RESEARCH ARTICLE

Kinetic model to describe the morphological evolution of filamentous fungi during their early stages of growth in the standard submerged and microparticle-enhanced cultivations

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Biosynthesis of metabolites and enzymes by filamentous fungi depends on their morphological form in submerged cultures. However, their early stages of growth lasting approximately 24 h, from the introduction of spores to the medium until the formation of stable morphological forms, such as clumps or pellets, have rarely been the objects of experimental and modeling studies. Microparticle-enhanced cultivation (MPEC) has been applied only to a few fungal species, mainly *Aspergilli*. Therefore, the objective of this work was to formulate the kinetic model to describe the early stages of the fungal evolution in the standard cultivation and MPEC for *Aspergillus terreus*, *Chaetomium globosum*, *Penicillium rubens*, and *Mucor racemosus*. These fungi exhibit various mechanisms of agglomerates formation in submerged cultures. The experiments were performed in batch shake flasks (parameters identification) and a stirred tank bioreactor (model verification). In the balance equation for fungal cells, the mean projected area of hyphal objects measured by the digital analysis of microscopic images was used as the dependent variable. The analysis of the experimental data and model solution revealed that the effect of the microparticles (aluminum oxide at 6 g L^{-1}) in MPEC toward the studied filamentous fungi was to the high extent species dependent. This effect was most evident in the case of spore coagulative *A. terreus* and noncoagulative *M. racemosus*.

KEYWORDS

bioreactor, filamentous fungi, kinetic modeling, microparticle-enhanced cultivation, morphology

1 | INTRODUCTION

For decades, various models of microbial growth have been proposed, starting from the simple unstructured kinetic modeling with the use of Monod equation only, up to more complicated multiparameter structured mathematical models of microbial metabolism [1]. It is difficult to use standard unstructured kinetic approach to describe the growth of filamentous fungi, as fungal morphology and hyphal differentiation have to be taken into account [2]. The classical approach

to model the growth of filamentous fungi that reflected the mechanisms of fungal proliferation in the form of dispersed hyphae was based on the quantification of hyphae elongation and branching with the use of total hyphal length and length of hyphal growth unit. Quantitative data obtained from the digital image analysis were next used for the purpose of modeling [3–5]. In another approach, the hyphae were divided into the zones of various metabolic activities, namely apical, sub-apical, and hyphal cells, and the morphological (physiological) differentiation of hyphae was taken into account in the modeling of fungal growth [2,6,7].

The attempts to model the initial stages of filamentous fungi growth were scarce, especially in the context of species

Abbreviations: ATCC, American Type Culture Collection; MPEC, microparticle-enhanced cultivation; OUR, oxygen uptake rate.

forming microscopic or macroscopic pellets. In the previous works, the growth of fungal pellets was proved to satisfy the so-called cube root law [8]. Only simple formulae were used by Chiu and Zjic to model the growth of fungal pellets [9]. Carlsen et al. showed that the increase of pellet radius in *Aspergillus oryzae* is linear in time between 15 and 45 h of the cultivation [10]. Grimm et al. quantitatively studied the mechanism of pellets formation in *Aspergillus niger*, using a simple exponential equation to describe their experimental data [11]. Nielsen and Krabben individually modeled the process of spore germination in *Penicillium chrysogenum* proving that five parameters, namely the viability of the spores, the time at which germination starts, time at which no more spores germinate, and the two parameters of the B-distribution determining the steepness and skewness respectively of the germination frequency, were sufficient to describe the overall process [12]. Bizukojc and Ledakowicz presented the model of the early stages of *A. niger* growth to predict biomass concentration in the submerged culture [13]. However, the three known mechanisms: (1) spore agglomeration (spore coagulative), (2) nonagglomeration (noncoagulative), and (3) hyphae aggregation (agglomeration) of pellet formation by filamentous microorganisms, including fungi, initially described by Takahashi et al. and Takahashi and Yamada [14,15] and confirmed by Metz and Kossen [16], have never been the subjects of any kinetic modeling efforts.

In the last decade, only a few papers on fungal growth modeling were published. At the same time, however, the modern methods of fungal cultivation, including morphological engineering techniques, were developed. Among various morphological engineering techniques, the microparticle-enhanced cultivation (MPEC) has been regarded as a particularly effective approach used toward promoting the development of favorable morphological forms [17–21]. It has been proved that the application of mineral microparticles to control the morphology of filamentous fungi in submerged cultures leads to a large increase of the titers of secondary metabolites and enzymes. Nevertheless, the majority of evidences for this positive action of mineral microparticles originated from the experiments involving filamentous fungi, mainly representing the *Aspergillus* genus, whose spores agglomerate to form pellets. The examples included the enzyme and secondary metabolites producers, such as *Aspergillus niger*, a lovastatin-producing *Aspergillus terreus* or other *Aspergilli* [18–22]. There are limited data concerning the fungal species that do not exhibit the agglomeration of spores during an initial phase of their morphological evolution [17,20]. Eventually, never was any MPEC process with the participation of filamentous fungi mathematically modeled on any level of complexity.

Previously, the significant differences during the spore-to-hyphae growth of *A. terreus*, *Chaetomium globosum*, *M. racemosus*, and *Penicillium rubens* were shown by

PRACTICAL APPLICATION

Microparticle-enhanced cultivation (MPEC) is a technique to increase productivity of filamentous microorganisms, including filamentous fungi, in the submerged bioreactor cultures. As the addition of mineral microparticles, like aluminum oxide or talc, is relatively cheap compared to the benefits from the increase of metabolite or enzyme titers, MPEC can be applied in the large-scale industrial bioreactors. Nevertheless, it is crucial to have a mathematical description of any process to be introduced in the large scale. The kinetic model presented here is the first attempt to mathematically describe the kinetics of the morphological changes of filamentous fungi in MPEC in comparison with the standard cultures. Furthermore, this article comprises the study of four fungal species exhibiting various mechanisms of morphological evolution, i.e. *Aspergillus terreus*, *Chaetomium globosum*, *Penicillium rubens*, and *Mucor racemosus*. The model well describes the growth of all these fungal species, which proves its universality and practical applicability for different fungi cultivated in the bioreactors.

Kowalska et al., who for the first time performed the detailed quantitative analysis of the early stages of fungal growth in MPEC [23]. It comprised the temporal changes of size and shape parameters for the aforementioned species in the standard and MPEC shake flask processes.

This work is aimed at the formulation of the kinetic model of the morphological evolution of four fungal species, *A. terreus*, *Ch. globosum*, *M. racemosus*, and *P. rubens*, in the early stages of their evolution in the submerged shake flask and stirred tank bioreactor cultivations. Fungal growth was tracked within the first 24 h after introducing the spores into the medium to collect the data for kinetic modeling. This approach was intended to concern solely the morphological development of the studied fungi (as quantified by digital image analysis) and no product formation was taken into account.

