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Assessment of Polysaccharide and Biomass Production from Three White-Rot Fungi by Solid-State Fermentation Using Wood and Agro-Industrial Residues: A Kinetic Approach

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Abstract: *Research Highlights:* For the first time, a model was developed and applied for polysaccharide production from *Trametes versicolor* grown in agro-industrial and woody residues under solid-state fermentation (SSF) conditions. *Background and Objectives:* Fungal biomass is an important biological resource for biotechnological applications. Basidiomycetes fungi can be grown and developed on lignocellulosic materials such as forestry, wood, and agro-industrial residues in order to produce value-added products like bioactive polysaccharides. The objectives of this study were to evaluate the effects of the C/N ratio and copper concentration on biomass and polysaccharide production during solid state fermentation (SSF), as well as on the consumption of cellulose and hemicellulose, and lignin degradation, and to propose and validate a mathematical model to describe the overall SSF process. *Materials and Methods:* This research was carried out by growing three Basidiomycetes species (*T. versicolor*, *Lentinula edodes*, and *Pleurotus ostreatus*) on twelve formulations of solid substrates using mixtures of different inexpensive lignocellulosic residues such as oak sawdust, coconut fiber (hairs), coffee husks, and corn bran plus soybean oil, calcium carbonate, and two levels of copper(II) sulfate. *Results:* The three fungal species grew well on all substrate formulations. The statistical analysis of experimental data showed no significant effects on polysaccharide production, in the range of C/N and copper concentrations evaluated. Taking into account that the best polysaccharide production was obtained with *T. versicolor* (96.09 mg/g solid substrate), a mathematical model was proposed for this fungus to describe the behavior of the fermentation system from the obtained data of all the resulting combinations to reach the highest polysaccharide production by the fungus. *Conclusions:* The mathematical model disclosed in this work enabled to describe the growth and development of a higher basidiomycete under solid-state fermentation conditions on lignocellulosic substrates as well as the production of value-added products like polysaccharides with medicinal properties.

Keywords: basidiomycetes; delignification; lignocellulosic waste; mathematical modeling; ruminant feed; substrate colonization

1. Introduction

Lignocellulosic biomass is the most abundant biological material on Earth. An important fraction of lignocellulosic biomass corresponds to waste from different economic activities, in particular, agricultural, and agro-industrial residues such as cereal straws, corn stover, coffee husks, coconut fiber, wood and forestry waste, palm press fiber, and palm kernel shells, among others. The disposal of this waste implies high costs and negative environmental impacts. Being a source of valuable sugars and

polymers, lignocellulosic biomass can be used for the production of a wide spectrum of chemicals and materials such as liquid [1], gaseous [2], and solid biofuels [3], enzymes [4], organic compounds [5], synthetic polymers [6], pharmaceuticals [7], and food products [8,9], among many others. In particular, lignocellulosic residues may be employed for animal feed, especially in the case of ruminants [10]. In this way, their utilization for feed production involves the exploitation of plant material not usable for human food, leading to the improvement of food security.

The main obstacle for the utilization of the lignocellulosic residues from agricultural, agro-industrial, and forestry activities by the rumen microorganisms is their high content of lignin compared to roughage [10]. In the lignocellulosic complex, lignin is bound to cellulose and hemicellulose, making them less accessible to the action of microbes in the rumen. To overcome this obstacle, the residues should be pretreated in order to degrade the lignin by chemical, physical, or biological methods. The application of chemical and physical methods is limited due to the need of expensive equipment and chemicals, which can cause environmental pollution. The biological pretreatment of lignocellulosic waste has the potential to upgrade such residues through its delignification. For this, the addition of ligninolytic enzymes has been proposed, but the results have not been conclusive [11,12]. Fungal pretreatment is an attractive option not only to reduce the lignin content of lignocellulosic waste used for animal feed, but also to increase its protein content due to the growth of fungal biomass. In particular, white-rot fungi can selectively degrade lignin especially during the early phase of colonization as pointed out by van Kuijk et al. [10] and Thongklang and Luangharn [13]. These authors indicate that, for an appropriate treatment with fungi to obtain an optimal value in animal nutrition, the process should be stopped before carpophores are formed; thus, this means that timing is particularly important during treatment with fungi of lignocellulosics. Alternatively, the use of the spent substrate from the cultivation of white-rot Basidiomycetes may be another suitable option for small farmers, allowing them to generate income from the production of edible mushrooms and utilization of the residual delignified materials for ruminant feed in developing countries [14–17].

The white-rot Basidiomycetes *Pleurotus ostreatus* (known as oyster mushroom) and *Lentinula edodes* (known as shiitake) have been intensively used as edible mushroom, especially in China, Japan and other Asian countries. These fungi have demonstrated their good properties as lignin degraders as well as their lower cellulose consumption during the phase of substrate colonization in the case of wheat straw [10]. This feature is significant when different agro-industrial residues are pretreated using these fungi that exhibit high ligninolytic enzymatic activities. In a previous work [18], *L. edodes* showed 51.8% lignin degradation when grown on oak sawdust by solid-state fermentation (SSF) after 30-day incubation in bags among 10 Basidiomycetes evaluated. On the other hand, the medicinal mushroom *Trametes versicolor* is one of the fungi cultivated on wheat straw that have demonstrated a high improvement of the dry matter and organic matter digestibility compared to many other white-rot fungi [10]. In addition, this fungus has shown an important ability to produce ligninases on different solid materials like sugarcane bagasse and several agricultural residues [12]. This fact makes it attractive for pretreatment of lignocellulosic waste with delignification purposes as well.

Many mushrooms, such as *G. lucidum*, *T. versicolor*, *Hericium erinaceus*, *Armillaria mellea*, *Marasmius androsaceus*, *Tremella fuciformis*, and *L. edodes*, have been used as medicines and tonics in China, Korea, and Japan. The medicinal attributes of these fungi have led to the fast growth of the mushroom nutraceuticals market. These nutraceutical products are medicinal preparations from mushrooms, and they are functional foods that are enriched or modified and consumed as part of a normal diet for providing health benefits. They are extracts (refined or partially refined) marketed in the form of capsules or tablets, as a dietary supplement, and they have potential therapeutic applications [19]. In recent years, a variety of medicinal preparations in the forms of tablets, capsules, and extracts from mushrooms have been produced and commercialized. The presence of bioactive components in mushroom mycelium makes it an attractive ingredient that is now used as dietary supplements or nutraceuticals [20]. In most cases, the biological and pharmaceutical value of these nutraceutical products relies on the polysaccharides contained in the fungi employed for such medicinal preparations.

Polysaccharides are used industrially as gelling agents, thickeners, and stabilizers in food products. There is a rising interest in their biological functions, such as antitumor, antioxidant, or prebiotic activities [21]. Polysaccharide production is extensively distributed among fungi [22]. They have different functions when fungi are grown on natural substrates such as adhesion to surfaces, immobilization of secreted enzymes, prevention of hyphae from dehydration, and increases in nutrient residence time inside the mucilage [23]. Research efforts have been focusing on polysaccharides produced by edible and medicinal mushrooms because of their diverse biological and pharmacological activities. These include immunostimulating, antitumor, antibacterial, antiviral, hypocholesterolemic, antioxidant, and hypoglycemic activities [24–27]. Several mushrooms are characterized as a source of different bioactive polysaccharides. Most of them are homoglycans and some are heteroglycans, although they can also be found in combination with proteins, forming peptidoglycans or polysaccharide–protein complexes (proteoglycans).

β-glucans are the most studied polysaccharides from Basidiomycetes. In fungi, β-glucans are found in the intermediate layer of the cellular wall, adjacent to the plasmatic membrane; their function is to maintain the rigidity and shape of the cell. In the case of *Ganoderma lucidum*, for which more than 100 types of polysaccharides have been isolated, some β-*D*-glucans have shown immunomodulatory and immune-stimulating activities [19,28]. In general, water-soluble *G. lucidum* β-glucans have been studied extensively and have been shown to have a variety of immunostimulatory (activate immune responses) or immunomodulatory (regulate immune functions) effects [29]. Numerous studies have shown that *G. lucidum* polysaccharides exert their antitumor activity in the host immune system indirectly instead of through direct cytotoxic effects [30,31]. The three fungal species evaluated in this work synthesize important bioactive polysaccharides. Some of the best known fungal β-glucans in terms of pharmacological applications are lentinan and krestin (PSK), which are mostly β-(1→3, 1→6)-glucans [26]. Lentinan, which is obtained from *L. edodes*, has a triple helix structure and a molecular weight between 400 and 800 kDa. PSK is a proteoglycan made up of 25–38% protein residues and β-(1,4)-glucan with side chains of β-(1,6)-glucopyranosides, which is obtained from *Trametes versicolor*, and it has a molecular weight of 94 kDa [32,33].

