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Modelling of morphogenesis to support the design of fungal-based engineered living materials

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Abstract

To realize the potential of materials comprising living organisms, bioengineers require a holistic understanding of the reciprocal relationship between environmental conditions and the biochemical and biophysical processes that influence development and behaviour. Mathematical modelling has a critical part to play in managing the complexity of biological dynamical systems and attaining higher degrees of control over their trajectories and endpoints. To support the development of mycelium-based engineered living materials, this paper reviews the literature of growth models for filamentous fungi with emphasis on the connection between morphogenesis and metabolism.

Analysis

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Introduction

Engineered living materials (ELMs) are a new class of materials distinguished by integrating, or being fully composed, of living cells (Nguyen et al. 2018). ELMs promise innovative functionalities—such as regeneration for self-maintenance (Elsacker et al. 2023)—and offer advances in the capabilities of interaction, sensing and actuation, in a variety of fields of application, such as medicine, consumer electronics and product design. Growing research interest for ELMs is also motivated by their potential role as sustainable alternatives to otherwise environmentally detrimental materials, specifically synthetic polymers.

ELMs open for an expansion of design capacities and theories by reconsideration of the methods for describing, conceptualising and producing materials. Dynamic by nature, members of this novel material class cannot be defined as a static collection of properties, but exist in a constantly evolving state of flux and exchange with their environment. No material persists as a closed system, but the consequences are felt acutely when dealing with the *living*. Furthermore, the qualification, *engineered*, emphasizes the resolution to exert control over the organisms in question. In this review, we are particularly concerned with mycelium-based ELMs, although many of the arguments apply analogously to nearby fields of biotechnological engineering.

Low barriers to entry in terms of both materials and methods have contributed to an increasing popularity of mycelium production in recent years with considerable participation of grass-root communities and amateur mycologists (Steinhardt 2018). Mycelium materials are versatile and design targets vary greatly with applications: tensile strength, acoustic absorbance, fire resistance and thermal conductivity, for example, may be of the essence in an architectural material, whereas color, texture, hydrophobicity and elasticity are paramount performance parameters for mycelium textiles. These qualities rely greatly on the microstructural morphology of the mycelium, so to modulate them we must control morphogenesis. In materials where the mycelium remains alive in the use phase, performance may also comprise bioremediation or regenerative potential and the production of chemical compounds. Here, predicting metabolic behaviour becomes critical. By controlling the processes by which filamentous fungi are cultivated, bioengineers can influence both the morphological and metabolic components of the material production.

Filamentous fungi can be cultivated in solid-state fermentation (SSF), in which solid substrate—typically an agar medium, or, in the case of wood rot fungi, a lignocellulosic aggregate—is inoculated with the fungal strain. In the latter case, common practice is to cast the inoculated aggregate in a mould, resulting in a mycelium composite material of arbitrary shape and size, within certain limits.

In liquid-state fermentation (LSF), filamentous fungi can be cultivated either statically, resulting in the production of a mycelial mat on the surface of the liquid phase medium, or they can be cultivated in a submerged state, in which case agitation may cause fungal colonies to form pellets. In both SSF and LSF methods of production, the geometric specification of the setup can significantly influence fermentation processes through thermodynamic and fluid dynamic effects (Rigobello 2023 p.134). Cultivation protocols usually involve incubators or bioreactors for controlling environmental conditions.

Current production methods rely on the indeterminate nature of fungal growth to achieve a degree of homogeneity from a more or less well-mixed initial state and more or less controlled cultivation conditions. Many stochastic factors, along with the non-linear dynamics of living systems, means that achieving consistent material properties is a considerable process control challenge. Even more so as the processes governing mycelium formation are generally not well described. Tinkering and experimentation eventually lead to cultivation protocols suitable for specific materials, methods and applications, but do not generalize well and are therefore unable to produce more fine-grained results.

Computational methods promise an invaluable tool for handling the complexity implied in biological design and engineering without resorting to reductionism. As an initial step, mathematical modelling can help to elucidate the effect of cultivation parameters on material outcome, resulting in higher efficacy and less variability. In any scenario with prompt and somewhat reliable modelling results, biodesigners can replace experimental trial and error with rapid design iterations *in silico*. With higher resolution simulations, the potential of heterogeneity can be harnessed instead of eliminated by identifying the local interventions necessary to steer the cultivation process towards a spatial grading of material properties and a distribution of fungal tissue types. The ability to induce synthetic morphologies and engineer tissues through the processes of biological development have been explored in animal systems with predominantly bottom-up approaches built around genetic interventions (Davies and Cachat 2016; Davies and Levin 2023). In the context of fungi, and the top-down manipulation of the system by modulating biological responses through environmental stimuli, the notion is less explored. Taking inspiration from developmental biology, we can imagine that high-fidelity models could one day allow bioengineers to induce organized fungal morphologies across orders of scale, akin to those of the animal or plant kingdoms.

