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Research Article

Newly isolated wood-decay mushroom *Inonotus hispidus* – modeling of growth kinetics and utilization of plant-derived waste materials

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Abstract

This study is the first to investigate the growth kinetics of *Inonotus hispidus* GA4B, a newly isolated wood-decay mushroom from Bulgaria, under *in vitro* cultivation, as well as its ability to utilize plant-derived waste materials as complex growth media. Using logistic and reversed autocatalytic growth models, the effects of nine media were assessed, identifying the binary medium SDLSWB as the most effective for promoting fungal growth ($\mu_{max} = 0.480 \pm 0.019$ d^{-1}), though further optimization is needed to improve substrate efficiency. Similar growth potential was observed with WSWB and HERFWB media. Based on the kinetic analysis, it can be concluded that the optimal cultivation temperature for *I. hispidus* on SDLSWB medium is 28°C. A fourth-order polynomial model of the pre-exponential factor in the Arrhenius equation enabled accurate predictions of μ_{max} across a 19 - 31°C range, with strong agreement between theoretical and experimental results. Additionally, pH 5.5 was identified as optimal, and a pH-dependent kinetic model successfully predicted μ_{max} values between pH 5.0 and 7.5. Overall, the findings provide a solid foundation for future research aimed at optimizing the use of various plant-derived waste materials as culture media for the growth of basidiomycete fungi.

Keywords

Inonotus hispidus, Basidiomycetes, complex culture media, growth kinetics modeling, plant-derived waste materials

Abbreviations

ANOVA – analysis of variance; HERF – hexane-extracted rose flowers; HERFWB – hexane-extracted rose flowers with wheat bran; PS – pine sawdust; PSWB – pine sawdust with wheat bran; SDLS –steam-distilled lavender straw; SDLSWB – steam-distilled lavender straw with wheat bran; WB – wheat bran; WS – wheat straw; WSWB – wheat straw with wheat bran

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Introduction

Until the 20th century, humanity operated within a linear economic model – extracting resources, producing goods, using them, and then discarding the waste. This system has severely impacted the environment and public health, contributing to pollution, environmental degradation, and overwhelming waste accumulation (Geissdoerfer et al. 2017). In 1978, the United Nations laid the groundwork for sustainable development, introducing the concept of a circular economy. This alternative model promotes the reuse of production waste and the creation of new products, aiming to extend the life cycle of resources and reduce the environmental footprint of human activity. In the European Union alone, agricultural waste (also referred to as bio-waste) exceeds 100 million tons annually. Its application in biotechnological industries represents an innovative and increasingly important use of plant-based residues (Karimi et al. 2018).

Basidiomycete fungi are saprophytic and play a crucial role in the decomposition of wood and organic matter. Their enzyme systems facilitate the degradation of lignocellulose biomass, rendering them indispensable for ecosystems (Sandargo et al. 2019; Webster et al. 2007). The genus *Inonotus* is a small group xylotrophic basidiomycetes found throughout Europe, Asia, and North America. These organisms serve as a source of biologically active substances, with polymeric compounds being a notable component. *Inonotus hispidus* is a lesser-studied edible and medicinal mushroom with a long history as a health food and ancient folk medicine in Europe and East Asia. Research has revealed the richness of *I. hispidus* in metabolites such as polyphenols, triterpenoids, and polysaccharides, which have anticancer, antioxidant, antimicrobial, immunomodulatory, and inhibitory activities against lipase and α-glycosidase (Khatun et al. 2012; Zan et al. 2011; Zhang et al. 2022; Temesgen 2018; Wang et al. 2022).

The economic and therapeutic potential of various mushrooms has resulted in significant interest in their intensive cultivation. Different species of fungus demand divergent growth conditions, and even different strains of the same species can exhibit distinct requirements (Liu et al. 2024). The optimization process involves studying the physicochemical parameters that influence the process, including the culture medium, temperature, pH, carbon and nitrogen sources, and C/N ratio. Natural media consist of natural substrates such as grass or tree stems, seeds, leaves, cornmeal, wheat germ and oat kernels. These media are generally easy to prepare, however, their main drawback is the unknown and variable composition of the substrate (Basu et al. 2015). Temperature is a key abiotic factor for fungal mycelial growth and development because it has a direct influence on a number of metabolic processes. In the context of fungi, the temperature factor can be associated with a range of processes including assimilation and translocation of sugars and nitrogen, respiration and biosynthesis (Akinyele et al. 2005). The pH of the medium is crucial for fungal cultivation as it affects growth, changes in morphology, metabolite synthesis, salt solubility and nutrient uptake (Akinyele et al. 2005; Gorai and Sharma 2018). A significant quantity of by-products from diverse industrial sectors can be recovered and used to make culture media for mushroom cultivation (Abon et al. 2020). The biosynthesis of metabolites is often substrate dependent, with specific bioactive metabolites only being produced on certain substrates (Shang et al. 2012). The modification of substrates has been successfully employed to enhance the secondary metabolite production and bioactivity of the most prevalent medical polypores as Reishi and Chaga (Zan et al. 2011; Zhang et al. 2022).