In the case of feed for animals, especially ruminants, several reports indicate that polysaccharides from mushrooms contained in feed rations have the potential to improve animal health and nutrition [34,35]. Regarding this, the mixture of lignocellulosic waste materials colonized by fungal biomass (mycelium) is an attractive alternative for production of functional feed for ruminants. In particular, Kim et al. [36] pointed out that the glucans from *P. ostreatus* have a prebiotic function improving the nutritional value of the feed obtained from the spent substrate of this mushroom. These authors suggested that the glucan content of the sawdust-based spent substrate used for oyster mushroom cultivation could have beneficial effects on the rumen development of post-weaning calves. The effect of the water extract of the spent substrate from the white-rot fungus *Ganoderma lucidum* has been evaluated on the production performance and blood biochemical parameters of dairy cows [37], indicating that fungal polysaccharides can improve the health of ruminants. Polysaccharides from white-rot fungi have the potential to improve the microflora in the gut of other non-ruminant animals like broiler chickens; this effect could be explained by the increase in bacterial count and short chain fatty acids production in the large intestine in the case of chickens fed with polysaccharides from the submerged fermentation concentrate of the white-rot fungus *Hericium caput-medusae* [38]. On the other hand, β-glucans from mycelium biomass contained in the pretreated residue can be an additional glucose source in the rumen or replace part of the carbohydrates, hemicellulose, and cellulose, which are degraded by the fungus [10]. β-glucans from other types of fungal organisms like yeast have also shown their nutritional functions when added to feed as presented in [39] for the case of Holstein cows and Japanese black calves; the orally administered β-(1→3),(1→6)-*D*-glucan may affect the growth of particular bacterial strains, which are able to use it as a nutrient, and this may influence the formation of bacterial microbiota in the intestine. In addition, the increasement of glucanases activity may affect

oligosaccharides and glycoproteins production from feed in the rumen. Precisely, this type of glucans is frequently found in white-rot fungi [19].

Many factors influence the polysaccharide production from white-rot fungi by SSF. Besides the fermentation conditions (temperature, aeration, humidity, pH, CO_2 content, light), the substrate composition plays a crucial role. In particular, the carbon/nitrogen (C/N) ratio directly affects the behavior of the fungal culture. The content and types of carbon and nitrogen sources influence the mycelial growth and polysaccharide production by fungi. Basidiomycetes more readily assimilate organic nitrogen sources ensuring maximum mycelial biomass and polysaccharide formation in submerged cultures [40,41]. The presence and concentrations of some key metals have an important effect on the fungal growth and development as well [42]. In particular, the addition of copper salts to the culture medium is carried out to induce the activities of ligninases such as laccase and manganese peroxidase, two of the most important enzymes for the degradation of lignin contained in lignocellulosic biomass [43]. In fact, in a previous work [44], copper(II) sulfate was added to solid media as an inducer of the enzymatic activities of the oxidoreductases synthesized by *L. edodes*, *T. versicolor*, and *P. ostreatus*, obtaining good results. In this regard, the laccases belong to the group of blue copper oxidases which contain four copper atoms per molecule, distributed in three different copper binding sites [45]. Therefore, the presence of this metal in the culture media is always important for the induction and production of these enzymes [46]. These enhanced enzymatic activities lead to an increased biomass production and, therefore, to higher polysaccharide production, taking into account that these metabolites are mostly integrated to the cell wall of the white-rot fungi [42].

Thus, the quality and yield of fungal polysaccharides are greatly affected by the environmental and nutritional conditions, although most of the research on this subject was conducted under submerged fermentation conditions [22,47,48]. In a previous work [49], the ability of 10 species of Basidiomycetes to produce both extracellular and intracellular polysaccharides was tested; among the species screened for production of polysaccharides, the best results were obtained with *G. lucidum* and *P. ostreatus* by submerged fermentation (SmF), and *G. lucidum* and *Grifola frondosa* in the carpophores obtained by SSF. However, the assessment of the polysaccharide production from the solid substrates during the colonization stage before fructification was not performed.

Although a significant amount of reports have been published on the production of fungal polysaccharides, they are most oriented to the study of submerged fermentation conditions and not to the polysaccharide production during the vegetative stage under SSF conditions. In this latter case, besides the production of valuable polysaccharides, the white-rot fungi degrade the lignocellulosic materials, especially the lignin, improving their digestibility for ruminants. For this reason, a greater insight on the kinetics of biomass formation, polysaccharide production, nutrients consumption, and lignin degradation is required in such a way that the most significant features of SSF cultivation can be appropriately described. As far as the authors of this paper know, there are not available mathematical models describing neither the growth of white-rot fungi during the vegetative stage, nor the consumption of the substrate's components and production of different valuable compounds from them by SSF. Some kinetic profiles have been reported to describe the SSF process, including linear, logistic, and two-phase models (growth acceleration and deceleration) [50], but these models do not involve the effect of the concentration of the main components of the culture medium on the growth and do not consider the role of the lignocellulolytic enzymes related [51–53]. Therefore, it is necessary to develop efficient models to contribute to the implementation at the commercial level of this kind of process reducing, at the same time, the experimental work required to determine the optimum operating conditions and the data needed for scale-up research. For delignification of agro-industrial residues, it is particularly important to analyze the time-profile of lignin degradation by white-rot fungi. In this sense, modeling tools may be very valuable with this purpose as well. Unfortunately, there are no reports of such mathematical models.

The cultivation of white-rot fungi on agricultural, agro-industrial, and wood and forestry residues can significantly contribute to the production of animal feed in nutritional terms as follows: production of a protein-enriched feed material, synthesis of ligninolytic enzymes for the degradation of the lignin contained in the residues (delignification) to improve their digestibility, and synthesis of polysaccharides that could have potential benefits for ruminant health. This paper addresses the two latter issues, so the objectives of this work were: (i) to evaluate the polysaccharide production and biomass growth by *L. edodes*, *T. versicolor*, and *P. ostreatus*, using twelve formulations of solid media with different C/N ratios and two copper levels, and (ii) to study the time course of the polysaccharide production and lignin degradation during SSF by the fungal species with the highest synthesis of polysaccharides through a mathematical model proposed to describe these processes.

2. Materials and Methods

2.1. Microorganisms

Three Basidiomycetes species were employed for polysaccharide production by SSF: *Lentinula edodes* CICL54 provided by the National Coffee Research Center, Cenicafé (Chinchiná, Colombia), *Trametes versicolor* PSUWC430 supplied by the Pennsylvania State University (State College, PA, USA), and *Pleurotus ostreatus* UCC001 from the internal collection of the Universidad de Caldas (Manizales, Colombia). Pure cultures are deposited at the Culture Collection of Macrofungi at Universidad de Caldas. These species were maintained on potato dextrose agar at 4 °C with periodic transfer.

2.2. Spawn Preparation

Spawn of the three species studied was prepared on wheat grains (40% moisture content). The hydrated wheat was packed in 1-kg bi-oriented polypropylene bags. One square hole (2.54 cm side) was made at the top of each bag and covered with a microporous breather strip to allow for gas exchange. Bags were sterilized at 121 °C for 30 min. The sterilized grains were aseptically inoculated with 4% (wet basis) of each fungal species and incubated for 12–15 days at 25 °C and darkness until full colonization.

2.3. Culture Media and Fermentation Conditions

Twelve formulations of the solid media were assayed. All the substrates contained (dry basis): 40% oak sawdust, 20% coconut fiber, 2% soybean oil, and 2% calcium carbonate as fixed components. Different percentages of coffee husks and corn bran were tested to modify the C/N ratio (see Table 1 and Table S1 of the Supplementary Material). The C/N ratio was determined measuring organic matter by the method reported by Walkley and Black [54]. The total carbon amount corresponded to 58% of organic matter. The total organic nitrogen was determined by the Kjeldahl method [55]. The concentration of copper(II) sulfate was varied at two levels: 0.08% and 0.16%. The moisture content was adjusted at 60%. The substrates were packed in 200-g polypropylene bags and sterilized at 121 °C for 1 h. The bags containing the substrate were aseptically inoculated with 4% (wet basis) spawn and incubated at 25 °C in darkness for 49 days with 10-min air exchange daily. For each medium and each mushroom species, 15 bags were prepared (every bag represented one destructive sample). A total of 15 samples were taken for each treatment, the initial one and two samples per week for 7 weeks. This procedure was performed by triplicate and 1620 bags were tested. The experimental design is detailed in Section 2.7.

Table 1. Carbon/nitrogen ratios and copper(II) sulfate concentrations employed for formulations of twelve solid media.

Formulation	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
$CuSO_4$ (wt. %)	0.16	0.08	0.16	0.08	0.16	0.08	0.16	0.08	0.16	0.08	0.16	0.08
C/N ratio	92.6	89.7	97.6	93.6	100.9	91.8	85.3	84.9	55.1	51.5	117.1	111.1

Remarks: The solid media contained (dry basis) 40% oak sawdust, 20% coconut fiber, and 2% soybean oil. In addition, the media contained coffee husks and corn bran, which were used to adjust the carbon/nitrogen (C/N) ratio.

2.4. Polysaccharide Measurement

Since the target polysaccharides are β-glucans and related carbohydrates, the extraction procedure was chosen in such a way that cellulose and chitin were not measured as done elsewhere [19]. For the determination of these polysaccharides, 0.1 of dry solid was mixed with 5 mL of concentrated reagent grade ethanol and extracted with an ultrasound-assisted Elmasonic E Elma extractor (Elma Hans Schmidbauer GmbH & Co., Singen, Germany) for 20 min, with a subsequent 24-h rest contact between the sample and the solvent. The extract was filtrated at reduced pressure by using a preweighed glass microfiber filter (Whatman GF/F) and then centrifuged for 20 min at 14,000 rpm. The precipitate was suspended in 1-M sodium hydroxide solution at 60 °C for 1 h. Total carbohydrates were determined in this suspension using the phenol-sulfuric method [56]. The concentration of polysaccharides as total carbohydrates was expressed in mg per g dry solid (ds) as well as the concentrations of biomass, reducing sugars, cellulose, hemicellulose, and lignin.