The Fungateria EU EIC Pathfinder project is one of the current vessels for the development of ELMs. Its principal goal is to cultivate a mycelium-based material to serve as a scaffold for the subsequent growth of functionalized bacteria. The resulting material can thereby incorporate novel properties, such as activating specific bacterial metabolic pathways in response to environmental cues. As the mycelium may be living while the bacteria is introduced, the research looks to ways to develop a symbiotic relationship between the consortium species. The capacity to describe the development of the biological agents, and their interactions, is critical in this project. For this reason, the design, implementation and validation of a simulation model is a central activity that aims at providing vectors of control that link design targets to interventions in the cultivation process.

The modelling activities comprise two principal axes for the description of this ELM. The first axis considers the ELM as the result of the metabolic activities of the microorganisms, and needs to describe the reciprocal relationship between organisms and environment. The second axis considers the ELM as a material, for which mechanical properties are a fundamental model output. As an initial step in developing an integrated model that bridges material properties and microbial metabolic activities, this article presents a review of the state-of-the-art of the models for growth of filamentous fungi that have been described in the literature, leaving the bacterial agent outside the scope for the moment.

The proposed applications of existing fungal growth models are many and varied, and consider the organism on scales ranging from the ecological study of populations to the molecular mechanics of cellular processes. At the population scale, the incentive to study fungal growth include prevention of crop damages (Almeida et al. 2019; Rossman 2009; Paterson 2007), implementation of crop control (Shah and Pell 2003), prevention of building decay (Andersen et al. 2011; Schmidt 2007) and the study of how fungi contribute to various ecosystem services (Chen et al. 2018; Willis et al. 2013; van der Heijden et al. 2015). At the scale of fungal individuals or colonies, interest lies in understanding fungal pathogens as well as harnessing and optimising the capabilities of the fungal metabolism to synthesise and degrade chemical compounds, such as for industrial penicillin production (Martin and Demain 1980), bioremediation of polluted soil (Deshmukh et al. 2016), or production of materials (Rigobello and Ayres 2023). At cellular scale, filamentous fungi are also receiving increasing interest within material research (Haneef et al. 2017). Interestingly, from the perspective of developmental biology, fungal morphogenesis has been proposed as an avenue to a more general understanding of networked structures (Davidson et al. 1996) and tissue formation (Edelstein 1982). For synthetic biology and ELMs, an interesting aspect of working with filamentous fungi is that, compared to other organisms of indeterminate form, they can achieve considerable macroscopic scale and a range of differentiated structures.

This review aims to provide comprehensive knowledge about the state of the modelling activities for filamentous fungi at an intermediate scale that rhymes with mycelium production practices, and an analysis of potential contributions in the development of a new simulation model for ELMs within the framework of the Fungateria project. The review focuses on the conditions of morphological differentiation in filamentous fungi, taking particular interest in the role of the metabolism as coupled to the environment.

Methods

A semi-systematic method was followed in this review (Snyder 2019). The article search has been conducted by tracking references backwards (and to a lesser extent forwards) from known influential articles within and adjacent to the scope, and complemented by database queries using combinations and variations of the keywords: *filamentous fungi*, *model*, *modelling*, *simulation*, *mycelium* and *growth*. Due to this method, there is a potential bias towards earlier articles, and articles that do not appear in the references of others could have been missed.

Scale has profound implications for modelling (Davidson 2007) and will be a recurring theme below. For this review, only publications specifically addressing morphogenesis at the scale of an aggregate of hyphae have been included, as this corresponds to the development of mycelium materials; that is to say, models focusing on dynamics at single hypha or ecological scale are not included. Though we are interested in both SSF and LSF cultivation, models describing submerged LSF have not been included since these are concerned with chemical yields rather than morphogenesis. Another reason for exclusion has been where the same author reports incremental changes to the same model throughout a series of publications. In these cases, the articles contributing the most comprehensive model or unique features have been selected. One article might be considered outside of the scope of morphogenesis, but contributes to the description of translocation and cord-formation processes which are considered

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of great importance for morphological dynamics and features (Heaton et al. 2010).