2 | MATERIALS AND METHODS

2.1 | Strains and sporulation conditions

Four fungal strains obtained from the American Type Culture Collection (ATCC) were tested in the study: *A. terreus* ATCC 20542, *P. rubens* ATCC 9178, *Ch. globosum* ATCC 6205, and *M. racemosus* ATCC 7924. The strains were maintained

on agar slants prepared according to the recommendations of ATCC, as described in detail by Kowalska et al. [23]. The slants were incubated for 10 days and then applied for inoculation (about 10^9 spores per liter were used each time).

2.2 | Cultivation conditions

The input data used for the purpose of modeling were the time changes of mean projected area of hyphal objects measured with the use of image analysis. These data originated from the previous experiments conducted in shake flasks [23] and from the bioreactor experiments performed in the current study. The shake flask cultures in flat-bottomed flasks of 150-mL working volume were propagated in the thermostated rotary shaker Certomat BS-1 at 28°C (B. Braun Biotech International, contemporarily Sartorius Stedim, Germany) at the shaking speed equal to 110 min^{-1} [23]. The radius of deflection of the plate with flasks was 25 mm. Due to the technical reasons, dissolved oxygen could not be tracked in the shake flask culture. The bioreactor cultivations were performed in a fully automated stirred tank 5.7-L working volume bioreactor (Sartorius Stedim, Germany). The aeration and stirring conditions, namely the stirring speed of 220 min^{-1} and the air flow rate equal to $2\text{ l}_{\text{air}}\text{ min}^{-1}$, were kept constant throughout the study to maintain the same level of impact exerted by aeration and agitation toward the morphological events. The bioreactor was equipped with standard measurement and control devices, including dissolved oxygen, pH, and redox potential probes.

All processes were single-stage cultivations started from the spores washed out from the slants and suspended in the cultivation medium. The individual shake flask run lasted for 24 h [23]. It is the typical time of precultures for fungal cultivations. Furthermore, the morphological development of the fungus lasts approximately 24 h too. After that time, the stable hyphal elements (pellet, clumps, or dispersed hyphae) are formed. On the other hand, the bioreactor experiments took over 100 h. The prolongation of the time of cultivation was proposed due to the fact that different hydrodynamic and aeration conditions in the stirred tank might have influenced

the initial stage of the development of the fungi from spores to pellets. Nevertheless, the most significant morphological changes of hyphae in the bioreactor runs also took place within the first 24 h of the cultivation and only these data (excluding *Ch. globosum* that grew slower in the bioreactor) were used as the basis for modeling. Both standard and MPEC runs were performed over the course of the study. For each fungus, three independent experiments in shake flasks and a single experiment in the stirred tank bioreactor were performed. All the experiments are summarized in Table 1.

2.3 | Liquid cultivation media

Media compositions were dependent on the species and were the same as the ones used previously [23]. For *A. terreus* ATCC 20542 and *Ch. globosum* ATCC 6205, the medium contained lactose (10 g L^{-1}), yeast extract (8 g L^{-1}), KH_2PO_4 (1.51 g L^{-1}), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.51 g L^{-1}), NaCl (0.4 g L^{-1}), $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (1 mg L^{-1}), $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$: 2 mg L^{-1} , and biotin 0.04 mg L^{-1} . Additionally, 1 mL L^{-1} of the trace elements solution containing $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ (100 mg L^{-1}), MnSO_4 (50 mg L^{-1}), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (250 mg L^{-1}), and $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ (50 mg L^{-1}) was added. For *P. rubens* ATCC 9178 (originally deposited in ATCC as *P. notatum*), this medium was modified with regard to the carbon sources and contained lactose (7.5 g L^{-1}) and glucose (7.5 g L^{-1}). *Mucor racemosus* ATCC 7924 was cultivated in preprepared Sabouraud medium (BTL Ltd, Poland) containing peptone (5 g L^{-1}), casein peptone (5 g L^{-1}), and glucose (20 g L^{-1}). Autoclaving time depended on the volume of culture: 30 min for shake flasks and 90 min for the bioreactor.

For the MPEC experiments, 6 g L^{-1} of sterile (separately autoclaved at 121°C for 30 min as dry powder) aluminum oxide (Al_2O_3) microparticles of a mean diameter equal to $10\text{ }\mu\text{m}$ were added to the cultivation media.

2.4 | Image processing and analysis

The samples from each shake flask and bioreactor experiments (standard and MPEC runs) were subjected to the

TABLE 1 Origin of the experimental data used for kinetic modeling

Type of cultivation ^a	Filamentous fungus				Source of data for modeling
	<i>A. terreus</i>	<i>Ch. globosum</i>	<i>P. rubens</i>	<i>M. racemosus</i>	
ShF standard cultivation ShF MPEC	Data used to determine of model parameters and sensitivity analysis (experimental runs performed in two or three replicates)				Partly from Kowalska et al. [23]
STB standard cultivation STB MPEC	Data used for the verification of the model (with the use of the unchanged parameters) and for the determination of the parameters in the extended version of the model.				This work

^aShF, shake flask; STB, stirred tank bioreactor.

immediate microscopic observations. The vital (unfixed and unstained) slides were prepared. The light microscope (phase contrast) OLYMPUS BX53 (Olympus Corporation, Tokyo, Japan) was used for the observations. The microscope was equipped with the high resolution RGB digital camera OLYMPUS DP27 and controlled by the computer with the image analysis software OLYMPUS cellSens Dimension Desktop 1.16 (Olympus Corporation). The details of slide observation conditions and image processing and analysis were described elsewhere [23].

2.5 | Modeling tools

The algorithms embedded in AQUASIM 2.0 software (EAWAG, Dübendorf, Switzerland) were applied to solve the differential equations, identify the parameters of the model and perform the identifiability (sensitivity) analysis. Equations were integrated numerically in time with the use of the algorithm called differential algebraic system solver. It is based on the implicit (backward differencing) variable-step, variable-order Gear integration technique. The parameters were estimated by minimizing the sum of the squares of the weighted deviations between measurements and calculated model results with the use of simplex method. For the purpose of identifiability analysis, the absolute relative sensitivity function was used [24].

3 | RESULTS AND DISCUSSION

Fungal morphology is the key factor influencing the performance of the bioreactor process and its productivity regarding the formation of metabolites (primary and secondary) and enzymes. Both dispersed and pelleted fungal morphology in the submerged culture cause more or less adverse effects on the run of the bioreactor processes regarding stirring, mixing, and aeration of the broth. All these ultimately determine the yield of the bioreactor process. The development of a particular morphological form is to a large extent dependent on the species of interest. At least three (spore agglomerative, hyphae agglomerative, and nonagglomerative) species-dependent mechanisms of fungal morphological evolution are known. The formation of the ultimate, stable fungal morphology in the submerged cultivation usually lasts not longer than about 24 h from the introduction of spores to the sterile medium [23].