2.5. Quantification of Fungal Biomass in Solid Substrates

The whole solid media were dried in an oven at 101 °C until constant weight, ground in a mortar, and stored until they were used for chitin determination. The fungal biomass contained in the dry solid media was estimated based on the quantification of the structural chitin component, the N-acetyl-*D*-glucosamine (NAGA) released after hydrolysis with 6 N HCl according to [57]. Analytical-grade NAGA served as reference. The NAGA content per gram of mycelium for each one of the three species grown in liquid medium was determined in parallel; for this, 250-mL flasks containing 100 mL liquid medium were used during 25-day incubation at 100 rpm. The liquid medium used for these purposes had the following composition (in g/L): glucose 30, yeast extract 6, $MgSO_4 \cdot 5H_2O$ 0.5, and $CaCl_2$ 0.1. The NAGA content for the mycelium grown in the liquid medium was determined according to [57] as well. In this way, the obtained values for conversion calculations (in μg NAGA/mg dry mycelium) were: 158.3 for *P. ostreatus*, 141.5 for *T. versicolor*, and 100.8 for *L. edodes*. Water-soluble compounds were extracted during the hydrolysis with 6 N HCl for three hours.

2.6. Quantification of the Fiber Components and Reducing Sugars

Cellulose, hemicellulose, and lignin (fiber components) for all the 15 samples collected during the cultivation of each one of the fungal species and for each one of the formulations of the solid media, as well as the soluble fraction, were quantified by using the results of the determination of neutral detergent fiber, acid detergent fiber, and acid detergent lignin. For this, each dried and ground solid sample underwent three hydrolysis series for 70 min each: hydrolysis with sodium lauryl sulfate and others; hydrolysis with ammonium bromide in 1 N sulfuric acid solution; and hydrolysis with 72% (w/v) sulfuric acid. At the end of each hydrolysis, samples were washed and dried at 105 °C until constant weight [58]. The concentration of reducing sugars as glucose was determined by the DNS method [59].

2.7. Experimental Design

To find the most appropriate combination of the three mushroom species and 12 medium formulations having the best performance in terms of polysaccharide production, a randomized factorial experimental design was used with three levels for the first factor (fungal species) and twelve levels for the second factor (formulation of the solid media). In addition, 15 levels of incubation time were analyzed. The formulations varied their C/N ratio and the copper(II) sulfate concentration as shown in Table 1; sampling frequency was described in Section 2.3. The response variables were the concentrations of polysaccharides and fungal biomass throughout the SSF process in the bags. Three replicas of all the experiments were performed.

All the statistical analyses for this work were carried out by using the software Matlab® 2010b (MathWorks, Natick, MA, USA). An analysis of variance was firstly performed for all the data obtained from the experimental design considering all the fungus/formulation combinations and the fermentation time (5% significance level). For this, the anovan Matlab function was applied. The normality distribution and variance homoscedasticity tests were conducted. As a normal distribution of the data was not obtained, a comparative Kruskal Wallis analysis was performed. This analysis for polysaccharide production was carried out for each one of the three fungal species studied and the twelve medium formulations by using the kruskalwallis and multcompare functions.

2.8. Time Profiles of the SSF Process

To get more insight on the kinetic behavior of the SSF of the lignocellulosic residues, the time profile of the main variables studied was obtained for each one of the fungal species grown on all the twelve medium formulations. For this, the data obtained from the experimental design described above were employed for building the time profiles for each one of the variables studied. These variables were as follows: concentrations of fungal biomass, polysaccharides, reducing sugars, and fiber components (content of cellulose, hemicellulose, and lignin). Samples for each fungus/formulation combination were taken during 15 different incubation times until the end of the SSF process (49 days). Three replicas of all the experiments were performed.

2.9. Mathematical Modeling of Fungal Growth and Polysaccharide Production

Different mathematical expressions were proposed and tested to describe the fungal growth and polysaccharide production under batch SSF conditions. The selected model included 12 ordinary differential equations. Matlab® was also used to solve the mathematical model proposed in this work. For this, the ode45 function based on an explicit Runge–Kutta (4,5) formula using a Dormand–Prince pair [60] as well as the ode15s function based on a variable-order formula employing numerical differentiation were applied. The model parameters were determined by non-linear regression from the experimental data by using the nlincon and fmincon Matlab functions. Some of the kinetic parameters were taken from one previous work on the modeling of lignocellulolytic enzymes produced during the cultivation of the same white-rot fungi evaluated in this work and under the same conditions [44].

To assess whether the proposed mathematical model adequately describes the experimental data, the one-tailed F test was performed. This test compares the variance of the sum of squared residuals (i.e., deviations of the experimental data related to the values calculated by the model) with the variance of the experimental data for each variable measured (it depends on the accuracy of the analytical method employed). For this test, vartest and vartest2 Matlab functions were applied.

3. Results and Discussion

3.1. Effect of C/N Ratio and Cupric Sulfate on Growth and Polysaccharide Production

The C/N ratio in the twelve substrate formulations assayed ranged from 52 to 143 corresponding to a fluctuation in the nitrogen content between 0.29% and 0.80% on a dry basis. For these same formulations, two concentrations of $CuSO_4$ were also varied (0.08% and 0.16%). The three species of fungi were capable of growing in all the substrates assayed, as shown in Figures S1 and S2 of the Supplementary Material (where all formulations are presented) and in Figure 1 (representing a subset of Figures S1 and S2). In this regard, it is necessary to point out that the modification of the nitrogen content and carbon source was ensured by using the same materials (oak sawdust, coconut fiber, coffee husks, corn bran, and soybean oil) and not by modifying the nitrogen and carbon sources.

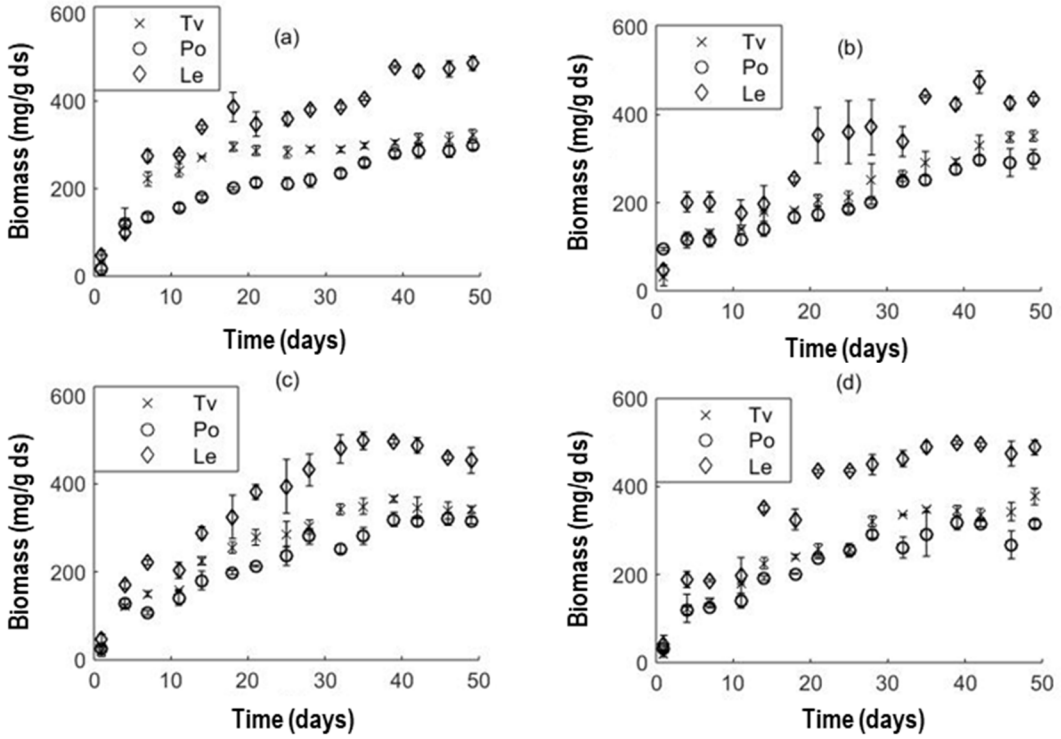

Figure 1. Biomass production of *T. versicolor* (Tv), *P. ostreatus* (Po), and *L. edodes* (Le) grown on different solid media based on oak sawdust, coconut fiber, coffee husks, corn bran, and soybean oil for 49 cultivation days under solid-state fermentation (SSF) conditions. (**a**) Biomass growth on F2 medium. (**b**) Biomass growth on F4 medium. (**c**) Biomass growth on F10 medium. (**d**) Biomass growth on F11 medium. The formulation for each medium is deciphered in Table 1; ds: dry solid.

The C/N ratio is a crucial factor affecting the synthesis of many metabolites, especially mycelial growth, and polysaccharide production, in fungal cultures. In this work, the fact that the three species studied were able to grow on all the media with significant polysaccharide production indicates that the selected high C/N ratios were appropriate for the synthesis of these carbohydrates. Other white-rot fungi exhibit a similar behavior. In particular, Lin and Chen [61] found that a relatively high C/N ratio can favor both mycelial growth and polysaccharide production by the basidiomycete *Antrodia cinnamomea*. The optimum ratio determined by these authors was 40. Nevertheless, it has been established that high nitrogen concentrations (low C/N ratios) promotes the degradation of cellulose and hemicellulose in lignocellulosic substrates favoring their faster colonization by basidiomycetes, while low nitrogen levels in the substrates stimulate lignin degradation [10,14,62]. Bento et al. [14] pointed out that the range of C/N ratios for cultivation of *L. edodes* should be about 20–25, since this fungus is more exigent in its nutritional requirements compared to *P. ostreatus* whose growth requirements are simpler. For *L. edodes*,

these authors supplemented several agro-industrial residues (including the coconut fiber) by adding urea, but they did not reach the complete colonization even during 60 days of cultivation; this indicates that a more appropriate and balanced C/N ratio should be employed. In contrast, *P. ostreatus* could grow easily in lignocellulosic residues without supplementation (high C/N ratios). The results obtained in the present work showed complete substrate colonization for all the three species, including *L. edodes*, for 49 days using a balanced mixtures of different lignocellulosic residues. These outcomes suggest the suitability of the range of C/N ratios evaluated as well as the proper selection of the media components.