In the analysis of the state-of-the-art, four main aspects were considered for model comparison: the description of metabolic processes, internal and external transport processes, the description of microstructural dynamics (branching, anastomosis, recycling) and the description of propagation (hyphal elongation rate, tropism). The articles considered in the state-of-the-art are presented in Tables 1 and 2. In comparison of the models under these criteria it is useful to consider that fungal biology is a rapidly evolving field, and some biological mechanisms are yet to be described. Therefore, model dynamics are more often than not descriptive rather than mechanistic, and a model exhibiting more detailed dynamics may, in a sense, appear more realistic though not actually incorporating more biological understanding.

In addition to these four categories, each model's mode of representation is presented for comparison, along with the characteristics of the spatial domain (number of dimensions, environmental heterogeneity), and the model species considered in each study. Note that, in the case of the model with zero spatial dimensions, biomass distribution (in the form of volume) is still described with regard to time (Heaton et al. 2016). The studies with one spatial dimension consider radial biomass distribution, thus implicitly considering a two-dimensional distribution through the assumption of circular symmetry.

State-of-the-art review

Representation

Fungal growth models in the literature can be categorized by whether biomass distribution is represented as discrete or continuous. In the former category, mycelial microstructure is represented explicitly as a network of hyphal filaments. Models in this category are referred to here as agent-based models (ABMs), as they are generally achieved by tracing the trajectory of agents navigating the simulation space. By contrast, coarse-grained representations use a density or probability field as a continuous measure of biomass averaged over the aggregate of hyphae, and are therefore often referred to as continuum-models. These models can be called implicit in the sense that hyphal tips and other microstructural details may be implied by the model dynamics although they are not represented individually. A continuum representation is generally expressed as a system of partial differential equations (PDE), describing the evolution of the variables under study in time and space. While it may be tempting to reduce the distinction between these modelling approaches to continuous vs. discrete models or implicit vs. explicit microstructure, there is, within many models, considerable hybridization of discrete and continuous modes of representation (see Figure 2), and microstructural topology may be implicit while microstructural dynamics are detailed explicitly and vice versa. For this reason, we prefer the distinctions ABM and PDE in the following.

Various authors have employed ABM to demonstrate qualitatively that characteristic rates of branching and growth can reproduce 2D network topologies that resemble those of mycelium grown under laboratory conditions. These models may even conform quantitatively to the experimental results they seek to replicate. Although these models focus on microstructure, the diversity of research interests means that the category displays a considerable range of included dynamics, the most common being hyphal tip extension, apical and lateral (sub-apical) branching (see Figure 5), translocation and substrate uptake and depletion. However, most models only consider a subset of dynamics and at varying levels of detail. Many of the models in this category have unique features: Du and Perré (2020) consider a planar domain where the mycelium can also expand in the vertical direction and respond thigmotropically to obstacles, Meškauskas et al. (2004) demonstrate the formation of structures resembling fungal fruiting bodies by modulating the flocking dynamics of hyphal tips, Boswell et al. (2007) argue that continuous representations are not well suited to describe growth in heterogeneous environments (because of the stochastic nature of exploration) and models hyphae navigating a granular 3D environment, Fuhr et al. (2011) represent wood rot mycelium spreading through pores and channels in wood microstructure as probabilistic propagation through a random lattice. Arguably, the most comprehensive model of the ABM type can be found in Vidal-Diez de Ulzurrun et al. (2017).

The strength of these models lies in qualitatively replicating microstructure in a visually convincing manner, and, in doing so, demonstrating the plausibility of their descriptions of fungal behavior. However, while these models represent microstructure explicitly, the processes governing the formation of microstructure often rely on stochastic variables and implicit mechanisms. For example, a model may assume that hyphal tips in proximity can sense each other, while the mechanism by which the signal is mediated is not accounted for (Meškauskas et al. 2004). Similarly, the navigation of hyphal tips is usually modelled as a biased random walk, and branch initiation as a stochastic event, relying on experimentally derived shape parameters and probability distributions to match simulations to in vitro fungal growth. Fitted models should replicate the data from which they are constructed, but this also implies that they cannot be relied upon to generalize beyond the specific study range for which the experiment was designed. Sensitivity analysis performed on the fitted parameters can help to alleviate some of these concerns, by showing that the overall dynamics are robust to variation. Regardless, the highly non-linear nature of living systems means that applying these models predictively requires an experimental set covering the same range of conditions that the model is to cover, making model predictions less straightforwardly useful. Since ABM models are necessarily stochastic (unless you assume perfect knowledge of initial conditions and mechanics), results can only be evaluated as ensemble averages, and, furthermore, quantitative comparison with in vitro-derived results requires the extraction of some descriptive bulk measures of the topology. Through these procedures discrete microstructural information is lost.