Mushrooms are the fruiting body of basidiomycetes, which play a key role in forest ecosystems. Their ability to decompose organic substances helps with nutrient cycling (Gbolagade et al. 2006). The utilization of plant waste as a significant nutrient source for the cultivation of basidiomycetes has garnered mounting interest from the scientific community (Chan et al. 2018). This approach presents a valuable opportunity to reduce the ecological footprint of industry – especially when products not only serve as nourishment but also promote consumer health (Ibarz and Augusto 2015). Additionally, utilizing bio-waste in production is expected to lower manufacturing costs for mushroom-based products, ultimately making the final product more affordable (Liu et al. 2024). Notable drawbacks include the high lignin content and the irregular chemical composition, which varies between different plant species and between individuals of the same species throughout the seasons. Other concerns relate to the potential presence of inhibitory substances and the possibility of heavy metal contamination. Therefore, the development of an appropriate plant-based waste substrate should take these factors into account, with necessary measures implemented to minimize or eliminate any negative effects on fungal growth.

Bulgaria is a significant exporter of wheat and wheat-based products and is internationally recognized for the exceptional quality of its rose and lavender oils. The country's extensive forests also offer favorable conditions for the sustainable harvesting of timber. A common feature of these industries is the large amount of plant-based waste they produce. This study aims to investigate the potential of utilizing such plant-derived waste materials as a complex growth medium for *Inonotus hispidus* cultivation, and to model the growth kinetics of a newly isolated strain of this fungus from Bulgaria.

Materials and Methods

Fungal strain. The fungal strain *Inonotus hispidus* GA4B is a part of the fungal collection of the Department of Microbiology and Biotechnology, University of Food Technology, Plovdiv, Bulgaria. The species was isolated from an apple stem from Haskovo region, Bulgaria. The strain was cultivated at 28°C on Malt Extract Agar (MEA), containing gL^{-1}: malt extract – 30.0, peptone – 3.0 and agar – 2.0, pH = 6.5. The fully grown culture was stored at 4°C and subcultured every 60 days.

Media composition. The nine-culture media used in this study were divided into two groups. The first group consisted of media prepared with a single-component plant-derived biowaste: hexane-extracted rose flowers (HERF), wheat bran (WB), steam-distilled lavender straw (SDLS), wheat straw (WS), and pine sawdust (PS). Each medium in this group was prepared with 40 $g.L^{-1}$ of the respective biowaste and 20 $g.L^{-1}$ of agar. The second group comprised media containing combination of two plant wastes in 1:1 ratio: hexane-extracted rose flowers with wheat bran (HERFWB), steam-distilled lavender straw with wheat bran (SDLSWB), wheat straw with wheat bran (WSWB), and pine sawdust with wheat bran (PSWB). Each of these media contained 20 $g.L^{-1}$ of WB and 20 $g.L^{-1}$ of the respective biowaste, alongside with 20 $g.L^{-1}$ of agar.

Cultivation and modeling of the kinetics of the process. Inoculation was performed by transferring agar disks (d = 10 mm) of a fully grown culture. Surface cultivation was conducted under static conditions in a thermostat at 25°C for 21 days. Mycelial diameter was measured daily, and growth density was recorded. Each culture medium was inoculated in five replicates (n = 5).

The resulting data were then utilized for the modeling of the growth kinetics by applying the logistic curve model (Eq. (1) and the reverse autocatalytic growth model (Eq. (2),

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

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

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

where parameters 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 (biomass yield rate constant, d^{-1}), and S_0' (initial substrate quantity in cell units described with the diameter of the mycelia, mm) were identified as parameters of interest.

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 (Choi et al. 2014; Kemmer et al. 2010).

Determination of the temperature optimum. The growth of *I. hispidus* GA4B was evaluated at seven different temperatures (19°C, 22°C, 25°C, 28°C, 31°C, 34°C, and 37°C). The growth and mycelial development of the colonies were observed and recorded over a 21-day period, with colony diameter measured regularly.

Determination of the pH optimum. To determine the optimal pH for the growth of *I. hispidus* GA4B, culture media were prepared with six different pH levels (4.5, 5.0, 5.5, 6.0, 6.5, and 7.0). The strain was cultivated at 28℃ for 21 days and optimal growth was assessed based on mycelial colony diameter.

Statistical analysis. 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's test); the value of $p < 0.05$ indicated a statistical difference (Bower 1998).

Results and Discussion

Cultivation on different natural culture media and colony morphology of *I. hispidus*. Biotechnological approaches in ecology represent a rapidly advancing branch of classical biotechnology. Increasing emphasis is being placed on the application of these methods for the valorization of agricultural and industrial waste, enabling the production of bioactive compounds synthesized by specific fungal producers. Common agricultural residues such as rose flowers, wheat bran, lavender straw, wheat straw and pine sawdust not only hold significant potential but are also readily available and cost-effective raw materials. Despite their potential value, plant-based waste biomass is frequently neglected and thrown away. These substrates can be efficiently utilized through solid-state cultivation of basidiomycetes, including *I. hispidus*. The fungal strain *I. hispidus* GA4B used in this study is the first identified representative of this species in Bulgaria and was officially reported in 2022 (Stefanova et al. 2022). In order to determine the most suitable natural culture media for the development of *I. hispidus*, a series of experiments were carried out to study the development of the strain on the following media: HERF, WB, SDLS, WS, PS, HERFWB, SDLSWB, WSWB, PSWB. The substrates used were harvested in one season and their chemical composition was determined previously (Angelova et al. 2021; Ganeva et al. 2025). The increasing diameter of the mycelium was the main observed parameter characterizing the growth ability of *I. hispidus* on different nutrient media. The variation of the diameter of the mycelium was monitored over 21 days of cultivation of *I. hispidus* on each medium and the results of these experiments are presented in Fig. 1 and Fig. 2.