In this work, coffee husks and corn bran were used as nitrogen sources for SSF. Coffee husks are one of the most important residues generated during the handling and processing of coffee. In this material, the caffeine and tannin content is about 1.3 and 10%, respectively, on a dry weight basis [63]. Due to the presence of these toxic compounds, there are no cost-efficient uses for this kind of residue and its disposal implies a major environmental problem [64]. However, the use of this residual material along with corn bran as a nitrogen source for cultivation of white-rot fungi may become an attractive way for producing ruminant feed. In fact, coffee husks and corn bran have demonstrated to be appropriate nitrogen sources for producing polysaccharides through SSF by the three species of fungi tested. Nevertheless, a clear correlation among the C/N ratio, mycelial growth, and polysaccharide production could not be established under the conditions evaluated for the three fungal species tested. On the other hand, copper is a metal that can enhance the production of peroxidases, which are key enzymes for delignification. From the statistical analysis, the different copper concentrations assayed did not affect biomass and polysaccharide production for all the three fungal species. Future studies for media optimization should evaluate wider ranges of copper concentration in order to obtain an optimum value for both polysaccharide production and delignification.

3.2. Time Course of Polysaccharide Production and Lignin Degradation during SSF

From the experimental kinetic data corresponding to the dynamics of SSF under the studied conditions, a short lag phase was observed in all the formulations followed by a stage of exponential growth (see Figure 1). All the formulations tended to reach the stationary phase after 30 days of incubation when the substrates were fully colonized. Biomass and polysaccharide production attained different values among the formulations, as inferred from Table 2 and Figures 1 and 2. The plots of the kinetic data corresponding to the polysaccharide production of the three fungal species grown on all the formulations are presented in Figures S3 and S4 of the Supplementary Material (Figure 2 is a subset of Figures S3 and S4). In general, higher polysaccharide concentrations were obtained when growing *T. versicolor* on F2, F4, and F11 media (see Figure 2), although these substrate formulations were not the most effective for enhancing mycelial growth. In this regard, the highest biomass concentrations were obtained on F11 medium for *T. versicolor* (377.1 mg/mg ds at 49th incubation day), F10 medium for *P. ostreatus* (320.8 mg/g ds at 46th incubation day), and F6 medium for *L. edodes* (514.7 mg/g ds at 49th incubation day).

The data obtained from the time course of the 49-day SSF in bags enabled us to observe a clear correspondence between the behavior of the fungal biomass (Figure 1) and the synthesis of polysaccharides (Figure 2). In fact, as the biomass concentration increases for all the species, the polysaccharide content in the substrates also increases according to the time trends of the experimental data. This indicates that polysaccharide synthesis is directly related to the cell growth rate in most cases, i.e., the polysaccharides are primary metabolites that are essential for the fungi during the colonization of the substrate. In some cases, the polysaccharide production is indirectly related to the growth rate (there is a slight displacement in the tendency of the data for biomass and polysaccharides) as can be observed in Figures 1a and 2a for the F2 medium in the case of *T. versicolor*. Tang and Zhong [65] pointed out that the exopolysaccharides of white-rot fungi can immobilize the lignocellulolytic enzymes that they release. Moreover, the gel formed by these biopolymers avoids the dehydration of the hyphae allowing the adherence of other fungal cells to the substrate surface [66], and enabling the selective uptake of the molecules from the surrounding environment [67]. In this way, these exopolysaccharides

play an important role for the growth and development of the biomass of white-rot fungi as primary metabolites. Precisely, the intense reproduction of cells during the colonization stage makes possible the filling of the void spaces in the solid matrix with fungal biomass, resulting in a compact material that may be pelletized during the formulation of feed for ruminants. In addition, the polysaccharide content of such a feed has the potential to improve not only the nutrition, but also the health of the animals.

Table 2. Fungal biomass (mg/g dry solid) and polysaccharide (mg/g dry solid) concentrations and specific polysaccharide production (μg polysaccharide/g dry mycelium) by *T. versicolor*, *P. ostreatus* and *L. edodes* grown on different formulations by SSF at 49th incubation day.

Medium	*T. versicolor*			*P. ostreatus*			*L. edodes*		
	Biomass	**POL**	**SPP**	**Biomass**	**POL**	**SPP**	**Biomass**	**POL**	**SPP**
F1	328.39 ± 2.11	63.03 ± 1.40	191.85	298.24 ± 1.32	65.68 ± 0.42	220.23	484.98 ± 1.61	40.74 ± 0.60	84.00
F2	323.79 ± 1.64	96.08 ± 1.24	296.74	297.07 ± 1.16	70.67 ± 1.30	237.89	485.90 ± 1.59	63.15 ± 1.01	129.97
F3	208.71 ± 1.55	80.41 ± 0.72	385.27	201.26 ± 0.58	49.49 ± 0.31	245.90	301.29 ± 0.84	87.45 ± 0.36	290.25
F4	351.90 ± 1.77	91.06 ± 0.71	258.77	299.09 ± 3.45	88.21 ± 0.24	294.93	434.21 ± 0.74	57.76 ± 1.38	133.02
F5	240.93 ± 2.50	59.43 ± 0.30	246.67	198.90 ± 0.74	88.72 ± 0.38	446.05	309.60 ± 3.15	86.98 ± 0.18	280.94
F6	360.81 ± 0.60	76.73 ± 0.73	212.66	313.49 ± 1.54	90.78 ± 0.38	289.58	514.70 ± 2.24	75.06 ± 0.23	145.83
F7	336.94 ± 2.48	68.33 ± 17.18	202.80	258.86 ± 6.71	26.41 ± 0.18	102.02	488.67 ± 1.43	87.45 ± 0.49	178.96
F8	346.91 ± 1.47	61.08 ± 0.46	176.07	308.82 ± 0.90	47.59 ± 0.24	154.10	462.83 ± 1.32	37.56 ± 0.76	81.15
F9	351.40 ± 1.54	64.46 ± 0.55	183.44	312.35 ± 1.80	6.69 ± 0.56	21.42	483.14 ± 3.33	50.80 ± 0.59	105.15
F10	341.42 ± 1.14	40.92 ± 1.50	119.85	316.25 ± 1.17	24.43 ± 2.88	77.25	453.60 ± 2.95	73.62 ± 0.43	162.30
F11	377.13 ± 2.65	93.00 ± 1.86	246.60	314.70 ± 1.38	52.62 ± 0.30	167.21	488.67 ± 1.71	51.40 ± 0.59	105.18
F12	362.14 ± 0.92	61.80 ± 2.38	170.65	296.47 ± 1.54	23.28 ± 0.36	78.52	492.70 ± 1.47	78.71 ± 0.63	159.75

POL: polysaccharide concentration; SPP: specific polysaccharide production. The formulation for each medium is deciphered in Table 1. The values are the mean of three samples.

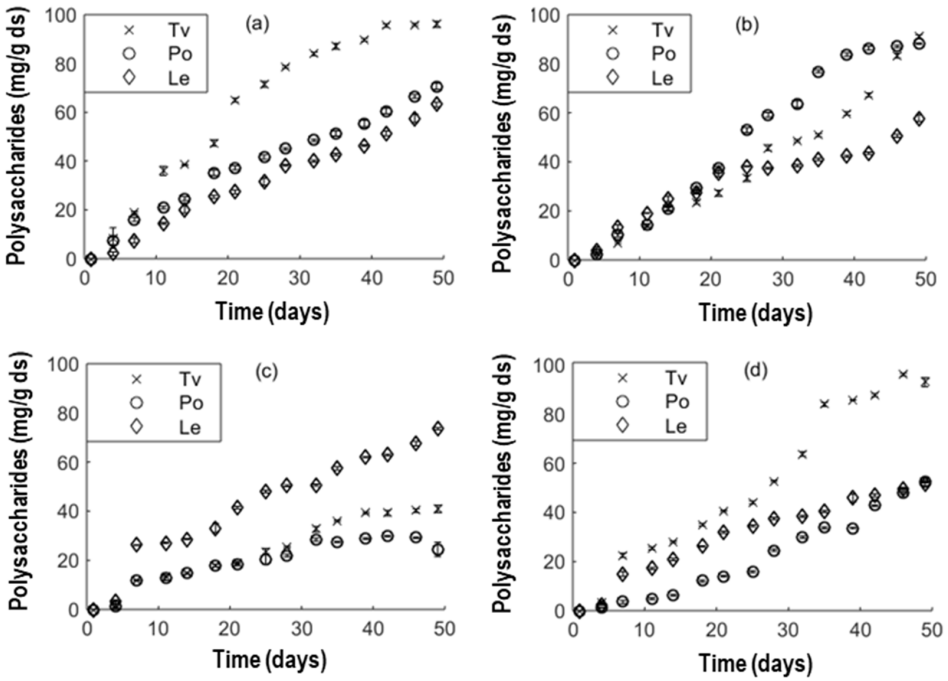

Figure 2. Polysaccharide production of *T. versicolor* (Tv), *P. ostreatus* (Po), and *L. edodes* (Le) grown on different solid media based on oak sawdust, coconut fiber, coffee husks, corn bran, and soybean oil during 49 cultivation days under solid-state fermentation (SSF) conditions. (**a**) Polysaccharide concentration in F2 medium. (**b**) Polysaccharide concentration in F4 medium. (**c**) Polysaccharide concentration in F10 medium. (**d**) Polysaccharide concentration in F11 medium. The formulation for each medium is deciphered in Table 1; ds: dry solid.

