869667 \times pH^2 - 8.129333 \times pH^3 + 0.705333 \times pH^4 - 0.024533 \times pH^5$$

Using the derived polynomial model, theoretical μ_{max} values for *F. pinicola* were calculated across the tested pH range and compared with the experimentally obtained data, as illustrated in Figure 8.

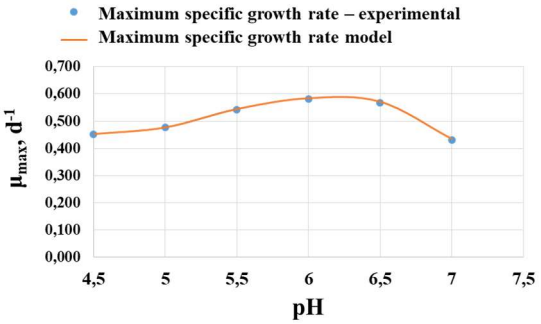

Fig. 8. Comparison of the maximum specific growth rate (μ_{max}) of *F. pinicola* on WSWB medium, obtained from the polynomial model with the experimentally determined value.

The comparison reveals a high degree of concordance between the modeled and observed values, accurately capturing the dependence of μ_{max} on pH within the studied interval. Minimal deviations between predicted and measured values confirm the robustness and predictive reliability of the polynomial model.

Consequently, the developed regression function can be effectively used to estimate the maximum specific growth rate of *F. pinicola* within the pH range of 4.5 – 7.0 and to predict the strain's growth behavior under similar cultivation conditions. This modeling approach not only provides a precise understanding of the physiological response of *F. pinicola* to environmental pH but also serves as a reliable tool for optimizing culture conditions in biotechnological and applied mycological research.

4 Conclusion

This study represents the first systematic application of plant-derived waste materials as complex growth media for the cultivation of *F. pinicola* and provides a quantitative description of the growth kinetics of this newly isolated Bulgarian strain. Using logistic and reverse-autocatalytic models to analyze growth across nine natural media, the binary WSWB substrate was identified as the most effective, although further refinement is needed to improve substrate utilization efficiency. Optimal cultivation conditions were established, with 28°C identified as the ideal temperature for growth on WSWB. By integrating the Arrhenius equation with a fourth-order polynomial model for the pre-exponential factor, the maximum specific growth rate (μmax) was accurately predicted over the 19 – 31°C range, showing strong concordance with experimental results. Additionally, pH 6.0 was confirmed as the most favorable for growth, and a pH-dependent kinetic model was developed that reliably predicts μmax between pH 4.5 and 7.5. Collectively, these results demonstrate the feasibility of using plant-derived waste as an effective cultivation medium for *F. pinicola* and establish robust kinetic models for predicting growth under varying temperature and pH conditions. This study establishes a strong basis for refining cultivation strategies and utilizing agro-industrial by-products as nutrient-rich substrates in basidiomycete fungal biotechnology.

This research was funded by National Science Fund of Bulgaria: Contract No. КП-06-Н86/7 from 06.12.2024, "Controlled *in vitro* cultivation of a wild medicinal mushroom *Inonotus hispidus (Basidiomycota)* and complete genomic characterization: promising approaches for bioprospecting and sustainable production of new therapeutically active biomolecules".

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