The analysis of the contemporary knowledge available in literature reveals two important issues. First, there is a need to develop the quantitative description and the possibility to predict the behavior of various fungal species with regard to their morphology in the submerged culture. Second, it is desirable to control the fungal morphology and, out of several available morphological engineering techniques, the

microparticle-enhanced cultivation is viewed as the most promising approach. The kinetic models presented below are the adequate responses to solve the two aforementioned problems.

3.1 | Formulation of the kinetic model

The microscopic observations of the growing fungal species *A. terreus*, *Ch. globosum*, *P. rubens*, and *M. racemosus* allowed for distinguishing the three phases of their growth, from the introduction of spores into the liquid medium to the formation of regular fungal elements (either clumps or pellets). These were spore swelling, spore or hyphae agglomeration, and mycelial growth [23]. Out of the studied fungi, *A. terreus* belongs to the spore agglomerative (coagulative) species that may form macroscopic pellets, *P. rubens* and *Ch. globosum* display hyphal agglomeration (aggregation) mechanism of pellets formation, while *M. racemosus* is a nonagglomerative (noncoagulative) species. These specific behaviors of the tested fungal species are schematically visualized in Figure 1.

Actually, only in the case of *A. terreus* these three phases could have been clearly distinguished. No sooner had the germ tubes emerged to start the phase of mycelial growth in this species, than the spores formed the distinctive large agglomerates that could have contained more than 10 000 spores [23,25]. In the case of *Ch. globosum* and *P. rubens*, the mycelial growth was observed to be initiated both from spore agglomerates of medium size (often smaller than the ones of *A. terreus* considering the number of spores) and from the individual spores. Larger agglomerates were later formed from the germinated spores and larger hyphal elements. Ultimately, the pellets formed by *A. terreus* and *Ch. globosum*, despite various mechanisms of pellet formation, occurred to be of similar size and shape. In the case of *M. racemosus*, no spore agglomerates were observed and, at a later stage, the clumps were formed from the branched hyphae that emerged from individually germinated spores [23]. All the aforementioned distinctive phases of fungal growth were observed irrespective of the type of the culture, both in shake flask and bioreactor runs.

Upon these observations, the following assumptions of the kinetic model were proposed. Three aforementioned stages of fungal evolution were assumed and modeled: (1) spore swelling, (2) spore or hyphae agglomeration, and (3) hyphal growth. Fungal growth, from spore swelling to large mycelial objects, like clumps or pellets, was quantified by the state variable mean projected area A (μm^2) of the mycelial objects. Changes of A (a size parameter derived from digital analysis of the microscopic images) in time were the subject of modeling. Neither oxygen saturation at these early stages of fungal growth in the shake flask cultures, nor limitations coming from media components (carbon and

FIGURE 1 Schematic graph illustrating the differences in the morphological evolution of *A. terreus*, *Ch. globosum*, *P. rubens*, and *M. racemosus*; phases of morphological evolution were depicted

nitrogen source) were assumed. The latter were confirmed by the simple analysis of media composition. Practically, no changes in either carbon source (lactose, glucose) or nitrogen concentrations were observed (data not shown). With regard to oxygen levels, this assumption was somewhat forced by the lack of measurements of dissolved oxygen in the shake flask cultivations. The increase of the size of the mycelial objects was observed to be limited, which is an immanent feature of fungal growth in the submerged culture discovered upon the analysis of the microscopic images. It was, e.g. impossible to find any spore agglomerates larger than a certain size, in which no germ tubes were still observed. Also, the size of evolved pellets could not be higher than a certain value dependent on culture conditions and studied species.

Due to the presence of three stages of fungal evolution, the following rates to describe them were proposed. Spore swelling rate was directly proportional to the mean projected area of the mycelial object, A , and denounced as:

$$r_{sw} = k_{sw} \cdot A \quad (1)$$

Additionally, swelling time was estimated upon the images of the spores and germinating spores and denounced as t_{sw} . Swelling time was the time break-point of the model to simplify the modeling of the other phases.

The rates of spore agglomeration and mycelial growth were similar to Edwards equation for biomass growth that expresses the action of an inhibitor in the microbial system [26]. Its

original form to express volumetric biomass growth rate of biomass was:

$$r_X = \mu_{\max} \cdot \frac{c_S}{c_S + K_S} \cdot \exp\left(-\frac{c_I}{K_I}\right) \cdot c_X \quad (2)$$

where: $\mu_{\max}$ is the maximum biomass specific growth rate, c_S is the substrate concentration, K_S is the Monod constant, c_I is the inhibitor concentration, K_I is the inhibition constant, and c_X is the biomass concentration.

Our equation differed to a certain extent from the original Edwards one: first by omitting, in this specific case, the unnecessary (upon the assumptions of the model above) substrate limitation term $\frac{c_S}{c_S + K_S}$. The measure of biomass amount and the method to track its growth in these experiments was the increase of mean projected area of the mycelial objects A . Thus, A was used instead of biomass concentration c_X . The most important difference is that the inhibitor concentration c_I was also replaced by mean projected area of the mycelial objects A . Actually, there was no inhibitor in the studied system but, as mentioned above, it was the size of the mycelial objects that could not be higher than a certain value. All in all, the rates of spores agglomeration r_A ($\mu\text{m}^2 \text{h}^{-1}$) and mycelial growth r_G ($\mu\text{m}^2 \text{h}^{-1}$) were described by Equations (3) and (4).

$$r_A = k_A \cdot \exp\left(-\frac{A}{K_A}\right) \cdot A \quad (3)$$

$$r_G = k_G \cdot \exp\left(-\frac{A}{K_G}\right) \cdot A \quad (4)$$

It is important to mention here that the stage of mycelial growth is understood here as the growth of filaments from emerging germ tubes, elongation of hyphae, and their branching up to the formation of either dispersed hyphae, or clumps, or pellets. The parameters k_A and k_G are agglomeration and growth rate constants, respectively and they are analogous to maximum biomass growth rate from Equation (2). Referring again to the presence of the “inhibition” exponential term from Equation (2) $\exp(-\frac{c_I}{K_I})$ and “inhibition” constants K_A (μm^2) and K_G (μm^2) in Equations (3) and (4), it must be mentioned here that from the biological point of view this “inhibition” was not like the one caused by the presence of the toxic substance in the broth. However, the use of this exponential term was justified by the observation that the hyphal elements of all tested species reach a certain maximum size at the end of each development stage. Furthermore, this mathematical form allowed for the accurate representation of the respective experimental data. Ultimately, these constants were called the agglomeration limitation K_A and growth limitation K_G constants to better reflect their meaning in this specific model.

The most general model was proposed for the hyphae aggregating species, in which the phases of spores agglomeration and mycelial growth occur simultaneously. It is expressed by the equation:

$$\frac{dA}{dt} = \begin{cases} r_{SW} & \text{if } t < t_{SW} \\ r_A + r_G & \text{otherwise} \end{cases} \quad (5)$$

Total volumetric growth rate of the mycelial object dA/dt is then the sum of the rate of spore agglomeration r_A (where most of them are already germinated spores) and rate of mycelial growth r_G .