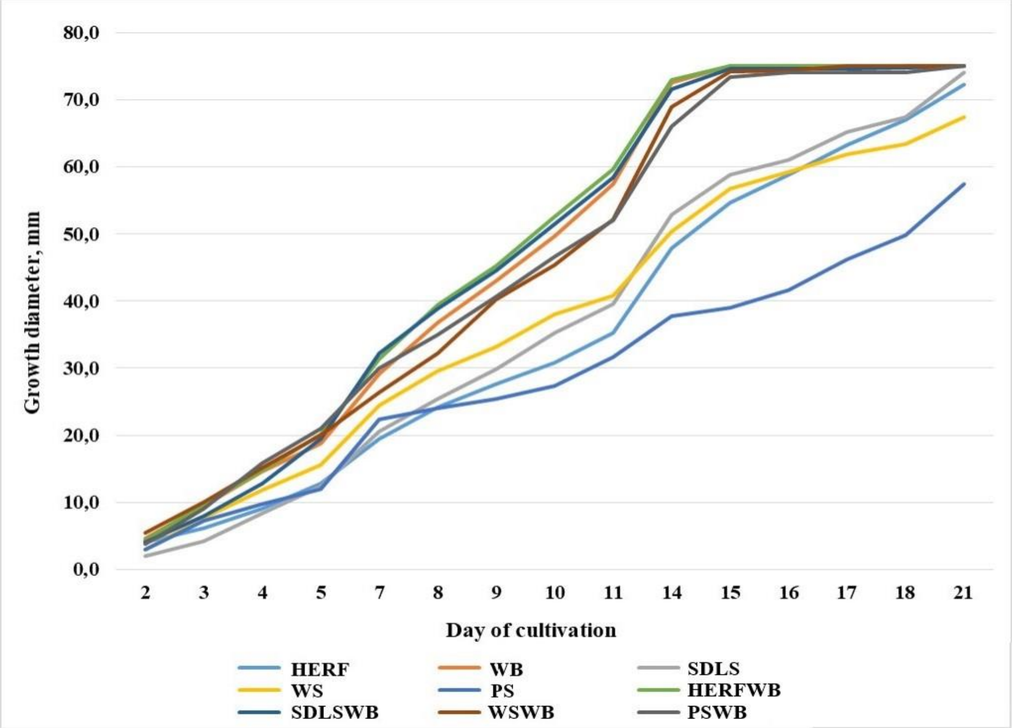

Figure 1. Mycelial growth of *I. hispidus* on different complex culture media

The recorded growth curves indicate comparable and more intensive development of the strain on the media WB, HERFWB, SDLSWB, WSWB and PSWB. The growth rate of *I. hispidus* cultivated on single-component media such as HERF, SDLS, WS and PS is significantly lower compared to the same media supplemented with wheat bran. This suggests that wheat bran contains a wide range of potentially assimilable substrates, thereby enriching the composition of the media to which it is added.

When cultured on SDLSWB (Fig. 2A), *I. hispidus* formed dome-shaped colonies characterized by soft, fluffy, and dense mycelium. The hyphae were white, with localized brown pigmentation near the agar block. On WSWB (Fig. 2B), dome-shaped colonies with soft, fluffy, but loosely arranged mycelium were observed. The hyphae remained white, with pigmentation confined to the area surrounding the agar block.