PDE models rely on more analytical descriptions of fungal dynamics. For example, the radial density profile of hypothetical colonies under various regimes was studied by formulating a set of equations describing branching and propagation of hyphal tips (Edelstein 1982), and subsequently extended to a 2D domain (Edelstein and Segel 1983). Without explicitly representing microstructural details, this approach is able to formulate quantitative constraints on microstructural dynamics for colony propagation. Similarly, a later model shows that parallelization of hyphal fibres may arise spontaneously from branching and anastomosis dynamics (Edelstein-Keshet and Ermentrout 1989). The details of PDE models depend greatly on what hypotheses they were formulated to test. In a rare effort to include differentiation, a model was presented to describe the division of biomass into distinct classes: vegetative, competent, conidiophore and conidia (Georgiou and Shuler 1986). Another model discriminates between vegetative and reproductive aerial hyphae with distinct

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Table 1. Overview of fungal morphogenesis models from the literature

Reference	Representation	Environment	Metabolism	Transport	Microstructural dynamics	Propagation	Model species
Edelstein 1982	PDE; Hyphal density; Tip density	1D	None	None	Lateral & apical branching; Stochastic anastomosis; Tip decay; Autolysis	Constant speed	Generic
Edelstein and Segel 1983	PDE; Hyphal density; Tip density	1D	Growth-limiting substrate; Michaelis-Menten uptake; Constant maintenance; Linear biomass yield	External diffusion; Internal diffusion; Steady internal flow	Generic branching; Stochastic anastomosis; Autolysis	Extension $\propto$ nutrients	Generic
Davidson et al. 1996	PDE; Biomass density; Tip density	2D	Growth-limiting substrate; Growth $\propto$ nutrients; Substrate replenishment	External diffusion	Tip decay	Diffusion	Generic
Davidson 1998	PDE; Biomass density	2D; Heterogeneous	Growth-limiting substrate; Monod-like uptake; Monod-like biomass yield	External diffusion; Internal diffusion	Tip decay	Diffusion $\propto$ nutrients	Generic
Boswell 2008	ABM; Polyline network	3D lattice; Obstacles	Growth-limiting substrate; Linear biomass yield	Internal diffusion	Branching $\propto$ nutrients (lateral); Anastomosis	Biased random walk; Advection $\propto$ nutrients; Diffusion $\propto$ nutrients	*Rhizoctonia solani*
Carver and Boswell 2008	ABM; Polyline network	2D	Growth-limiting substrate; Linear biomass yield; Substrate replenishment	Internal diffusion	Branching $\propto$ nutrients (apical); Anastomosis	Biased random walk; Extension $\propto$ nutrients	Generic
Heaton et al. 2010	PDE; Polyline network	2D	Linear substrate depletion	Hydraulic translocation	Cord-formation	Constant speed	Generic
Fuhr et al. 2011	ABM; Graph	3D random lattice; Heterogeneous	Linear uptake; Linear biomass yield	Internal graph traversal	Nutrient-contingent branching	Nutrient-contingent growth; Function of pH; Function of temperature	*Physisporinus vitreus*
Hopkins and Boswell 2012	ABM; Polyline network	2D; Heterogeneous	Monod-like uptake	External diffusion; Internal diffusion; Constrained diffusion	Branching $\propto$ nutrients (lateral); Anastomosis	Biased random walk; Negative autotropism; Diffusing inhibitor	*Rhizoctonia solani*