For the other examined species, certain modifications of this basic equation were introduced. For the fungus exhibiting the agglomerative mechanism of pellet formation (*A. terreus*), the processes of spores agglomeration and mycelial growth actually do not overlap. Germ tubes generally did not emerge until the spores formed the agglomerates of the demanded size. Thus, the proposed model was modified into the following form:

$$\frac{dA}{dt} = \begin{cases} r_{SW} & \text{if } t < t_{SW} \\ \max[r_A; r_G] & \text{otherwise} \end{cases} \quad (6)$$

For the nonagglomerative species *M. racemosus*, in these runs in which no agglomeration of spores took place and no distinctive pellets were formed spore agglomeration rate was set to 0, making the model equation simpler:

$$\frac{dA}{dt} = \begin{cases} r_{SW} & \text{if } t < t_{SW} \\ r_G & \text{otherwise} \end{cases} \quad (7)$$

Ultimately, the formulated model had the following parameters: k_{sw} , swelling rate constant (h^{-1}); k_A , agglomeration rate constant (h^{-1}); K_A , agglomeration limitation constant (μm^2); k_G , growth rate constant (h^{-1}); K_G , growth limitation constant (μm^2). They were all identified upon experimental data from shake flask experiments and verified upon the experimental data from the bioreactor runs.

3.2 | Mycelial growth in the standard and microparticle-enhanced shake flask and stirred tank bioreactor cultivations

The selected data from the experimental runs (shake flask cultures) described in the Materials and methods section served as the input data for the estimation of the model parameters. These were both the standard and microparticle-enhanced cultivations. The results of the simulations and identified parameters of the model were subjected both to the interspecies and standard cultivation versus MPEC comparisons.

FIGURE 2 Experimental data (scattered points) versus model curves (solid lines) in standard (A, C, E, and G) and MPEC (B, D, F, and H) shake flask runs with: *A. terreus* (A and B), *Ch. globosum* (C and D), *M. racemosus* (E and F), and *P. rubens* (G and H); dashed lines represent error bounds calculated upon Equation (9)

To estimate the parameters, the sum of the squares of the weighted deviations between measurements and calculated model results was minimized:

$$\chi^2(p) = \sum_{i=1}^n \left(\frac{A_{\text{measured},i} - A_i(p)}{\sigma_{\text{measured},i}} \right)^2 \quad (8)$$

where $A_{\text{measured},i}$ is the i^{th} measurement of mean projected area of hyphal element, $\sigma_{\text{measured},i}$ is its standard deviation, $A_i(p)$ is the calculated value of mean projected area of hyphal element corresponding to the i^{th} measurement, $p = (k_A; K_A; k_G; K_G)$ are the estimated model parameters, and n is the number of data points. Spore swelling rate k_{sw} and time of swelling t_{sw} were assumed to be constant upon the experimental data and were not estimated upon Equation (8). In Figure 2, the experimental data obtained from the shake flask experiments [23] were shown together with the simulation curves. Experimental data from three (Figures 2A–D, G and H) or two (Figures 2E,F) independent experiments were used to determine the parameters. The error propagation formula, being a part of uncertainty analysis of the model, was based upon the linearized model to find the approximate standard deviation σ_A of the model result:

$$\sigma_A = \sqrt{\sum_{i=1}^4 \left(\frac{\partial A}{\partial p_i} \right)^2 \cdot \sigma_{p_i}^2} \quad (9)$$

where p_i are the uncertain model parameters and σ_{p_i} are their standard deviations. The dashed lines in Figure 2 indicate error bounds limiting the range of values of the result plus and minus one standard deviation value.

The values of the fitted parameters together with their standard errors are presented in Table 2. They clearly indicated on different mechanisms of fungal evolution, excluding the value of spore swelling rate, which was an individual parameter for each of the tested species. Agglomeration and growth rate constants indicated on the kinetics of the processes of agglomeration and growth, while agglomeration and growth limitation constants referred to the ability of the given fungus to form the agglomerates (clumps and pellets of the given size).

In the case of *A. terreus* and *Ch. globosum*, whose pellets were visually the most similar to each other in the standard cultivation, the agglomeration rate constants k_A were close in their values (1.79 and 1.69 h⁻¹, respectively), while the agglomeration limitation constant K_A was almost six times higher for *Ch. globosum* (Table 2). This fact was related to the participation of hyphal agglomeration in the stage of agglomeration and consequent formation of larger objects from spores and germinated spores in *Ch. globosum*, unlike in *A. terreus*, whose agglomerates were formed of spores only. The differences in parameters describing the stage of hyphal growth (k_G and K_G) in these two species were connected

TABLE 2 Values of kinetic parameters for the studied fungi in the standard cultivation and MPEC in shake flasks

Fungus	Culti-variation	<i>A. terreus</i>		<i>P. rubens</i>		<i>Ch. globosum</i>		<i>M. racemosus</i>	
		STD	MPEC	STD	MPEC	STD	MPEC	STD	MPEC
k_{sw} (h ⁻¹)		0.5	0.5	0.3	0.3	0.35	0.35	0.5	0.5
k_A (h ⁻¹)		1.79 ± 0.06	1.34 ± 0.18	3.99 ± 0.95	1.83 ± 0.42	1.69 ± 0.28	2.097 ± 0.53	-	0.076 ± 0.005
K_A (μm ²)		31157 ± 2215	38932 ± 5659	25234 ± 4230	327 ± 53	184192 ± 7530	88609 ± 9830	-	644.7 ± 88.8
k_G (h ⁻¹)		0.63 ± 0.07	0.26 ± 0.09	0.51 ± 0.04	1.21 ± 0.05	0.152 ± 0.029	0.169 ± 0.086	2.2 ± 0.6	2.35 ± 0.58
K_G (μm ²)		1.211 · 10 ⁶ ± 5.11 · 10 ⁵	1.297 · 10 ⁶ ± 1.03 · 10 ⁵	6.998 · 10 ⁵ ± 3.02 · 10 ⁴	3.345 · 10 ⁵ ± 2.02 · 10 ⁴	1.343 · 10 ¹¹ ± 4.21 · 10 ¹⁰	1.019 · 10 ⁷ ± 5.6 · 10 ⁶	4.667 · 10 ³ ± 7.3 · 10 ²	1.068 · 10 ⁵ ± 2.01 · 10 ⁴
Type of evolution		Spore agglomerating		Hyphae agglomerating		Hyphae (and spore) agglomerating		Nonagglomerating	Hyphae agglomerating
Model equation		Equation (6)		Equation (5)		Equation (5)		Equation (7)	Equation (5)

mainly with the limiting size of the object, which was much bigger for *Ch. globosum* than for *A. terreus*. Furthermore, the models describing these two species were different (Equation 5 vs. Equation 6), as *Ch. globosum* exhibited to a certain extent the hyphae agglomeration mechanism, while *A. terreus* is purely spore agglomerative. Thus, despite the formation of the visually similar objects, the differences in the identified parameters, especially K_A , indicated on different mechanisms of the morphological evolution of these two fungal species.