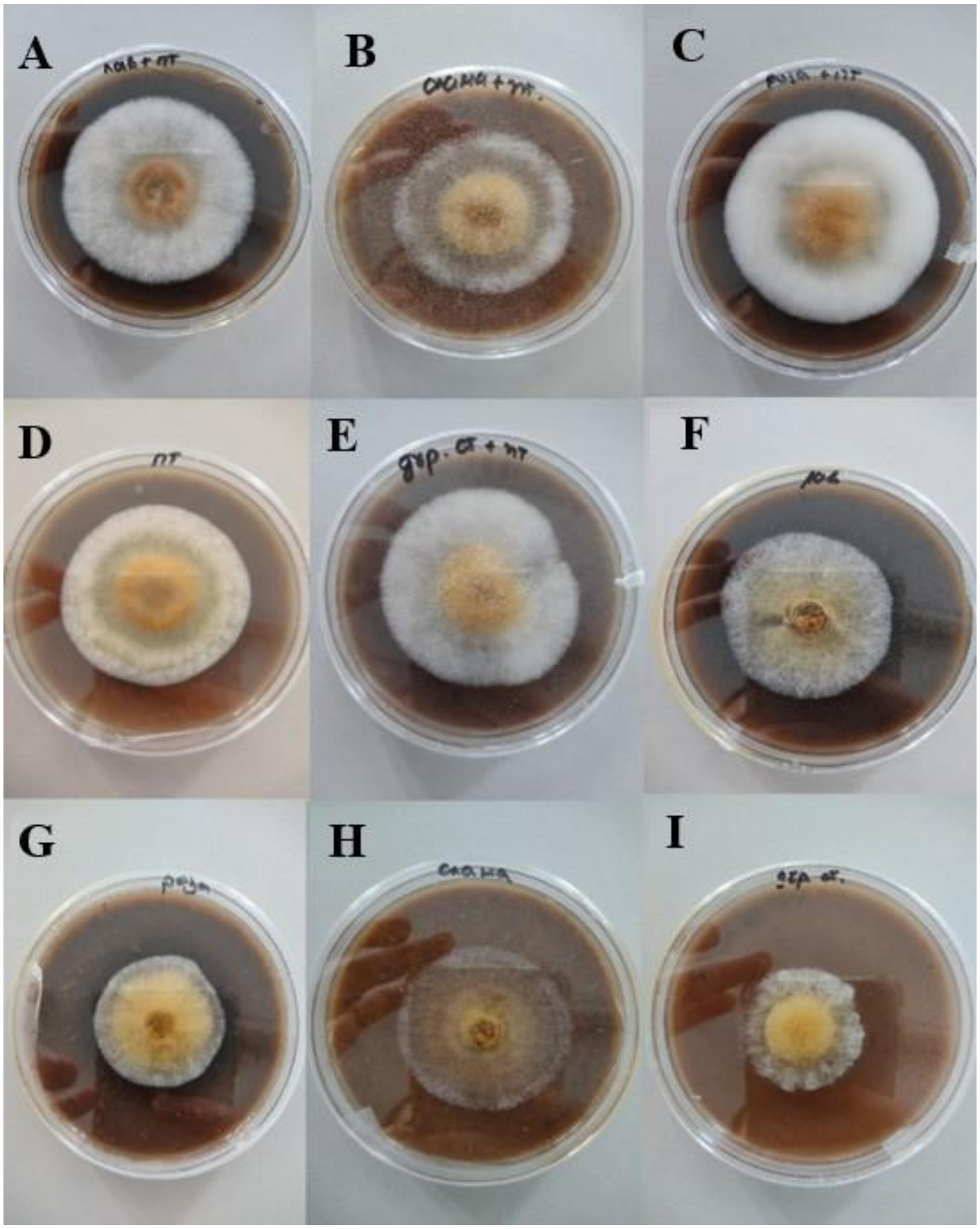

Figure 2. Growth of *I. hispidus* on complex culture media: **SDLSWB** (A); **WSWB** (B); **HERFWB** (C); **WB** (D); **PSWB** (E); **SDLS** (F); **HERF** (G); **WS** (H); **PS** (I)

Growth on HERFWB (Fig. 2C) also resulted in dome-shaped colonies with dense, soft, fluffy white mycelium. In this case, the central hyphae exhibited a yellow-brown pigmentation. On WB medium (Fig. 2D), the colonies retained a dome-shaped morphology with dense, soft, and fluffy mycelium. The hyphae were white at the margins, pale yellow along their length, and yellow in the central region. On PSWB (Fig. 2E), dome-shaped colonies developed, consisting of dense, soft, and fluffy mycelium. The hyphae were predominantly white, with yellow pigmentation localized around the agar block. In contrast, flat colonies were formed on SDLS (Fig. 2F), characterized by dense mycelium that was white at the periphery and yellow at the center. Cultivation on HERF (Fig. 2G) produced flat colonies with soft, loosely organized mycelium. The hyphae exhibited yellow pigmentation along most of their length, except at the tips, which remained white. On WS medium (Fig. 2H), flat colonies with sparse mycelial growth were observed, with pigmentation limited to the vicinity of the agar block. Finally, colonies grown on PS (Fig. 2I) exhibited a dome-shaped morphology, with soft, fluffy, and friable mycelium. The hyphae were pale yellow centrally and white at the margins.

Modeling of the kinetics of the process. A comprehensive understanding of process kinetics is essential for the successful industrial-scale solid-state cultivation of *I. hispidus*. Among the key factors influencing the strain's kinetic behavior is the composition of the culture medium. To evaluate the kinetics of the cultivation process, a combination of the logistic growth model and the reversible autocatalytic model was employed. Model parameter identification and estimation of key kinetic parameters were performed. The results of these analyses are summarized in Table 1 and show that both selected mathematical models are characterized by high correlation coefficients, which vary between 0.9442 and 0.9948. These results indicate that the models provide an excellent fit to the experimental data and are suitable for the kinetic characterization of *I. hispidus* growth on the complex media evaluated. The data in Table 1 show that the growth inhibition coefficient δ, which is the ratio of the maximum specific growth rate to the maximum diameter, has also appropriate values for the different types of media and varies between 0.0037 ± 0.0003 mm.d^{-1} and 0.0069 ± 0.0011 mm. d^{-1}. These parameter values are significantly lower than 1, indicating that all types of media are free of growth inhibitory factors for the cultivated strain. Among the single-component media tested, the highest maximum specific growth rate was observed on WB medium, with a μmax value of 0.465 ± 0.032 d^{-1}. The next single-component medium on which the strain developed with a relatively high growth rate was SDLS, for which μmax was 0.342 ± 0.026 d^{-1}. Whereas, lower and corresponding values of the maximum specific growth rate of I. hispidus were observed on HERF, PS and WS media with μmax assumed values of 0.299 ± 0.037 d-1, 0.229 ± 0.012 d-1 and 0.290 ± 0.022 d^{-1}, respectively. The addition of wheat bran to these media resulted in a marked alteration in the kinetic behavior (Table 1). When the strain was cultivated in the binary complex medium SDLSWB, a significant increase in the maximum growth rate of the fungus was observed (μmax = 0.480 ± 0.019 d^{-1}). This represents a notable change compared to the growth of *I. hispidus* on SDLS alone, where the maximum specific growth rate was 0.342 ± 0.026 d^{-1}. A similar effect of wheat bran supplementation was observed in other binary complex media – HERFWB, WSWB, and PSWB, resulting in increased maximum specific growth rates of 0.465 ± 0.054 d^{-1}, 0.380 ± 0.050 d^{-1}, and 0.378 ± 0.016 d^{-1}, respectively. The results of the kinetic modeling confirm the earlier conclusion that wheat bran provides a wide range of substrates essential for the growth of the tested strain. The obtained data are in agreement with other authors. Research on the valorization of wheat bran through solid-state fermentation by *I. obliquus* demonstrated that fermentation increased the levels of soluble dietary fiber, total phenolic content, and antioxidant activities of wheat bran, indicating the beneficial effects of wheat bran supplementation on fungal growth and metabolite production (Li et al. 2022).