Table 2. Overview of fungal morphogenesis models from the literature

Reference	Representation	Environment	Metabolism	Transport	Microstructural dynamics	Propagation	Model species
Heaton, Jones, and Fricker 2016	PDE; Biomass	0D	Growth-limiting substrate; Linear biomass yield; Substrate recalcitrance	Implicit translocation (hydraulic); Function of topology;	Crowding effect; Biomass recycling	Implicit; Volume $\propto$ biomass	Various
Vidal-Diez de Ulzurrun et al. 2017	ABM; Polyline network	3D; Heterogeneous; Obstacles	Growth-limiting substrate; Linear uptake; Linear biomass yield	Internal diffusion	Apical branching; Anastomosis	Biased random walk; Thigmotropism; Nutrient-contingent extension	Generic
Du et al. 2018	ABM; Polyline network	2D lattice; Obstacles	None	None	Lateral & apical branching; Anastomosis	Biased random walk; Thigmotropism; Constant speed	*Postia placenta*
Sugai-Guérios et al. 2019	ABM; Polyline network; Reproductive hyphae; Vegetative hyphae	3D lattice	None	None	Apical branching; Crowding effect; Differentiation	Random walk; Length-dependent inactivation; Constant speed	*Rhizopus oligosporus*
Du, Tran, and Perré 2019	PDE; Biomass density; Tip density	1D	None	None	Lateral & apical branching; Anastomosis	Diffusion	*Postia placenta*
Du, Perré, and Turner 2020	PDE; Biomass density	1D	Generic growth function	None	None	Fractional diffusion	*Postia placenta*
Du and Perré 2020	ABM; Polyline network	3D lattice; Obstacles	None	None	Laterial & apical branching; Anastomosis	Biased random walk; Thigmotropism; Constant speed	*Postia placenta*

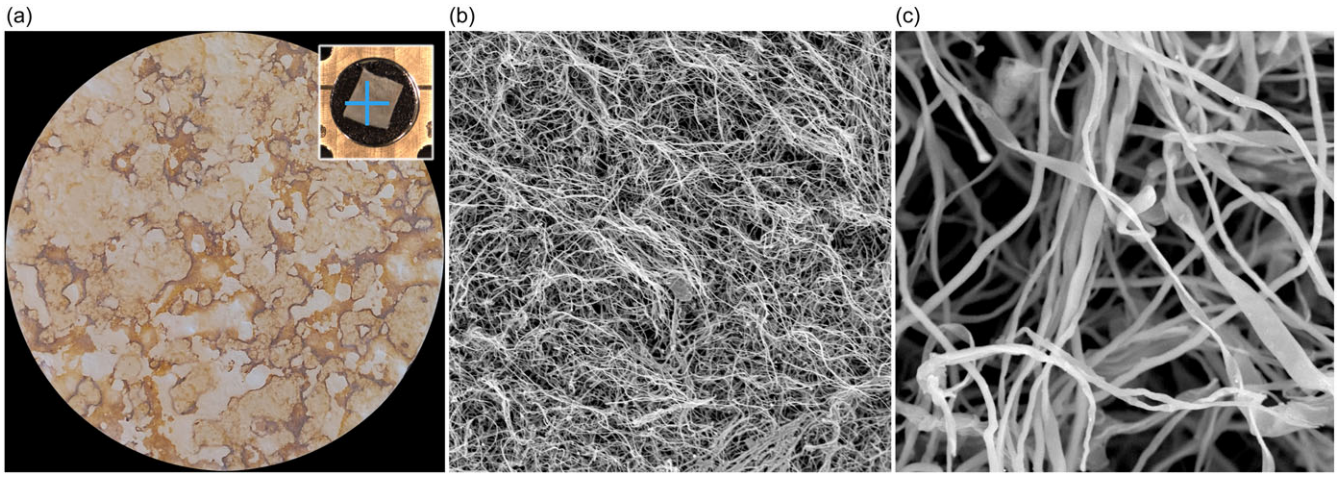

Figure 1. Pure mycelium sheet of *Ganoderma lucidum* grown on beech wood imaged at various magnifications. The properties of the macroscopic material depend on the microscopic structure. The salient question for modelling is how much detail needs to be represented to capture the variation in the properties of interest. **a)** Sheet of diameter 14 cm. The inset shows a 5 mm square slice on microscope stage with cross-hairs marking position of focus. **b)** Material imaged at 630x magnification, cropped to a 500 μm square. **c)** Material imaged at 6300x magnification, cropped to a 50 μm square.

microstructural behavior to model the density profile of hyphae extending into the gas phase (Sugai-Guérios et al. 2019). Disregarding microstructure completely, a framework was proposed where structure is the result of a simple reaction-diffusion system (Davidson et al. 1996). Interestingly, a reaction-diffusion system is also able to produce fractal structures that resemble discrete hyphal networks (Regalado et al. 1996).