Two studied fungi, i.e. *Ch. globosum* and *P. rubens*, were attributed as hyphae agglomerative and were described by the same Equation (6). Astonishingly in these two cases, the differences among the values of the parameters were even larger than the ones between *A. terreus* and *Ch. globosum*. Agglomeration rate constant for *P. rubens* was equal to 3.99 h^{-1} and was the highest k_A value out of all found, while for *Ch. globosum* it equaled only 1.69 h^{-1} . It proved that even if the same mechanisms of pellet formation were theoretically observed and attributed to the species, there might be other species-dependent factors that influence growth of the individual fungus. It was even seen with naked eye that *P. rubens* pellets were on average of different size, structure, and shape compared to the ones of *Ch. globosum*. Furthermore, *P. rubens* is believed to be the textbook example of the species of hyphal agglomeration to form pellets. *Penicillium rubens* pellets were mainly formed from the individually germinated spores being the unbranched or slightly branched hyphal elements which agglomerated. In the case of *Ch. globosum*, this mechanism was not so pure as some spore agglomerates and agglomerates formed from germinated spores were observed. Ultimately, pellet formation in *Ch. globosum* actually cannot be unequivocally attributed to the three literature mechanism of pellet formation. The agglomeration of hyphal elements and ungerminated spores of *Ch. globosum* was observed but the pellets formed were ultimately more similar, as mentioned above, to those formed by *A. terreus*. The case of *M. racemosus* was much simpler to discuss. Even when the model, including the agglomeration equation (Equation 5) had been first used for the determination of kinetic parameters, the two parameters connected with agglomeration phase k_A and K_A were identified as close to zero. Therefore, they were omitted and Equation (7) was used in this case. It clearly indicated on the nonagglomerative mechanism of the fungal development. As no agglomeration took place in this case, growth rate constant was higher than for the other studied species and equal to 2.2 h^{-1} . But although the hyphae of *M. racemosus* grew faster, they formed smaller clump-like agglomerates as growth inhibition constant K_G was the lowest out of all studied species.

Even more interesting findings could be spotted in the comparison between standard and MPEC cultivations. The decrease of k_A to 1.34 h^{-1} and increase of K_A to $38932 \text{ }\mu\text{m}^2$

in the case of MPEC with *A. terreus* was the clear evidence that microparticles interacted with the agglomerating spores, aggravating this process to ultimately lead to the smaller pellets. The growth rate constant of smaller agglomerates also occurred to be lower (0.63 vs. 0.26 h^{-1} in standard and MPEC cultivation, respectively). This confirmed all the previous findings connected with the action of microparticles toward spore agglomerating species of the genus *Aspergillus*, especially the verbal model of the action of microparticles toward *A. niger* presented by Driouch et al. [18].

Moreover, the aggravation of agglomeration process was also observed in *P. rubens* by more than two-fold decrease of the agglomerate rate constant k_A . Also, the size of agglomerates was somewhat more limiting (upon value of K_A , which was extremely low at $327 \text{ }\mu\text{m}^2$). This effect on model parameters dealing with the agglomeration process was even stronger in *P. rubens* than in *A. terreus*. On the other hand, the microparticles made the smaller agglomerates grow faster in the phase of growth compared to standard culture (compare Figure 2G,H).

These changes of growth kinetics of *A. terreus* and *P. rubens* caused by microparticles may have several, mainly positive, consequences on the run of the process with morphologically engineered pellets. First, the chance that oxygen deficiency would occur due to mass transfer resistance in the smaller and slower growing hyphae is much less likely in MPEC than in the standard culture. If a metabolic product formation were oxygen dependent, one could expect its higher titers [17–22]. A negative effect would take place when smaller hyphal objects formed more non-Newtonian suspensions, which may aggravate mixing in the bioreactor [18].

Astonishingly, the microparticles did not aggravate the agglomeration and growth of *Ch. globosum* at all. In fact, they somewhat facilitated these processes and the size of pellets were ultimately unchanged. But the formation of core-shell pellets by *Ch. globosum* indicated on the change of structure [23], which cannot be modeled with the proposed equations. Despite the lack of change of size of *Ch. globosum* pellets, the positive effects of MPEC were possible as the pellets with mineral core are usually better penetrated by oxygen [19] and, thus, the positive effect of MPEC could be realized.

The proposed model clearly indicated and described the drastic change of morphology of *M. racemosus* due to the presence of microparticles. Within the calculations performed for the identifications of model parameters (Equation 5), it occurred that the agglomeration stage parameters were not equal to zero as happened in the standard culture and they were here identified as $k_A = 0.076 \text{ h}^{-1}$ and $K_A = 644.7 \text{ }\mu\text{m}^2$. The model predicted, in this way, the change of the mechanism of agglomerate formation (from its lack to its occurrence) in this fungus that was confirmed by the microscopic observations. Pellet-like objects and agglomeration

of hyphae were clearly seen in the microscopic images. Similar phenomenon was previously observed for another nonagglomerative species *Rhizopus oryzae* treated with microparticles [27].

Additionally, the sensitivity analysis of the model parameters was performed. Absolute relative (AbsR) sensitivity was calculated from:

$$\delta_{A,p}^{AbsR} = p \frac{\partial A}{\partial p} \quad (10)$$

and the error contribution for the individual parameter from

$$\delta_{A,p}^{err} = \frac{\partial A}{\partial p} \sigma_p \quad (11)$$

where ∂A is the change of the state variable (mean projected area of the hyphal element) and p and ∂p are the value and change of the value of each of the parameters identified, respectively. The value of σ_p is standard deviation for the given parameter. The results of the sensitivity analysis are presented in Figure 3 in the form of the changes of relative sensitivity in time and the averaged values of error contributions are listed down in the appropriate graphs beside the parameters which they concern.

The feature of the values of absolute relative sensitivity function is that they do not depend on the unit of the parameter, which makes the quantitative comparisons of the effect of a given parameter on the variable A possible. The higher are the values of sensitivity functions and the more pronounced the differences are in the shape of the sensitivity functions, the more accurately are the parameters identifiable. It was satisfied for all the curves connected with the parameters describing the growth of the studied fungi, namely growth rate constant k_G and growth limitation constant K_G (Figure 3). In the case of the agglomeration parameters, namely agglomeration rate constant k_A and agglomeration limitation constant K_A , the shapes of the curves were more similar, excluding the ones for the spore agglomerative *A. terreus*. In this case, the identifiability of the parameters was worse.