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 among the following media: HERF, SDLSWB, WB, WS, PS, HERFWB, PSWB, and WSWB.

Table 1. The kinetic parameters of *Inonotus hispidus* cultivation models on different complex 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**	**D_m, mm**	**K/1+K**	**R^2**
HERF	0.299 ± 0.037	0.0040 ± 0.0005	0.9851	0.0032±0.0002	96±2	77±2.00	0.8078±0.0186	0.9782
WB	0.465 ± 0.032	0.0063 ± 0.0004	0.9669	0.0035 ± 0.0003	90 ± 2	82 ± 0.91	0.9184 ± 0.0213	0.9897
SDLS	0.342 ± 0.026	0.0046 ± 0.0003	0.9540	0.0040 ± 0.0008	111 ± 5	65 ± 4.38	0.5853 ± 0.0637	0.9543
WS	0.290 ± 0.022	0.0069 ± 0.0011	0.9442	0.0050 ± 0.0005	86 ± 3	74 ± 3.82	0.8601 ± 0.0283	0.9691
PS	0.229 ± 0.012	0.0037 ± 0.0003	0.9434	0.0040 ± 0.0003	88 ± 3	61 ± 3.02	0.7008 ± 0.0571	0.9324
HERFWB	0.465 ± 0.054	0.0062 ± 0.0007	0.9786	0.0045 ± 0.0005	104 ± 2	73 ± 1.68	0.7009 ± 0.0269	0.9945
SDLSWB	0.480 ± 0.019	0.0064 ± 0.0003	0.9673	0.0048 ± 0.0003	100 ± 3	76 ± 2.17	0.7598 ± 0.0423	0.9948
WSWB	0.380 ± 0.050	0.0051 ± 0.0004	0.9777	0.0042 ± 0.0003	92 ± 1	84 ± 0.88	0.9201 ± 0.0141	0.9922
PSWB	0.378 ± 0.016	0.0050 ± 0.0002	0.9702	0.0043 ± 0.0002	104 ± 1	72 ± 1.12	0.6886 ± 0.0161	0.9873

While there is limited direct research on the growth kinetics of *I. hispidus* across various culture media, several studies provide valuable insights into its growth patterns, optimization strategies, and metabolic production (Petre et al. 2021; Zhang et al. 2021). Xu et al. (2014) evaluated the effect of lignocellulose degradation in wheat straw, rice straw, and sugarcane bagasse and demonstrated that wheat straw was the most effective lignocellulose in enhancing the mycelia growth, accumulation and antioxidant activity of *I. obliquus* polysaccharides.

To further investigate the influence of media composition on the growth of *I. hispidus*, the reversible autocatalytic growth model was applied. This model incorporates key kinetic parameters, including the rate constant for biomass formation (k_1), the initial substrate concentration in cellular units (S_0'), the theoretical maximum mycelial diameter (D_m), and the substrate utilization efficiency coefficient (K/(1+K)). As shown in Table 1, the biomass formation rate constant (k_1) was comparable across the tested media, ranging from 0.0032 ± 0.0002 to 0.0050 ± 0.0005 d^{-1}. Of particular interest are the values for the initial concentration of balanced substrate components (S_0') and the substrate utilization efficiency coefficient. Among the single-component complex media, WB exhibited the highest K/(1+K) value (0.9184 ± 0.0213), indicating superior substrate utilization efficiency compared to the other media. In this medium, the initial substrate reserve (S_0'), expressed as mycelial diameter, was 90 ± 2 mm, while the theoretical maximum mycelial diameter (D_m) was 82 ± 0.91 mm. The high K/(1+K) value and the close proximity between S_0' and D_m suggest that this medium supports the most complete nutrient assimilation by the strain. Although SDLS exhibited a relatively high substrate reserve (S_0' = 111 ± 5 mm), it showed a much lower D_m (65 ± 4.38 mm) and a significantly lower substrate utilization coefficient (K/(1+K) = 0.5853 ± 0.0637). This indicates an imbalanced nutrient composition that supports selective assimilation of specific components, after which growth ceases. This medium may be more suitable as a single substrate for other basidiomycete species or different *I. hispidus* strains. Comparable K/(1+K) values were recorded for the single-component media HERF (0.8078 ± 0.0186) and WS (0.8601 ± 0.0283), with corresponding S_0' values of 96 ± 2 mm and 86 ± 3 mm, and D_m values of 77 ± 2 mm and 74 ± 3.82 mm, respectively. These results suggest a moderate efficiency of substrate utilization in both media. Similar conclusions can be drawn for PS which showed K/(1+K), S_0', and D_m values of