This behavior with the time has been found for other polysaccharide-producing fungi. *A. cinnamomea* and *G. frondosa* exhibited an increase in polysaccharide production that is parallel

to the biomass growth [68,69]. Nevertheless, in those works, *T. versicolor* and *Gloeophyllum trabeum* showed an inverse relationship between polysaccharide production and biomass growth; in these cases, a reduced biomass production was associated with a high polysaccharide production and vice versa. For this reason, the specific polysaccharide yield (milligrams of polysaccharide per gram of dry mycelium) can provide more insight on how different cultivation conditions influence the increase in polysaccharide production [22]. Table 2 shows the specific polysaccharide production obtained after 49 days of SSF. The highest specific polysaccharide productions were obtained in the F3 and F2 formulations for *T. versicolor*, in F3 and F5 media for *L. edodes*, and in the F5 medium for *P. ostreatus*. The specific polysaccharide production compares favorably to previous reports. A maximum of 139 mg polysaccharide/g ds was obtained by *Ganoderma lucidum* in an optimal medium for polysaccharide production [22], in contrast to the outcomes of this work where higher values were reached for the three fungal species tested.

The degradation of lignin during the cultivation process has paramount importance for animal feed. The lignocellulosic residues contained in the 12 media employed for solid cultivation would primarily provide polysaccharides and simultaneously provoke a significant delignification with feed purposes. At the same time, the cultivation would contribute to the disposal of these residues. The percentage of lignin degradation at the end of the SSF for the F1 medium (the formulation with the highest degradation of lignin for the three fungal species tested) reached 65.22% compared to 37.83% for cellulose, and 37.86% for hemicellulose by *T. versicolor*. In the case of *P. ostreatus*, the lignin degradation reached 46.01%, while degradation of cellulose and hemicellulose attained 29.49% and 35.65%, respectively. Finally, the corresponding data for *L. edodes* were 50.39% for lignin degradation, 27.83% for cellulose consumption, and 34.45% for hemicellulose consumption. In this regard, the F1 medium was the substrate with the highest C/N ratio (143) of all the ratios evaluated. For lignin degradation, high C/N ratios are required. The degradation of lignin occurs under conditions of nitrogen deficiency in nature; consequently, nitrogen addition to the fungal culture can restrain the ligninolytic effect [10]. The high C/N ratios selected have demonstrated to be suitable for colonization, delignification, and polysaccharide production. The results obtained in this work for lignin degradation of the three white-rot fungi studied were significantly higher than those of Bento et al. [14] in the case of *P. ostreatus* cultivated on coconut fiber (21.73%); likewise, the values of cellulose consumption were higher than those of the mentioned work (16.01%), and hemicellulose consumption was comparable (36.05%). In the case of the species/medium combination that reached the highest polysaccharide concentration (*T. versicolor*/F11), lignin degradation was also significant (53.94%) and higher than in the work cited [14]. This outcome suggests that further research on media optimization should be carried out in order to reach significant degradation of lignin but with lower consumption of cellulose, especially for the case of *T. versicolor*, although other white-rot fungi could be screened. In this way, the colonized substrate resulting from such an optimized medium could offer an enhanced content of structural carbohydrates, increased concentration of polysaccharides with potential nutritional and health benefits, and reduced lignin content for a proper formulation of a valuable and digestible ruminant feed. Logically, this kind of experimentation efforts should be complemented with in vitro and in vivo evaluations of digestibility. Our research group will tackle this challenge in future works.

As shown in Figure 3, the time profile of the components of the neutral detergent fiber (cellulose, hemicellulose, and lignin) shows a more notable decrease in the case of the lignin (Figure 3c,d) than in the case of cellulose and hemicellulose (Figure 3a,b). In fact, a slower pace of hemicellulose consumption during the first 10–15 days of cultivation can be observed in all the medium formulations for the three species studied (see Figure 3b). This behavior is not exhibited by lignin since it is degraded at almost the same rate during the whole cultivation process. It is known that white-rot Basidiomycetes have a strong lignin-modifying system allowing them to break the lignin seal covering the cellulose bundles within the lignocellulosic biomass particles. In terms of ruminant feed production, delignification is crucial to enhance the digestibility of many agricultural, agro-industrial, and woody residues with great availability and low cost, but with fewer possibilities to be utilized by ruminants. The outcomes

obtained in the present work indicate that the consumption of the structural carbohydrates (cellulose and hemicellulose) was not very high, especially if considering that the cultivation was stopped before the beginning of the fructification stage where these two polysaccharides are more intensively hydrolyzed and consumed by the fungal biomass. Data from other authors [70,71] show that the consumption of cellulose during fructification is significantly higher and the resulting spent substrate, though delignified, has much less cellulose (less nutritional value) than the colonized substrate before the fruitbody formation. For production of ruminant feed from agricultural, agro-industrial, and woody residues as those analyzed in this work, it is essential that the hydrolysis and consumption of these carbohydrates be as low as possible since they are the main energy source for ruminants. In particular, van Kuijk et al. [10] emphasized that the variations in the content of lignin during fungal pretreatment of lignocellulosic residual materials can be used to predict the effectiveness of a specific fungus/substrate combination. Undoubtedly, modeling tools may play a crucial role in this purpose to save costly experimental work. In general, the behavior shown in Figure 3 of the three mushrooms evaluated is quite similar. These mushroom species are white-rot Basidiomycetes whose growth and development is carried out on lignocellulosic biomass, mostly on woody materials, in nature. All the substrate formulations proposed in this work are based on the same carbon (lignocellulosic residues) and nitrogen sources. Therefore, the time profiles for the degradation of the fiber components had similar trends, specially taking into account that the operating conditions were not changed for the three mushrooms and the mechanism they utilize to attack the lignocellulosic biomass is the same as described elsewhere [72].

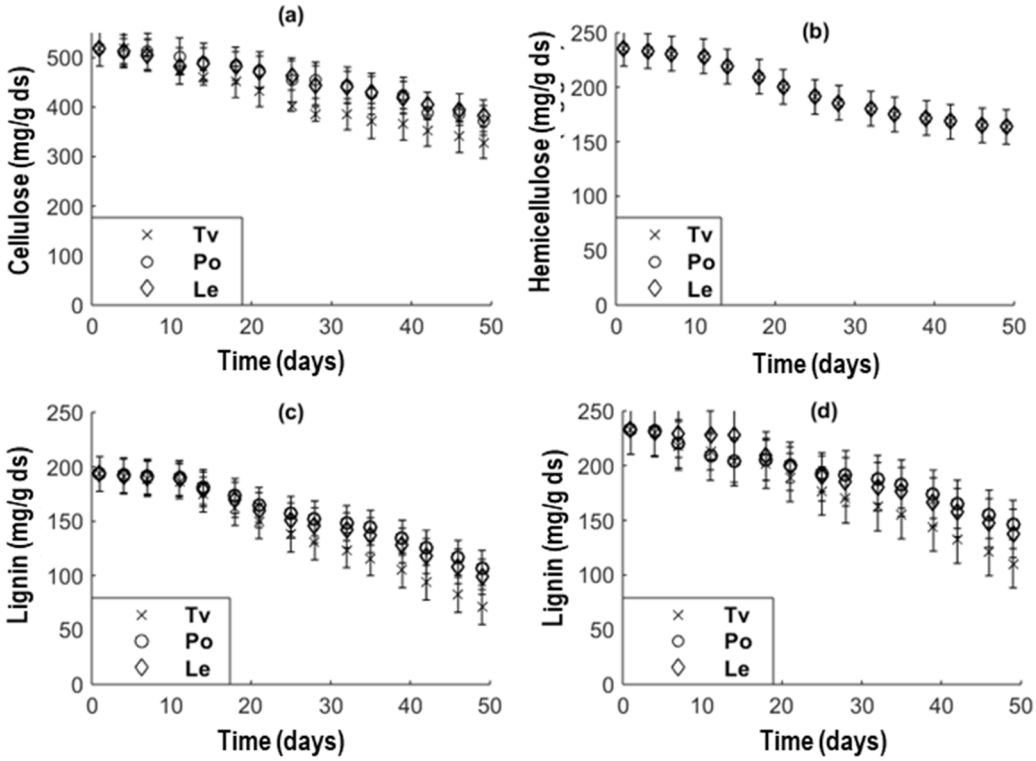

Figure 3. Consumption and degradation of the main components of the lignocellulosic substrates by *T. versicolor* (Tv), *P. ostreatus* (Po), and *L. edodes* (Le) grown on different solid media based on oak sawdust, coconut fiber, coffee husks, corn bran, and soybean oil during 49 cultivation days under solid-state fermentation conditions. (**a**) Cellulose consumption for F11 medium. (**b**) Hemicellulose consumption for F4 medium. (**c**) Lignin degradation for F1 medium. (**d**) Lignin degradation for F12 medium. The formulation for each medium is deciphered in Table 1; ds: dry solid.

3.3. Selection of Fungus/Medium Combination for Mathematical Modeling

The experimental data obtained in the present paper enabled the selection of the most suitable medium formulation and the most appropriate white-rot-fungus from the three species evaluated regarding their polysaccharide concentration. This selection is intended to choose the species/medium combination whose experimental data will be used for the development of the mathematical model of the cultivation kinetics. The performed three-way analysis of variance (i.e., considering the fungal species, the medium formulation, and the time) showed that there were statistically significant differences (p-value < 0.05) among all the species/medium combinations for the production of polysaccharides obtained for each one of the 15 times measured during the 49-day cultivation with a 5% significance level. Then, for each fungal species, the maximum value of the polysaccharide concentration with dependence on the time was selected considering all the formulations. The comparative analysis carried out among all the maxima by using the Matlab multcompare and kruskalwallis functions indicated that there existed statistically significant differences among the maximal values (p-value < 0.05) and that the best species/medium combination corresponded to *T. versicolor* grown on the F11 medium followed by the F2, F4, and F7 media for this same fungus. These four combinations with improved performance underwent a new comparative analysis in order to select the best one according to their polysaccharide production. As this comparative analysis showed no statistically significant differences (p-value = 0.212), the subsequent analysis by using mathematical modeling tools is presented below for these four *T. versicolor*/medium combinations, although it was applied to all combinations involving this fungus.