Sensitivity analysis to a certain extent confirmed the attributed mechanisms of fungal growth. It is clearly seen in Figure 3 that between 7- and 10-h the sensitivity of agglomeration-bound parameters was higher than those describing the hyphal growth. This effect was even stronger in the case of *A. terreus* MPEC (Figure 3B) than in the respective standard cultivation of this fungus (Figure 3A). In the case of *Ch. globosum* and *P. rubens* (hyphal agglomerative species), both agglomeration and growth parameters were simultaneously sensitive within the whole time of the cultivation. (Figure 3C,D,G,H). Nevertheless, microparticles aggravated the agglomeration of *P. rubens* hyphae as the sensitivity of growth parameter much more dominated in the MPEC than in the standard cultivation. Due to the lack or weak

agglomeration of spore or hyphae in *M. racemosus*, the growth parameters were much more sensitive than the agglomeration ones, even in the MPEC runs. The axis break in the graph had to be used to see the sensitivities of k_G and K_G in the MPEC run (Figure 3E,F) as the values of these parameters, their absolute relative sensitivities, and averaged error contributions were low. What is also important, it was found for *Ch. globosum* that in the standard runs the sensitivity of growth limitation constant was much lower than for all the other species and so was averaged error contribution for this parameter (Figure 3C). Other averaged error contributions were on the similar levels, except for agglomeration limitation constant for both standard and MPEC runs of *P. rubens*, which can be attributed to the mechanism of hyphal agglomeration mechanism of growth.

To check the universal applicability of the model, the determined parameters were also used to describe the run of the process in the bioreactor cultivations (Figure 4). Few parameters for the individual species had to be changed for these simulations (Figure 4A,B). For unknown reasons, the spore swelling rate in the bioreactor runs in *A. terreus* occurred to be slower ($k_{sw} = 0.25 \text{ h}^{-1}$) than in the respective shake flask runs. Generally, the accuracy of the fit confirmed the good quality of the model (Figure 4A–F), although the worst correlation between simulated curve and experimental data occurred to be recorded for *P. rubens*. That is why some modifications of model parameters were proposed and the model was recalculated. The spore agglomeration rate constant and the growth rate constant were identified anew at $k_A = 0.900 \text{ h}^{-1}$ and $k_G = 0.398 \text{ h}^{-1}$ (Figure 4G,H).

Nevertheless, this model can be treated as insufficient for the processes run in the bioreactor. Better control of the bioreactor processes (relatively to shake flask runs) and the tracked dissolved oxygen curves revealed that to the certain extent oxygen might have been a limiting factor even in the early stages of mycelial evolution. Therefore, the model was modified, which was shown in the next section.

3.3 | Extension of the model to regard oxygen utilization

As mentioned above, due to the experimental limitations in the shake flask culture it was impossible to take the effect of oxygen saturation into account. As already stated, within 24 h neither carbon nor nitrogen source were utilized by the studied fungi (data not shown) in the shake flask runs, nor were they utilized in the bioreactor cultivation. Also, the bioreactor runs clearly indicated that for spore swelling and spores agglomeration, oxygen was hardly required. But when germ tubes started to grow, the oxygen saturation curve sharply decreased in some cases, indicating on various oxygen uptake rates (OURs) (Figure 5), which were in turn dependent on the strain and type of cultivation (standard vs. MPEC).

FIGURE 3 Absolute-relative sensitivity functions of a calculated mean projected area of hyphal elements of the studied fungi: *A. terreus* (A and B), *Ch. globosum* (C and D), *M. racemosus* (E and F), and *P. rubens* (G and H) with respect to four key model parameters: k_A , K_A (black lines), k_G , K_G (red lines), and their averaged error contributions shown in the legends of the graphs

FIGURE 4 Experimental data (scattered points) versus model curves (solid lines) for standard (A, C, E, and G) and MPEC (B, D, F, and H) bioreactor cultures; simulations were performed with the model parameters determined from shake flask culture collected in Table 2, dashed lines represent error bounds as in Figure 2; for *A. terreus* (A and B) the spore swelling constant was decreased to 0.25 h^{-1} to better fit experimental data; for *P. rubens* (G and H) the fit was unsatisfactory (short-dashed line) and parameters were identified anew ($k_A = 0.9 \text{ h}^{-1}$ and $k_G = 0.398 \text{ h}^{-1}$)

FIGURE 5 Time changes of oxygen uptake rate (OUR) for the studied species: *A. terreus* (A), *Ch. globosum* (B), *M. racemosus* (C), and *P. rubens* (D) in standard and MPEC bioreactor cultivations

Analyzing Figure 5, it can be said that in each case the high peaks of OUR values were in the cultivation and their occurrence was usually attributed to the exponential growth phase of microorganisms. Here, due to the complicated morphological development, this exponential growth phase was not as simply seen as it could be for any unicellular microorganisms. That is why the phases of fungal growth were named otherwise in this work. However, comparing the graphs in Figure 5 with those in Figure 4 a certain correlation can be observed. Only in *M. racemosus* standard cultivation, the almost linear increase of mean projected area in the semilogarithmic coordinates system and the parallel exponential increase of OUR were observed (Figure 5C). However, it should be remembered that it was the strain that generally do not form the distinctive agglomerates. For the other species, this type of correlation was not straightforward.

With regard to the effect of microparticles on the changes of OUR, it was clearly seen that only in the case of spore agglomerating *A. terreus* the addition of microparticles stimulated the metabolism of the fungus, as the OUR drastically increased until achieving its maximum at 10 h of the run

(Figure 5A). For the standard cultivation, the maximum value of OUR occurred about 5 h later and was three times lower than that for MPEC (Figure 5A). For the other species, no clear effect of microparticles on the increase of OUR in time was seen (Figure 5B,D). Even in the standard cultivation, the OUR values were higher than those in MPEC. For *M. racemosus*, after the main peak of OUR some additional peaks were observed in MPEC (Figure 5C). All in all, the studied species differed to the high extent with regard to the values of OUR. *Aspergillus terreus* showed the highest respiration activity (Figure 5A) and *Ch. globosum* the lowest (Figure 5B), although the size of the fungal agglomerates of these two species were comparable (Figure 2A–D).

As the oxygen concentration data were available from the bioreactor runs, the extension of the model was proposed. It was connected with the change of the expression for growth rate r_G , in which oxygen limitation term, replacing size limitation term, was introduced:

$$r_G = k_G \cdot \left(\frac{c_{oxy}}{c_{oxy} + K_{OXY}} \right) \cdot A \quad (12)$$

Additionally, the oxygen concentration balance was added:

$$r_{OXY} = k_{La} \cdot (c_{oxy}^* - c_{oxy}) - \frac{1}{Y_{A/OXY}} \cdot r_G \quad (13)$$

where k_{La} (h^{-1}) is the convective mass transfer coefficient estimated at the level of 180 h^{-1} upon previous works [21], c_{oxy}^* is the maximum oxygen solubility in the bioreactor equal to $0.0072 \text{ g O}_2 \text{ L}^{-1}$ (temperature of the cultivation and the presence of dissolved medium components taken into account for the estimation of this value), $Y_{A/OXY}$ ($\mu\text{m}^2 \text{ g O}_2^{-1}$) is the yield coefficient expressing the increase of the size of the hyphal element (expressed in μm^2) per 1 g O_2 assimilated, and K_{OXY} is the oxygen saturation constant ($\text{g O}_2 \text{ L}^{-1}$).