0.7008 ± 0.0571, 88 ± 3 mm, and 61 ± 3.02 mm, respectively. The addition of wheat bran to HERF led to an increase in substrate reserve (S_0' = 104 ± 2 mm), however, the theoretical mycelial diameter was 73 ± 1.68 mm, and a slight reduction in K/(1+K) to 0.7009 ± 0.0269 was observed compared to the single-component medium. This may be due to accelerated growth and selective substrate consumption, followed by growth cessation. The decrease in K/(1+K) indicates that the current ratio of rose waste to wheat bran is suboptimal, and further experiments are necessary to determine the optimal component ratio. Supplementation of SDLSWB significantly increased the substrate utilization coefficient to 0.7598 ± 0.0423, compared to the single-component lavender straw medium. Additionally, the mixed medium reduced the disparity between S_0' (100 ± 3 mm) and D_m (76 ± 2.17 mm), supporting the hypothesis that this mixture approaches an optimal composition. Nevertheless, additional optimization experiments are required to improve the ratio of these components. In the two-component medium WSWB, a notable increase in K/(1+K) to 0.9201 ± 0.0141 was observed, along with increases in S_0' (92 ± 1 mm) and D_m (84 ± 0.88 mm). The proximity of K/(1+K) to 1.0 suggests that this medium has an optimal composition for *I. hispidus* growth. Table 1 also shows that adding WB to PS resulted in increases in both S_0' (104 ± 1 mm) and D_m (72 ± 1.12 mm), compared to the single-component sawdust medium. Given the similar K/(1+K) values between the single- and two-component media, a statistical significance test was conducted using the Tukey HSD from real statistics software package for MS Excel 2016. The analysis confirmed no statistically significant difference in substrate utilization efficiency between the two media, indicating equivalent assimilation performance. Based on the conducted kinetic analyses, the binary medium SDLSWB currently appear to be the most suitable for *I. hispidus* cultivation as it supports high maximum specific growth rates. Nevertheless, it requires further optimization to improve substrate utilization efficiency. Similar conclusions could be applied to the mixed media WSWB and HERFWB.

Determination of temperature optimum. Furthermore, the binary medium SDLSWB was used to investigate the effect of temperature on the development of *I. hispidus* grown. As a result of these studies, the optimal value for temperature was determined, along with the activation energy for growth. The primary growth parameter observed was the diameter of the mycelium. Fig. 3A illustrates the dynamics of mycelial diameter changes during cultivation on SDLSWB medium within a temperature range of 19 to 31℃. Based on the presented data, it is evident that *I. hispidus* exhibits significantly higher growth intensity at cultivation temperatures of 28℃ and 25℃, compared to the other temperatures tested (Fig. 3A). A maximum mycelial diameter of 75 mm was reached on day 13 and day 14 of cultivation at 28℃ and 25℃, respectively. A similar mycelial diameter of 75 mm was also achieved at 22℃ and 33℃, but only around day 20 and day 21, indicating a lower growth rate at these temperatures. The lowest biomass accumulation was observed at 19℃, where a maximum mycelial diameter of only 51 mm was reached after 21 days of cultivation. Since the growth intensity on SDLSWB medium at 28℃ and 25℃ was comparable, the maximum specific growth rate (μ_{max}) was determined at each temperature to identify the optimal temperature for growth (Fig. 3B). The maximum specific growth rate increased with temperature up to 28℃, where μ_{max} reached its peak value of 0.560 d^{-1}. Beyond this point, further increases in temperature led to a decline in μ_{max}, with a value of 0.262 d^{-1} recorded at 31℃. Based on the kinetic analysis, it can be concluded that the optimal cultivation temperature for *I. hispidus* on SDLSWB medium is 28℃. Similar data have been obtained by other authors. Choi and Kim studied another member of the *Inonotus* genus, *Inonotus mikadoi*. The team reported an optimal growth temperature of 27℃ (Choi and Kim 2004).

The data of the maximum specific growth rate at different temperatures allows to determine the activation energy for the growth of *I. hispidus* on SDLSWB medium, which is 97507 $J.mol^{-1}$. With a known activation energy, the pre-exponential factor in the Arrhenius equation can be determined and used for the theoretical calculation of μ_{max} at temperatures other than those studied in the range 19-31℃.

For this reason, the pre-exponential multipliers in the Arrhenius equation were determined for the