Sufficiently simple PDE models are open to mathematical treatments like linear stability analysis and phase plane analysis. Modellers are often able to make predictions that should apply for any equivalent system where the underlying assumptions hold true. With moderately complex systems, analytical methods may not be applicable, requiring instead simulation by numerical integration. Prohibitive computational expenses associated with numerical methods may explain why PDE models seem more popular in early fungal growth literature. For reference, when Gillespie presented his method for stochastic simulation, he reports expenses up to USD 80 for a single simulation run of the Oregonator model with only a couple of million iterations (to be adjusted for inflation) (Gillespie 1977). Notably, a disadvantage with PDEs is that they scale with the grid resolution, so that the steps from 1D to 2D and from 2D to 3D imply very considerable increases in the demand for computing power, although computational complexity is constant in time. ABMs, meanwhile, scale with the number of agents (hyphal tips), which, for biological growth models, is generally exponential in time, but limited for small colonies. As computers became increasingly ubiquitous but remained limited in computational power, ABMs may have presented an economical alternative for achieving detailed outputs. As computational capacity continues to increase, complex PDE models may become more feasible.

Disregarding ensemble averaging, the relative computational efficiency of an ABM or PDE model will depend on the required resolution and scale of the model. In general, ABMs may be simpler to implement in terms of programming, since they are less mathematically formal and rely on more intuitive descriptions of system dynamics. This makes them particularly useful for generating small-scale mycelium networks to examine the effect of a particular subset of microstructural dynamics on local morphology. PDEs are arguably more rigorous and able to produce more general results, but this comes at the expense of being less straightforward both in mathematical derivation and numerical implementation. PDEs are likely to be more useful when the properties of interest for the modelling emerge from large quantities. In the context of materials, it is useful to consider the scale of microstructural dynamics in comparison to the scale of the material to assess the relevance and feasibility of explicit representation for describing functional properties, see Figure 1.

Metabolism

Growth-medium optimization has long been an important strategy for improving the productivity of biochemical processes. From the perspective of material design the implications are straightforward: changes in growth medium result in different material properties, and variation of growth medium in space results in a spatial distribution of material qualities. In fungi, the nutrients needed for growth include organic carbon (most commonly in the form of mono- or disaccharides), nitrogen, phosphorus, sulphur, potassium ions, calcium, magnesium and other trace minerals (Watkinson et al. 2015). The availability and quality of water significantly influences metabolic activity (for wood rot, see Rigobello 2023 p.66), as does oxygen availability (for wood rot, see Rigobello 2023 p.134).

Taking into account known features of the metabolic behavior of the species under study, a growth model must account for several stages in the metabolic process. First, fungi produce and secrete enzymes to digest extracellular resources. Nutrients in the growth medium must then diffuse to the organism and be transported across the cell membrane before they can be used to synthesize useful components (including biomass), which in turn need to be directed to their proper place. This description may seem fastidious, but it is in itself a gross simplification, and it is important to be aware that there are typically many biochemical and biophysical processes involved in the metabolism. For growth modelling, the literature is mostly occupied with simplified descriptions of biomass synthesis.

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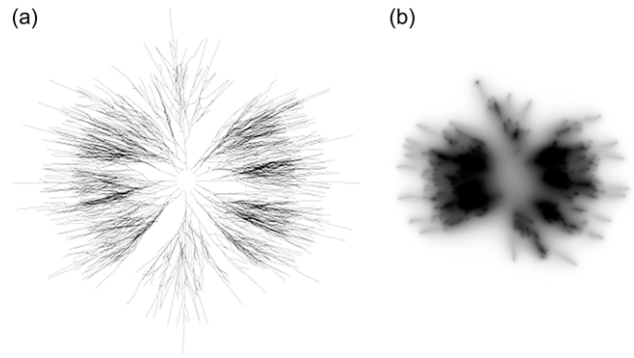

Figure 2. Example output of a generic lattice-free discrete-continuum hybrid model in a 2D domain. **a) A discrete hyphal network** generated as trails left by agents propagating by lateral branching and a random walk biased by a chemical gradient in the external environment. Each hypha is generally represented as an array of the coordinate visited in each iteration. One of the advantages of discrete representation is that it allows for topological network analysis. **b) A continuous chemical concentration** caused by secretion from the tips and subsequent diffusion in the medium, resulting in a smooth gradient. The evolution of the chemical concentration is calculated with a PDE, in which the dynamics are consistent with physical phenomena.

In mycelium, the hyphae are effectively the biomass, making the connection between metabolism, biomass growth and propagation explicit—every unit of biomass produced implies a proportional increase in hyphal length. From this perspective, simple models may assume biomass production a direct result of a growth front of hyphal tips propagating at a constant speed and branching rate. More detailed approaches couple biomass production and micro-structural dynamics to environmental conditions by limiting hyphal extension rate and branching rate to a function of resources available at the hyphal tip (Carver and Boswell 2008). In the absence of resources, hyphae cannot extend. Some species are able grow locally by redirecting resources recycled from the established network by autophagy, but for growth in a global sense, external nutrients must be internalized for hyphal extension, which leads to the discovery of new sources of nutrients. This loop motif leads to colony propagation as a wave-like front.