3.4. Mathematical Modeling

All the fungal species studied in this work grew well on all media formulations. The response obtained was assessed by the production of polysaccharides for several fungus/medium combinations. A mathematical model was proposed to describe the production of biomass and polysaccharides as well as the consumption/degradation of the main components of the lignocellulosic matrix, considering the data obtained of all the cultivations conducted on the different media formulations for *T. versicolor*, since this fungus was the one with the highest ability to produce polysaccharides and degrade the lignin of the three species studied. In this paper, the modeling results for the F2, F4, F7, and F11 formulations are shown considering that these cultivation media exhibited a better behavior in terms of polysaccharide production compared to the other ones. The results for the rest of the formulations are shown in Figures S5–S12 of the Supplementary Material.

The model proposed in this work is based on the description of the growth of fungal biomass and the degradation of lignocellulosic materials under conditions of SSF. In a previous work [44], such a description was developed considering not only the degradation of cellulose, hemicellulose, and lignin, but also the production of lignocellulolytic enzymes related to the hydrolysis or oxidation of those substrate components. Thus, the model developed considered the synthesis of endoglucanase, exoglucanase, and β-glucosidase as cellulose-hydrolyzing enzymes releasing reducing sugars. For a description of the hemicellulose hydrolysis, the model involves one expression for endoxylanase production. Finally, for lignin degradation, the model considers the production of the main ligninases: laccase and manganese peroxidase (MnP). As reducing sugars (mostly glucose, cellobiose, and xylose) are formed from both cellulose and hemicellulose and simultaneously consumed by the fungal biomass, an expression is included to take this dynamics into account. In the present work, this model is complemented with one differential equation describing the synthesis of the polysaccharides (both exo- and endopolysaccharides) by *T. versicolor* grown on lignocellulosic substrates. In this way, the proposed non-structured, non-segregated, deterministic mathematical model comprised a system of 12 differential equations with the same amount of variables dependent on the time (see Table 3).

Table 3. Mathematical model describing the production of biomass and polysaccharides, and consumption of the components of the lignocellulosic matrix.

Variable	Equation	No.	Parameters and Variables
Biomass	$\frac{dC_b}{dt} = \mu_m C_b\left[1-\left(\frac{C_b}{C_{bm}}\right)^n\right]$	(1)	μ_m: Specific growth rate (day^{-1}) C_b: Fungal biomass concentration (mg/g ds) C_{bm}: Maximum biomass concentration (mg/g ds) $n < 1$ The organism is relatively sensitive to the autoinhibition; it occurs for very low values of C_b $n = 1$ Logistic equation $n > 1$ The organism is relatively resistant to the autoinhibition; it occurs only when $C_b \approx C_{bm}$ t: fermentation time (day)
Reducing sugars	$\frac{dC_{RS}}{dt} = q_P\mu_m\frac{dC_b}{dt} \times \left[1-(n+1)\left(\frac{C_b}{C_{bm}}\right)^n\right]$	(2)	q_p: Production coefficient for reducing sugars ($mg \times mg^{-1}$) C_{AR}: Reducing sugars concentration ($mg \times g\ ds^{-1}$)
Lignin	$\frac{dC_L}{dt} = -k_L C_{LAC} C_{MnP}$	(3)	k_L: Lignin degradation coefficient ($mg \times g\ ds \times day^{-1} \times U^{-2}$) k_{LAC}: Laccase production coefficient ($U \times g\ ds \times mg^{-1} \times mg^{-1}$) k_{MnP}: Mn peroxidase production coeff. ($U \times g\ ds \times mg^{-1} \times mg^{-1}$) μ_{LAC}: Inhibition coefficient for laccase ($U \times mg^{-1} \times day^{-1}$) μ_{MnP}: Inhibition coefficient for Mn peroxidase ($U \times mg^{-1} \times day^{-1}$) C_L: Lignin concentration ($mg \times g\ ds^{-1}$) C_{LAC}: Laccase activity ($U \times g\ ds^{-1}$) C_{MnP}: Manganese peroxidase activity ($U \times g\ ds^{-1}$)
Laccase	$\frac{dC_{LAC}}{dt} = k_{LAC}C_L\frac{dC_b}{dt} - \mu_{LAC}C_L$	(4)	
Manganese peroxidase	$\frac{dC_{MnP}}{dt} = k_{MnP}C_L\frac{dC_b}{dt} - \mu_{MnP}C_L$	(5)	
Hemicellulose	$\frac{dC_{HM}}{dt} = -k_{HM}C_{ENX}$	(6)	k_{HM}: Hemicellulose consumption coefficient ($mg \times day^{-1} \times U^{-1}$) C_{HM}: Hemicellulose concentration ($mg \times g\ ds^{-1}$)
Endoxylanase	$\frac{dC_{ENX}}{dt} = k_{ENX}C_{HM}\frac{dC_b}{dt} - \mu_{ENX}C_{RS}$	(7)	k_{ENX}: Endoxylanase production coefficient ($U \times g\ ds \times mg^{-1} \times mg^{-1}$) μ_{ENX}: Inhibition coefficient for endoxylanase ($U \times mg^{-1} \times day^{-1}$) C_{ENX}: Endoxylanase activity ($U \times g\ ds^{-1}$)
Cellulose	$\frac{dC_C}{dt} = -k_C C_{ENG} C_{EXG}$	(8)	k_C: Cellulose consumption coefficient ($mg \times g\ ds \times day^{-1} \times U^{-2}$) C_C: Cellulose concentration ($mg \times g\ ds^{-1}$)
Endoglucanase	$\frac{dC_{ENG}}{dt} = k_{ENG}C_C\frac{dC_b}{dt} - \mu_{ENG}C_{RS}$	(9)	k_{ENG}: Endoglucanase production coefficient ($U \times g\ ds \times mg^{-1} \times mg^{-1}$) μ_{ENG}: Inhibition coefficient for endoglucanase ($U \times mg^{-1} \times day^{-1}$) C_{ENG}: Endoglucanase activity ($U \times g\ ds^{-1}$)
Exoglucanase	$\frac{dC_{EXG}}{dt} = k_{EXG}C_C\frac{dC_b}{dt} - \mu_{EXG}C_{RS}$	(10)	k_{EXG}: Exoglucanase production coefficient ($U \times g\ ds \times mg^{-1} \times mg^{-1}$) μ_{EXG}: Inhibition coefficient for exoglucanase ($U \times mg^{-1} \times day^{-1}$) C_{EXG}: Exoglucanase activity ($U \times g\ ds^{-1}$)
β-glucosidase	$\frac{dC_{BG}}{dt} = k_{BG}\frac{dC_b}{dt} - \mu_{BG}C_{RS}$	(11)	k_{BG}: β-glucosidase production coefficient ($U \times mg^{-1}$) μ_{BG}: Inhibition coefficient for β-glucosidase ($U \times mg^{-1} \times day^{-1}$) C_{BG}: β-glucosidase activity ($U \times g\ ds^{-1}$)
Polysaccharides	$\frac{dC_{POL}}{dt} = k_{POL}\frac{dC_b}{dt} + bC_b$	(12)	k_{POL}: Polysaccharide production coefficient related to growth rate ($mg \times mg^{-1}$) b: Polysaccharide production coefficient related to biomass concentration ($mg \times mg^{-1} \times day^{-1}$) C_{POL}: Polysaccharide concentration (mg/g ds)

Equation (1) intended to describe the cell biomass dynamics that corresponded to a logistic model modified by Mitchell et al. [73] and was successfully applied to the growth of the white-rot fungus *G. frondosa* in a previous work [74]. The variation of the reducing sugars is described by Equation (2), involving a constant production factor that modifies the growth rate; thus, the change of these sugars had a better fit to the growth rate than to the cell biomass itself. Equations (3), (6), and (8) depicting the degradation of lignin, hemicellulose, and cellulose, respectively, were proposed as a function of the specific activities of the enzymes responsible for degrading the corresponding substrates. The variation of the cellulolytic, xylanolytic, and lignin-modifying specific enzymatic activities (equivalent to the concentration for the enzymes) was contemplated to be dependent on the concentration of the corresponding substrate and on the growth rate with an inhibition factor. This factor is proportional to the reducing sugars content for endoglucanase, exoglucanase, and xylanase as shown in Equations (9), (10) and (7). For the two lignin-modifying enzymes, this inhibition factor was related to the content of lignin in the medium as observed in Equations (4) and (5). The production of β-glucosidase (Equation (11)) was described as a dependence on the growth rate with an inhibition term affected by the reducing sugars concentration.

The production of polysaccharides, described through Equation (12), was expressed by the equation of Luedeking and Piret [75], which represents the production of metabolites associated with both the growth rate and biomass concentration during fermentation processes. Thus, the proposed model tries to depict the biomass production, consumption of cellulose and hemicellulose as carbon sources for fungal growth, reducing sugars as intermediate products consumed during the fermentation, lignin degradation by the fungus in order to attain access to the hemicellulose and cellulose, and the synthesis of both exo- and endopolysaccharides associated with the fungal growth and concentration.