Also, the effect of mechanical forces to destroy hyphal element was taken into account. The hyphae destruction rate r_D was expressed as:

$$r_D = k_D \cdot A \quad (14)$$

where k_D is the hyphae destruction rate constant (h^{-1}).

All in all, the full systems of differential equations for each previously presented version of the model were as follows.

First, the most general model for the fungi of hyphal agglomeration mechanism was in the form:

$$\begin{aligned} \frac{dA}{dt} &= \begin{cases} r_{SW} & \text{if } t < t_{SW} \\ r_A + r_D - r_D & \text{otherwise} \end{cases} \\ \frac{dc_{oxy}}{dt} &= \begin{cases} 0 & \text{if } t < t_{SW} \\ r_{OXY} & \text{otherwise} \end{cases} \end{aligned} \quad (15)$$

and used for *P. rubens* and *Ch. globosum*.

For the fungus of the agglomerative (coagulative) mechanism of pellet formation, it had the following form:

$$\begin{aligned} \frac{dA}{dt} &= \begin{cases} r_{SW} & \text{if } t < t_{SW} \\ \max[r_A - r_D; r_G - r_D] & \text{otherwise} \end{cases} \\ \frac{dc_{oxy}}{dt} &= \begin{cases} 0 & \text{if } t < t_{SW} \\ r_{OXY} & \text{otherwise} \end{cases} \end{aligned} \quad (16)$$

and was used for *A. terreus*.

For the nonagglomerative (noncoagulative) species, the model had the following form:

$$\begin{aligned} \frac{dA}{dt} &= \begin{cases} r_{SW} & \text{if } t < t_{SW} \\ r_G - r_D & \text{otherwise} \end{cases} \\ \frac{dc_{oxy}}{dt} &= \begin{cases} 0 & \text{if } t < t_{SW} \\ r_{OXY} & \text{otherwise} \end{cases} \end{aligned} \quad (17)$$

and, as in the previous subsection, was applied to the standard culture of *M. racemosus*.

In Table 3, the kinetic parameters of the extended model are collected. It is clearly seen than the previously found parameters connected with growth of the studied species were maintained on the same level, as the ones used for the

TABLE 3 Values of kinetic parameters for the studied fungi in the standard cultivation and MPEC in batch bioreactor cultivations

Fungus Cultivation	<i>A. terreus</i>		<i>P. rubens</i>		<i>Ch. globosum</i>		<i>M. racemosus</i>	
	STD	MPEC	STD	MPEC	STD	MPEC	STD	MPEC
k_{sw} (h^{-1})	0.25 ^a	0.25	0	0	0	0	0.5	0.5
k_D (h^{-1})	0	0	0	0	0	0	0	0.3
k_A (h^{-1})	1.79 ± 0.06	1.34 ± 0.18	0.9 ± 0.04	1.83 ± 0.42	1.69 ± 0.28	2.097 ± 0.53	-	0.076 ± 0.005
K_A (μm^2)	31157 ± 2215	38932 ± 5659	25234 ± 4230	327 ± 53	184192 ± 37530	88609 ± 9830	-	644.7 ± 88.8
k_G (h^{-1})	0.63 ± 0.07	0.26 ± 0.09	0.51 ± 0.04	0.4 ± 0.03	0.152 ± 0.029	0.169 ± 0.086	2.2 ± 0.6	2.35 ± 0.58
K_G (μm^2)	$1.211 \cdot 10^6 \pm 5.11 \cdot 10^5$	$1.297 \cdot 10^6 \pm 1.03 \cdot 10^5$	$6.998 \cdot 10^5 \pm 3.02 \cdot 10^4$	$3.345 \cdot 10^5 \pm 2.02 \cdot 10^4$	$1.343 \cdot 10^{11} \pm 4.21 \cdot 10^{10}$	$1.019 \cdot 10^7 \pm 5.6 \cdot 10^6$	$4.667 \cdot 10^3 \pm 7.3 \cdot 10^2$	$1.068 \cdot 10^5 \pm 2.01 \cdot 10^4$
K_{OXY} ($\text{g O}_2 \text{ L}^{-1}$)	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004
$Y_{A/OXY}$ ($\mu\text{m}^2 \text{ g O}_2^{-1}$)	$1.500 \cdot 10^5 \pm 4.10 \cdot 10^4$	$4.000 \cdot 10^2 \pm 1.50 \cdot 10^1$	$5.200 \cdot 10^6 \pm 2.10 \cdot 10^5$	$7.000 \cdot 10^4 \pm 4.00 \cdot 10^3$	$1.500 \cdot 10^6 \pm 3.50 \cdot 10^5$	$1.200 \cdot 10^6 \pm 4.50 \cdot 10^2$	$3.000 \cdot 10^3 \pm 2.00 \cdot 10^2$	$3.000 \cdot 10^4 \pm 4.60 \cdot 10^3$
Type of evolution	Spore agglomerating	Spore agglomerating	Hyphae agglomerating	Hyphae agglomerating	Hyphae (and spore) agglomerating	Hyphae agglomerating	Nonagglomerating	Hyphae agglomerating
Model equation	Equation (16)	Equation (16)	Equation (15)	Equation (15)	Equation (15)	Equation (15)	Equation (17)	Equation (16)

^aChanged model parameters (in relation to Table 2) are underlined.

simulation of bioreactor processes (compare Table 2 and the comments in the previous subsection on *A. terreus* and *P. rubens*). Here, the parameters connected with oxygen utilization were added. Oxygen limitation (saturation) constant K_{OXY} was set at the same level for each fungus and run and equal to $0.004 \text{ g O}_2 \text{ L}^{-1}$. Yield coefficients $Y_{A/OXY}$ occurred to be different for various species. It is not unusual as, generally, substrate over biomass yield coefficients depend on the microorganism and they are thermodynamically limited by the number of electrons available in the given biomass and substrate. In the studied case, these differences seem to be larger than one may expect; however, $Y_{A/OXY}$ refers to the mean projected area, not biomass concentration. Mean projected area is a morphological parameter connected with the size of hyphal object and the development of hyphal object, namely their size, is strictly connected with the mechanisms of morphological evolution, i.e. coagulative (agglomerative), noncoagulative (nonagglomerative), or hyphae agglomeration. Here, one can see that for *P. rubens* and *Ch. globosum* the values of $Y_{A/OXY}$ are of the same magnitude ($5.2 \cdot 10^6$ and $1.5 \cdot 10^6 \text{ } \mu\text{m}^2 \text{ g O}_2^{-1}$, respectively) and only for the spore-agglomerative *A. terreus* and nonagglomerative *M. racemosus* they are lower ($1.5 \cdot 10^5$ and $3.0 \cdot 10^3 \text{ } \mu\text{m}^2 \text{ g O}_2^{-1}$, respectively) than those for the other species.