Mathematically, the limiting effect of an environmental variable on an overall metabolic rate is commonly described by modified versions of the hyperbolic function suggested by Monod (Koch 1998). These functions approximate the rate of biochemical processes as a sigmoidal function fitted to experimental observations, varying between zero and a maximum rate depending on the local value(s) of one or more rate-limiting variables, see Figure 3. In the context of fungal growth models, biomass production rate is commonly limited by local availability of a generic carbon source or oxygen. Monod-like models may also include nitrogen as a growth-limiting factor as the element is required for protein synthesis and redox cofactors which are critical for many biochemical reactions (Lamour et al. 2001; Paustian and Schnürer 1987). Although mimicking Michaelis-Menten enzyme kinetics, Monod-like equations are non-mechanistic metabolic descriptions in that they are not explicitly based on the physical processes involved. Monod-like models clump a series of dynamics (that in reality are spatially compartmentalized) into a single step. Under certain conditions, the simplifications involved may be appropriate with the assumption of a rate-limiting reaction—the observation that with chemical reactions occurring in series, a good estimate for the overall reaction rate is the rate of the slowest step. However, in fungi, it is unclear whether, and under what

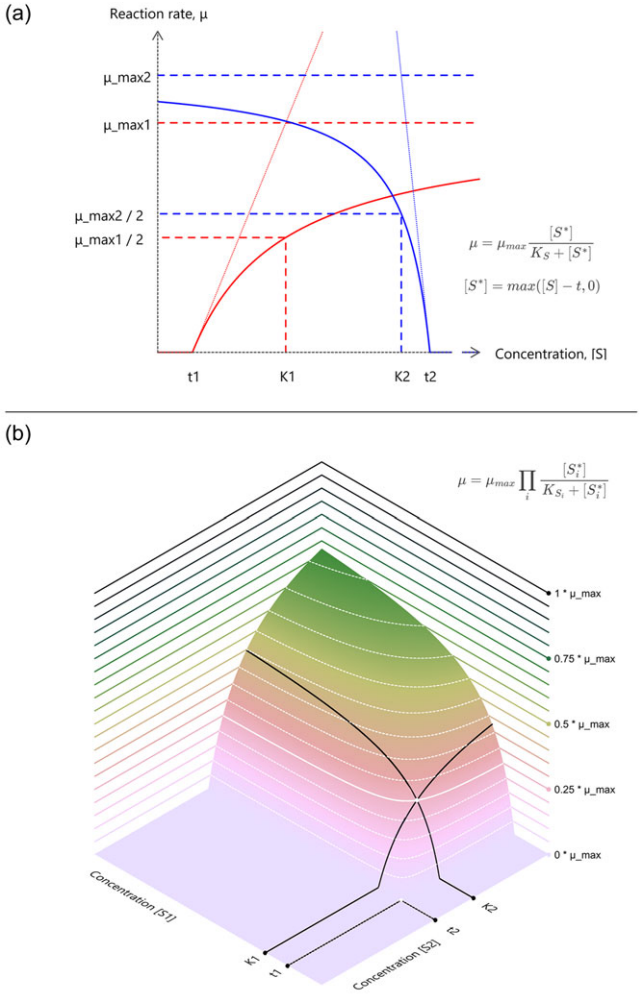

Figure 3. Examples of Monod-type functions. **a) For a single variable** a Monod equation is typically parameterized by a maximum reaction rate, μ_{max}, that the function approaches asymptotically, and a Michaelis constant, K_m, which is the concentration at which the reaction rate reaches half of the maximum. As the concentration goes to zero the slope approaches μ_{max}/K_m. Additional control can be achieved by modifying the function. In the example, the function is shifted by a threshold, t, below which the reaction rate is zero, and changing the sign makes the effect of increasing concentration inhibitory (blue). Monod-like functions can be useful for describing simple systems because the parameters are few, but capture important information about the overall behaviour of the system. In reality, an organism's reliance on any single variable is more complicated. For example, excessively high concentrations of useful compounds may be toxic, and while temperature increases the rate of chemical reactions generally, this also implies increasing maintenance costs. **b) For multiple variables** a straightforward approach is to use the product of a Monod-like expression for each variable, each with its own set of parameters. Metabolic behaviour is nonlinear and there is, of course, no guarantee that the interaction between variables is multiplicative, but it can serve as a simple descriptive model. While the properties of the resulting system are similar to the one-dimensional case it is important to consider that the asymptotic approach to the maximum rate becomes much slower, and the meaning of the Michaelis constant must be regarded in relation to the other variables. In a two-variable system, if both concentrations are at the level of their respective Michaelis constant, the reaction rate is one fourth of the maximum rate rather than half.