The values of the kinetic parameters of the model related to Equations (1)–(3), (6), (8) and (12) were calculated from the data obtained from the cultivation of *T. versicolor* grown on F2, F4, F7, and F11 media, corresponding to the time profiles of concentrations of biomass, reducing sugars, lignin, hemicellulose, cellulose, and polysaccharides, respectively (see Table 4). For this, non-linear regression was used to minimize the sum of squared residuals. However, as the data of the different enzymatic activities were not disclosed and available in this work, the parameters of Equations (4), (5), (7), (9)–(11) were directly taken from a previous report [44] in which *T. versicolor* was grown on the same lignocellulosic residues and whose enzymatic activities were measured. The model was solved for all the media formulations by using the ode45 and ode15s Matlab functions and considering the initial concentrations of all the involved variables.

Table 4. Values of the kinetic parameters and initial concentrations corresponding to the equations of the model proposed that describe the variables measured in this work.

Parameter	Media			
	F2	**F4**	**F7**	**F11**
μ_m	41.66400	43.25150	49.4620	28.62810
C_{bm}	314.16490	423.68590	348.12000	357.87150
n	0.00534	0.00252	0.00228	0.00407
C_{b0}	47.82610	12.34850	34.7000	27.98850
q_p	0.10000	0.76960	0.26860	0.70000
C_{AR0}	6.50000	7.50000	8.00000	5.20000
k_L	0.02072	0.00602	0.00784	0.00960
C_{L0}	211.62000	194.14000	200.00000	207.25000
k_{HM}	0.04294	0.03174	0.05629	0.06197
C_{HM0}	210.98000	200.87000	199.96000	205.55000
k_C	0.00531	0.06209	0.01002	0.01059
C_{C0}	493.29000	475.65000	499.35000	490.04100
k_{POL}	0.14130	0.06512	0.05862	0.10930
b	0.00506	0.00630	0.00403	0.00510
C_{POL0}	0.00000	0.00000	0.00000	0.00000

Remarks: The values of the kinetic parameters were obtained from the experimental data through non-linear regression. The equations describing the variables measured were as follows: (1)–(3), (6), (8), (12).

The modeling results obtained are presented in Figures 4 and 5 only for the media selected previously because of space constraints. In those figures, the experimental values of the variables measured are also shown as discrete points. Comparing the kinetic curves calculated by the model with the data obtained from the experimental design, it is possible to observe the good fitting of the model, meaning that the proposed mathematical description is adequate. The results of the one-tailed *F*-test, which compared the sum of squared residuals with the variance of the experimental data for each studied variable, allowed proving the validity of the model proposed in terms of the best description and representation for the experimental data obtained.

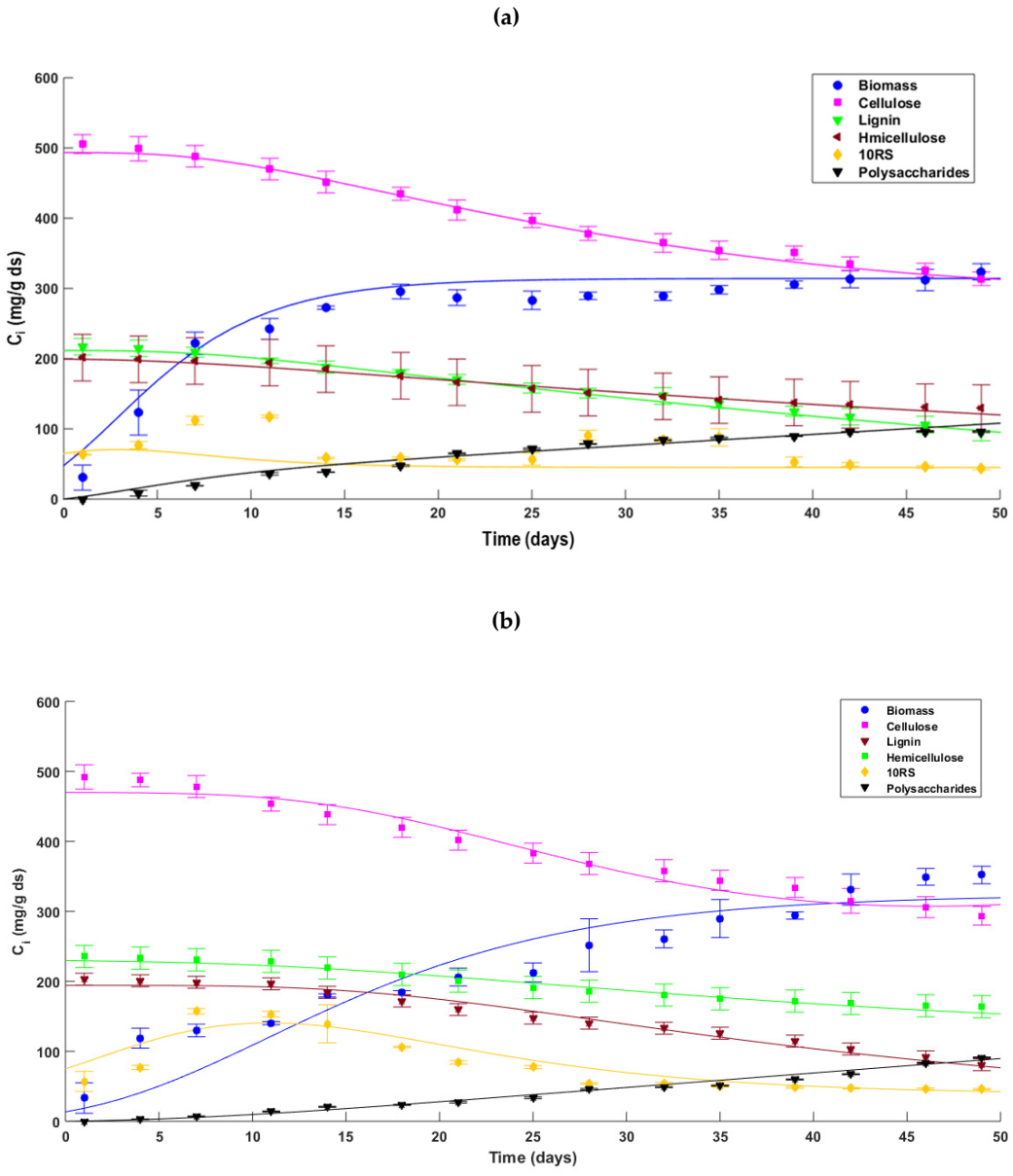

Figure 4. Time profile of cell biomass, polysaccharide production, and cellulose, hemicellulose, and lignin degradation by *T. versicolor* grown on different solid media. (**a**) Kinetic behavior for F2 medium. (**b**) Kinetic behavior for F4 medium. The continuous lines were calculated by the model proposed. The values for reducing sugars are multiplied by 10; C_i: concentration, ds: dry solid, RS: reducing sugars.

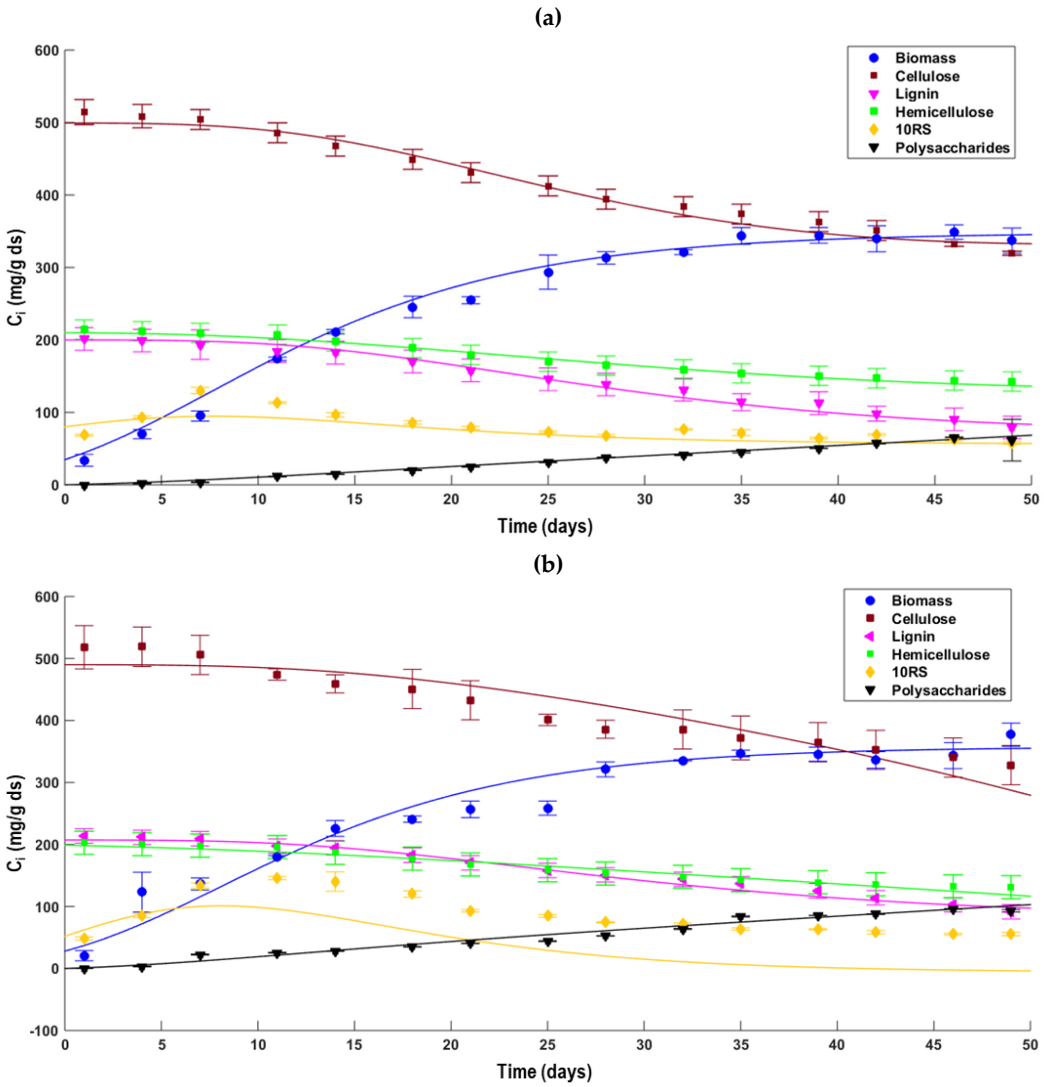

Figure 5. Time profile of cell biomass, polysaccharide production, and cellulose, hemicellulose, and lignin degradation by *T. versicolor* grown on different solid media. (**a**) Kinetic behavior for F7 medium. (**b**) Kinetic behavior for F11 medium. The continuous lines were calculated by the model proposed. The values for reducing sugars are multiplied by 10; C_i: concentration, ds: dry solid, RS: reducing sugars.