Furthermore, the effect of the addition of the microparticles was indicated by the yield coefficient. It occurred that for all species growing in MPEC excluding *M. racemosus*, $Y_{A/OXY}$ values were lower or equal to the ones in the standard cultivations. These decreases were even by two or three magnitudes (*A. terreus* and *P. rubens*). No change was observed for *Ch. globosum*, while only for *M. racemosus* yield coefficients increased by one magnitude. It was definitely connected with the fact that its mechanism of growth is nonagglomerative and the addition of microparticles caused hyphal agglomeration in MPEC of *M. racemosus*.

The simulation curves of the extended model were presented in Figure 6. The fit of model curves to the experimental data occurred to be satisfactory in the studied time domain up to about 24-h of the run (Figure 6A,B,E–H). Only in the case of *Ch. globosum*, the simulation was made for twice longer time due to slower oxygen utilization. But even in that case, the fit occurred to be satisfactory (Figure 6C,D). The effect of destruction of hyphae was only observed in the case of *M. racemosus* in MPEC. Only in that particular case, the destruction rate constant was identified as higher than zero.

The results of simulations with the use of these two models remained in agreement with the findings on the development of hyphae for *A. terreus*, *Ch. globosum*, *M. racemosus*, and *P. rubens* regarding the phases of evolution and mechanisms ruling them, which were all previously described by Kowalska et al. [23]. Introduction of oxygen balance made the proposed model more reliable, although it is a rare practice to include oxygen balances in the kinetic modeling of fungal

growth [28]. The results of the simulations were also in agreement with the previous findings about the influence of oxygen on the run of the early stages of fungal growth. It is clear that the demand of oxygen was not as high as one may have expected and much depended on the studied species. Here, only *A. terreus* indicated the highest values of OUR, especially when grown in MPEC. It confirmed that the best effect of MPEC was observed toward the spore agglomerating fungi and had much to deal with the availability and utilization of oxygen [18,19,21].

4 | CONCLUDING REMARKS

The presented kinetic model described the early stages of the evolution of four fungal species in the standard and microparticle-enhanced submerged cultures solely in terms of hyphal morphology and oxygen utilization (extended model). Thus, it did not take any carbon or nitrogen substrate uptake or metabolic product formation into account.

To formulate the kinetic model of the growth of fungal species that exhibit different mechanisms of morphological evolution, some variations in the forms of the kinetic equations have to be used to express these mechanisms. Nevertheless, the core of the model, namely the form of equations, remains unchanged, which is its undeniable advantage. To the best of our knowledge, any similar models have not been published so far.

The proposed model and its parameters determined for the replicated data from shake flask cultures are valid also for the scaled-up cultivations in the stirred tank bioreactor. Furthermore, when extending this model to account for the oxygen balance, there is no need to change the values of the majority of its important parameters. It all proves its universal applicability.

The form of the model equations only depends of the mechanism of morphological evolution for the given fungus. That is why the model is universal and its equations can be used for any fungi that display the given mechanisms of hyphal evolution in the early stages of their growth. Only the parameters might require certain adjustments. It can be expected that this model will be applied and verified for other fungal species. It is also worth testing, whether it would be applicable for actinobacteria, e.g. *Streptomyces* sp., due to the morphological similarity of these groups of microorganisms.

Upon the parameters of the model, the effect of the cultivation of the fungus in MPEC is clearly seen and the effect of microparticles addition to the given fungal culture can be predicted in the dependence on the fungal species studied.

All in all, it is crucial to quantify the early stages of growth of filamentous fungi as it is helpful to understand the action of

FIGURE 6 Experimental data (scattered points) versus model curves (solid lines) in standard (A, C, E, and G) and MPEC (B, D, F, and H) bioreactor experiments with: *A. terreus* (A and B), *Ch. globosum* (C and D), *M. racemosus* (E and F), and *P. rubens* (G and H); simulations were performed with the model parameters from Table 3

microparticles in MPEC, which are a modern and promising technique of controlling fungal morphology.

Nomenclature

∂A	[μm^2]	Change of mean projected area value (Equations 9–11)
∂p	[of parameter]	Change of parameter value (Equations 9–11)
A	[μm^2]	Mean projected area of hyphal objects
$A_{\text{measured},i}$	[μm^2]	i^{th} Measurement of mean projected area of hyphal element (Equation 8)
c_I	[g L^{-1}]	Inhibitor concentration
c_{oxy}	[$\text{g O}_2 \text{ L}^{-1}$]	Oxygen concentration
c_{oxy}^*	[$\text{g O}_2 \text{ L}^{-1}$]	Maximum oxygen solubility
c_S	[g L^{-1}]	Substrate concentration
c_X	[g L^{-1}]	Biomass concentration
k_A	[h^{-1}]	Agglomeration rate constant
K_A	[μm^2]	Agglomeration limitation constant
k_D	[h^{-1}]	Hyphae destruction constant
k_G	[h^{-1}]	Growth rate constant
K_G	[μm^2]	Growth limitation constant
k_{La}	[h^{-1}]	Convective mass transfer coefficient for oxygen
K_{OXY}	[$\text{g O}_2 \text{ L}^{-1}$]	Oxygen saturation constant
K_S	[g L^{-1}]	Monod constant
k_{sw}	[h^{-1}]	Swelling constant
n	[-]	Number of data points (Equation 8)
$p, p_i, \mathbf{p}$	[of parameter]	Parameter or i -th parameter value (Equations 8–11)
r_A	[$\mu\text{m}^2 \text{ h}^{-1}$]	Rate of spore agglomeration
r_D	[$\mu\text{m}^2 \text{ h}^{-1}$]	Hyphae destruction rate
r_G	[$\mu\text{m}^2 \text{ h}^{-1}$]	Rate of mycelial growth
r_{OXY}	[$\text{g O}_2 \text{ l}^{-1} \text{ h}^{-1}$]	Oxygen utilization rate
r_{sw}	[$\mu\text{m}^2 \text{ h}^{-1}$]	Swelling rate
r_X	[$\text{g l}^{-1} \text{ h}^{-1}$]	Volumetric biomass growth rate
t	[h]	Time
t_{sw}	[h]	Swelling time
$Y_{A/OXY}$	[$\mu\text{m}^2 \text{ g O}_2^{-1}$]	Yield coefficient (increase of mean projected area per oxygen utilized)
Greek symbols		
$\delta_{A,p}^{\text{err}}$	[μm^2]	Averaged error contribution (Equation 11)
$\delta_{A,p}^{\text{AbsR}}$	[-]	Absolute relative sensitivity (Equation 10)
$\chi^2(\mathbf{p})$	[-]	Sum of the squares of the weighted deviations between measurements and calculated model results (Equation 8)
μ_{max}	[h^{-1}]	Maximum biomass growth rate
σ_A	[μm^2]	Standard deviation (Equation 9)
$\sigma_{\text{measured},i}$	[μm^2]	Standard deviation of i -th mean projected area measurement (Equation 8)
σ_{p_i}, σ_p	[of parameter]	Standard deviations of the parameters (Equations 9, 11)

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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