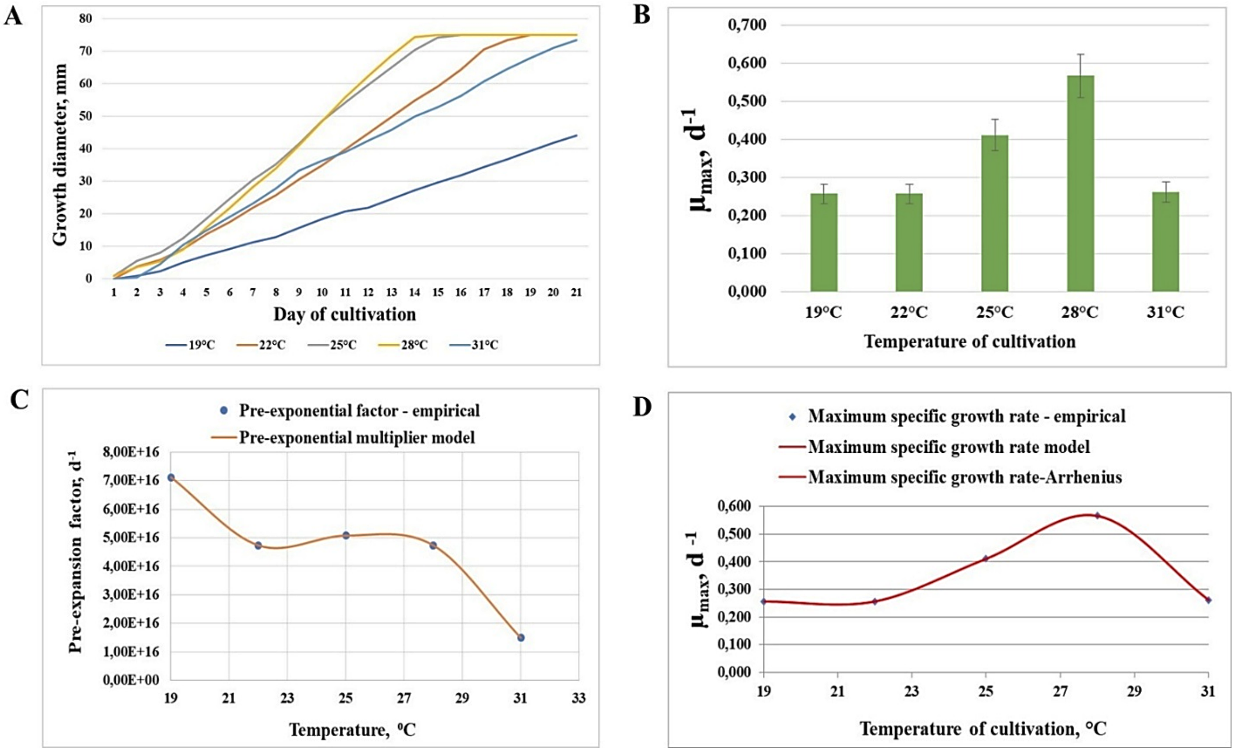

Figure 3. Cultivation of *I. hispidus* on SDLSWB medium at different temperatures (or Growth dynamics and modeling of *I. hispidus* on SDLSWB medium at different temperatures): Dynamics of mycelial growth (A); Variation in the maximum specific growth rate (μ_{max}) (B); Comparison of the experimentally obtained data for the pre-exponential multiplier in the Arrhenius equation and those from the polynomial model (C); Comparison of experimentally obtained and modelled μ_{max} values (D)

observed maximum growth rates of *I. hispidus* on SDLSWB medium at the temperatures tested. The temperature dependence of the pre-exponential multiplier, which is a fourth order polynomial, was derived. To calculate μ_{max} and thus predict the rate of development at different temperatures in the range 19 - 31℃, the model allows this parameter to be determined at temperatures other than those tested. In order to check the adequacy of the model, the pre-exponential factor was calculated. The results were compared with those obtained experimentally (Fig. 3C). The data indicate that the model accurately reflects the experimental results and can be effectively used to calculate the pre-exponential factor in the Arrhenius equation, allowing for theoretical determination of μ_{max} at various temperatures within the 19 to 31℃ range. Using this relationship along with the Arrhenius equation, the μmax rates of I. hispidus cultivated on SDLSWB medium were calculated for the temperatures studied.

$$\mu_{max} = -32{,}5053 + 6{,}31734 \times T - 0{,}448832 \times T^2 + 0{,}0139002 \times T^3 - 0{,}000157922 \times T^4 \qquad (3)$$

$$\mu_{max} = A_e^{-\frac{E_q}{RT}} \qquad (4)$$

The results obtained from the two models were then compared with the experimental data, as shown in Fig. 3D. The theoretical μ_{max} data from the two models exhibit significant overlap, indicating that both models are equally suitable for predicting the development of *I. hispidus* over a temperature range of 19 - 31℃. This is further confirmed by the high correlation coefficients for both models, which are close to one.

Determination of pH optimum. Series of experimental studies were conducted in order to elucidate the effect of pH value on the development of *I. hispidus* cultivated on SDLSWB medium. The dynamics of the mycelial growth of *I. hispidus* at different pH values is presented in Fig. 4A. The strain exhibits higher and relatively consistent growth intensity within the pH range of 5 to 6.5, compared to other pH levels. Regardless of the growth rate across all pH values, the maximum mycelial diameter of 75 mm is typically reached by around the 14th day of cultivation. For further investigation the effect of pH on the strain's development on SDLSWB, a logistic growth model was applied, and the maximum specific growth rate was calculated for each pH condition. The data presented in Fig. 4B show that the highest maximum specific growth rate (μ_{max} = 0.704 d^{-1}) was observed at pH 5.5, indicating that this pH level is optimal for the growth of *I. hispidus* on the SDLSWB medium. The figure also indicates that the μ_{max} values at pH levels 5.0, 6.0, and 6.5 are comparable, measuring 0.596, 0.594, and 0.589 d^{-1}, respectively. A similar trend is observed at pH 7.0 and 7.5, with maximum specific growth rate values of 0.513 and 0.511 d^{-1}, respectively.