The development and selection of the kinetic expressions and relationships for each one of the experimentally studied variables was carried out in such a way that the equations have some biological sense. The proposed model reasonably described all the variables despite the complexity of the process evaluated. From the results obtained, it is evident that the polysaccharides measured are primary metabolites as the curve representing their concentration is coupled with the curve corresponding to the biomass growth. In fact, the polysaccharides determined in this work are polysaccharides released by the fungal cells (exopolysaccharides) and polysaccharides accumulated inside the cells (endopolysaccharides), so they are synthesized at higher rates when the cell biomass is intensively growing and colonizing the lignocellulosic residues. In this sense, the low value obtained for the coefficient *b* in Equation (12) indicates that these polysaccharides are more directly related to the growth rate than to the biomass concentration itself.

From the curves calculated by the model, it can be corroborated that the lignin degradation continues to occur even when the biomass has reached the stationary phase. In fact, the model predicts that laccase synthesis continues after the biomass growth finishes; in the case of manganese peroxidase, the model predicts a maximum of its enzymatic activity at the end of the exponential

phase, with a slight decline until the end of the fermentation. These outcomes enable one to infer that the complete colonization does not decrease the process of lignin degradation since the enzymes oxidizing it are present in significant amounts for its degradation. The model proposed appropriately captures this behavior.

The model presented in this work was applied to all the formulations where *T. versicolor* was grown. The aim of any model is to be applicable to the higher possible amount of real systems. As far as the authors of this work know from the available literature, this is the first model developed and applied for polysaccharide production from the white-rot fungus *T. versicolor* grown on agro-industrial lignocellulosic residues under SSF conditions. In fact, the model is also applicable to the other two fungal strains and to other lignocellulosic residues. With the help of this model, it is possible to predict and evaluate not only the overall production of polysaccharides with potential health benefits for ruminants, but also the amount of fungal biomass formed (valuable to increase the protein content of the feed for ruminants) and the degree of lignin degradation when *T. versicolor* is used for pretreatment of the waste, which is necessary to improve the digestibility of the feed obtained. The model also may be used for scale-up studies intended to assess the length of the colonization process during the vegetative stage of fungal growth as well as the percentage of conversion of lignocellulosic biomass into an upgraded feed for ruminants for large scale cultivation in bags. Future work will be oriented to establish appropriate relationships between the kinetic expressions of the model and the cultivation conditions (temperature, pH, light intensity, CO_2 gaseous concentration) as well as to describe the quantitative changes in the substrate when fructification occurs.

4. Conclusions

The mathematical model proposed in this paper allows the description of the growth and development of a higher basidiomycete under SSF conditions on lignocellulosic substrates as well as the production of value-added products such as polysaccharides. The approach employed represents an attempt to provide powerful modeling tools to assess the implementation at the industrial level of this type of process, reducing, at the same time, the experimental efforts needed to determine the optimum operating conditions and the data necessary for scale-up research. In this work, by utilizing inexpensive lignocellulosic residues such as oak sawdust, coconut fiber, and coffee husks, a comparable high specific production of polysaccharides was obtained for all the Basidiomycetes assayed at the end of the cultivation process: 385.3 μg/g ds for *T. versicolor*, 446.1 μg/g ds for *P. ostreatus*, and 290.3 μg/g ds for *L. edodes*. Therefore, SSF in these solid residues is examined as a promising and attractive option for the cost-efficient production of polysaccharides from higher mushrooms during the colonization stage.

Supplementary Materials: The following are available online at http://www.mdpi.com/1999-4907/11/10/1055/s1, Figure S1: Biomass production of *T. versicolor* (Tv), *P. ostreatus* (Po), and *L. edodes* (Le) grown on different solid media based on oak sawdust, coconut fiber, coffee husks, corn bran, and soybean oil for 49 cultivation days under solid-state fermentation conditions. A. Biomass growth on F1 medium. B. Biomass growth on F2 medium. C. Biomass growth on F3 medium. D. Biomass growth on F4 medium. E. Biomass growth on F5 medium. F. Biomass growth on F6 medium. The formulation for each medium is deciphered in Table 1; ds: dry solid, Figure S2: Biomass production of *T. versicolor* (Tv), *P. ostreatus* (Po), and *L. edodes* (Le) grown on different solid media based on oak sawdust, coconut fiber, coffee husks, corn bran, and soybean oil for 49 cultivation days under solid-state fermentation conditions. A. Biomass growth on F7 medium. B. Biomass growth on F8 medium. C. Biomass growth on F9 medium. D. Biomass growth on F10 medium. E. Biomass growth on F11 medium. F. Biomass growth on F12 medium. The formulation for each medium is deciphered in Table 1; ds: dry solid, Figure S3: Polysaccharide production of *T. versicolor* (Tv), *P. ostreatus* (Po), and *L. edodes* (Le) cultivated on different solid media based on oak sawdust, coconut fiber, coffee husks, corn bran, and soybean oil during 49 cultivation days under solid-state fermentation. A. Polysaccharide concentration in F1 medium. B. Polysaccharide concentration in F2 medium. C. Polysaccharide concentration in F3 medium. D. Polysaccharide concentration in F4 medium. E. Polysaccharide concentration in F5 medium. F. Polysaccharide concentration in F6 medium. The formulation for each medium is deciphered in Table 1; ds: dry solid, Figure S4: Polysaccharide production of *T. versicolor* (Tv), *P. ostreatus* (Po), and *L. edodes* (Le) cultivated on different solid media based on oak sawdust, coconut fiber, coffee husks, corn bran, and soybean oil during 49 cultivation days under solid-state fermentation. A. Polysaccharide concentration in F7 medium. B. Polysaccharide concentration in F8 medium. C. Polysaccharide concentration in F9 medium. D. Polysaccharide concentration in F10 medium. E. Polysaccharide concentration in F11 medium. F. Polysaccharide concentration in F12 medium. The formulation for each medium is deciphered in Table 1; ds: dry

solid, Figure S5: Time profile of cell biomass, polysaccharide production, cellulose, hemicellulose, and lignin degradation by *T. versicolor* grown on F12 solid media. The continuous lines were calculated by the model proposed. The values for reducing sugars are multiplied by 10; C_i: concentration, ds: dry solid, RS: reducing sugars, Figure S6: Time profile of cell biomass, polysaccharide production, cellulose, hemicellulose, and lignin degradation by *T. versicolor* grown on F10 solid media. The continuous lines were calculated by the model proposed. The values for reducing sugars are multiplied by 10; C_i: concentration, ds: dry solid, RS: reducing sugars, Figure S7: Time profile of cell biomass, polysaccharide production, cellulose, hemicellulose, and lignin degradation by *T. versicolor* grown on F9 solid media. The continuous lines were calculated by the model proposed. The values for reducing sugars are multiplied by 10; C_i: concentration, ds: dry solid, RS: reducing sugars, Figure S8: Time profile of cell biomass, polysaccharide production, cellulose, hemicellulose, and lignin degradation by *T. versicolor* grown on F8 solid media. The continuous lines were calculated by the model proposed. The values for reducing sugars are multiplied by 10; C_i: concentration, ds: dry solid, RS: reducing sugars, Figure S9: Time profile of cell biomass, polysaccharide production, cellulose, hemicellulose, and lignin degradation by *T. versicolor* grown on F6 solid media. The continuous lines were calculated by the model proposed. The values for reducing sugars are multiplied by 10; C_i: concentration, ds: dry solid, RS: reducing sugars, Figure S10: Time profile of cell biomass, polysaccharide production, cellulose, hemicellulose, and lignin degradation by *T. versicolor* grown on F5 solid media. The continuous lines were calculated by the model proposed. The values for reducing sugars are multiplied by 10; C_i: concentration, ds: dry solid, RS: reducing sugars, Figure S11: Time profile of cell biomass, polysaccharide production, cellulose, hemicellulose, and lignin degradation by *T. versicolor* grown on F3 solid media. The continuous lines were calculated by the model proposed. The values for reducing sugars are multiplied by 10; C_i: concentration, ds: dry solid, RS: reducing sugars, Figure S12: Time profile of cell biomass, polysaccharide production, cellulose, hemicellulose, and lignin degradation by *T. versicolor* grown on F1 solid media. The continuous lines were calculated by the model proposed. The values for reducing sugars are multiplied by 10; C_i: concentration, ds: dry solid, RS: reducing sugars, Table S1: Percentage composition (on a dry basis) of the twelve solid media employed for the cultivation of *Trametes versicolor*, *Pleurotus ostreatus*, and *Lentinula edodes*.

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