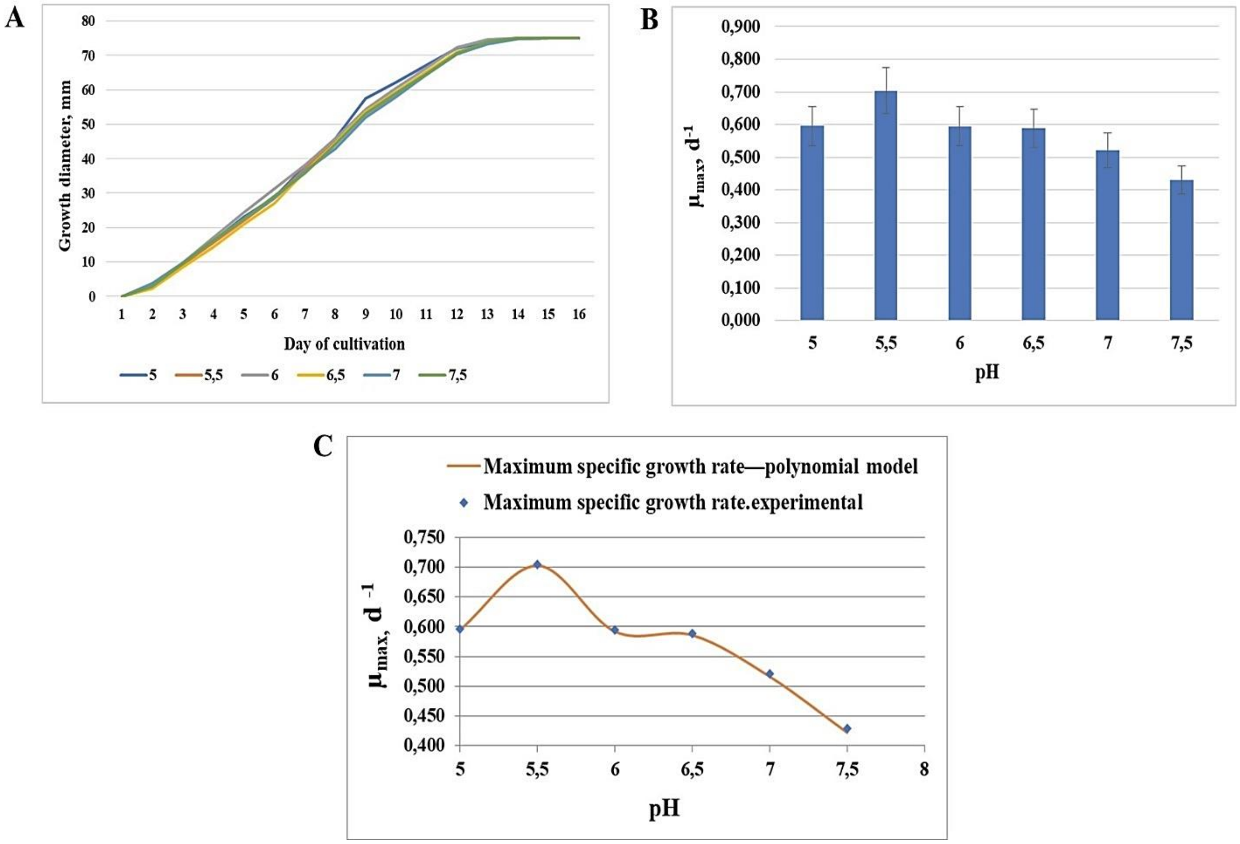

Figure 4. Cultivation of *I. hispidus* on SDLSWB medium at different pH values (или Growth dynamics and modeling of *I. hispidus* on SDLSWB medium at different pH values): Dynamics of mycelial growth (A); Variation in the maximum specific growth rate (μ_{max}) (B); Comparison of experimentally obtained and modelled μ_{max} values (C).

Similar results were obtained by Xu et al, who determined pH 6.0 as the optimal pH-value for the growth of *I. hispidus* (Xu et al. 2014). Slightly more alkaline conditions, with pH levels ranging from about 6.0 to 7.0, have also been reported by other researchers as favorable for the mycelial growth of *I. hispidus* (Song et al. 2020). Based on the kinetic studies, a pH-dependent relationship for μ_{max} was established, enabling the theoretical calculation and prediction of the maximum specific growth rate of *I. hispidus* cultivated on SDLSWB medium within the pH range of 5.0 to 7.5. The resulting equation is as follows:

$$\mu_{max} = -2081{,}958990669273 + 1681{,}845158536175 \times pH - 540{,}7268305092819 \times pH^2 + 86{,}52866617846982 \times pH^3 - 6{,}892666624679059 \times pH^4 + 0{,}2186666652299467 \times pH^5 \quad (5)$$

The maximum specific growth rate of *I. hispidus* on SDLSWB medium was calculated for the tested pH values. The μ_{max} values predicted by the model were then compared with those obtained experimentally, as shown in Fig. 4C. The model shows a strong agreement with the experimental data, evidenced by a high correlation coefficient close to 1. This indicates that the model reliably describes the observed results and can be used to theoretically calculate μ_{max} across the pH range studied.

Conclusions

This study pioneers the use of plant-derived waste materials as complex growth media for cultivating *Inonotus hispidus* GA4B, and introduces a model of the growth kinetics of this newly isolated Bulgarian strain. Employing logistic and reversed-autocatalytic growth models across nine natural media, the binary medium SDLSWB was identified as the most effective one. However, further optimization is necessary to enhance substrate utilization efficiency. Subsequent experiments established that 28°C is the optimal temperature for the growth of *I. hispidus* on SDLSWB. By applying the Arrhenius equation with a fourth-order polynomial model for the pre-exponential factor, the maximum specific growth rate was successfully predicted over a 19-31°C range, demonstrating strong alignment between model and data. The presented data confirmed that pH 5.5 is optimal for the growth of *I. hispidus* on the SDLSWB medium. A pH-dependent kinetic model was developed that accurately forecasts μ_{max} between pH 5.0 and 7.5. Together, these findings not only validate the use of plant-derived waste materials as media for cultivating *I. hispidus*, but also provide robust kinetic frameworks for predicting its growth under variable temperature and pH conditions. As such, this research lays essential groundwork for future efforts aimed at optimizing cultivation protocols and valorizing plant waste as a culture medium for the development of basidiomycete fungi.

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Author Contributions

Conceptualization, P.S., B.G.; methodology, P.S., D.B., A.G., B.G.; formal analysis B.K., A.G., G. A.; investigation, A.G., B.K.; resources, B.G., M. B.; data curation, P.S., D.B., G.A.; writing - original draft preparation, P.S., B.G. and G.A.; writing – A.G., B.K., B.G. and M.B., review and editing, P.S and M.B., visualization, , P.S and M.B.; supervision, D.B. and G.A.; project administration, P.S and M.B.; funding acquisition, P.S. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement

The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding authors.

Conflicts of Interest

The authors declare no conflicts of interest.

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