conditions, this limiting step would be the secretion of enzymes, degradation of substrate, translocation of external compounds across the cell membrane, or metabolic processes in the cytosol. Furthermore, Monod-like descriptions require parameter fitting, which leads to problems with generalization and experimental economy. The detail of a Monod-like model can be increased by

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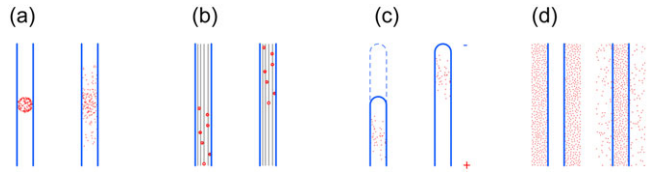

Figure 4. Transport mechanisms reported in fungi. The diagrams are read as a before and after state from left to right. **a) Diffusion** is one of the mechanisms by which particles are transported both within the mycelium and in the external substrate. The large number of particles and stochasticity involved, along with the fineness of the process compared to the scale of hypha, means it is usually represented as a continuous process. Diffusion can be represented as one-dimensional (longitudinal) between segments of discretized hypha. **b) Vesicular transport**, is the advection of particles gathered in vesicles within the hypha and drawn by molecular motors walking (unidirectionally) along cytoskeletal filaments. The moving vesicles can cause cytoplasmic streaming which drags smaller external molecules along with them. **c) Hydraulic transport** is caused by drops in pressure potential as water is lost locally either by exudation or volume increase as the tip extends. Particles are moved by the water flowing along the pressure gradient to replace the volume lost. **d) Absorption** of molecules from the external environment, and secretion in the opposite direction, is usually an active process. In most models this step is simply implied and baked into a total metabolic rate, but in reality metabolic processes occur in different compartments and in series of chemical reactions. Identifying rate limiting steps in these series can be an effective way of simplifying models.

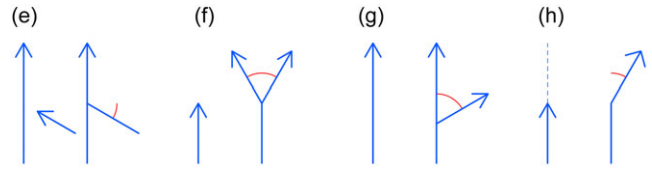

Figure 5. Common microstructural dynamics in ABM models. The diagrams are read as a before and after state from left to right. **e) Anastomosis** is the fusion of a hyphal tip with another hypha. Algorithmically this generally requires collision detection which is relatively computationally involved. Since the exact position of the hypha may be difficult to determine, and as the conditions required for anastomosis to occur may be unknown, the event is often simply assumed to have occurred with some probability when tips come within an arbitrary distance of other hypha (for example within the same cell of a simulation lattice). A collision angle may also be used to determine the likelihood of the event. **f) Apical branching** is relatively simple to represent as a hyphal tip simply becomes two tips, parameterized by a change in direction. A function of internal and/or external conditions determines the frequency of branching events—in the simplest case stochastic events can be drawn from a cumulative probability distribution with the desired properties (mean, std. deviation). It may be useful to consider that branching at a well-defined rate is equivalent to exponential growth in a continuum regime. **g) Sub-apical or lateral branching** is more subtle because the algorithm also needs to consider how far behind the tip and how far in front of the last branch new branches should appear. This can complicate the procedure by necessitating additional length calculations and the storage of more microstructural data. **h) Random-walk** is the most common way of representing tip propagation. At each iteration the position of the tip is updated by a propagation speed given by some function, and a new direction drawn from some probability distribution. If the update velocity is not uniformly distributed over the circle (2D) or sphere (3D) of possible directions the random walk is said to be biased, which can be used to represent various tropisms. For example, in chemotaxis the presence or absence of an external substance may bias the distribution of update velocities, causing growth away from or towards the substance concentration gradient. Notably, taking the continuum limit of randomly walking agents, or the expected value of displacement, is equivalent to diffusion.

representing more of the steps involved in digestion. For example, by including the secretion of digestive enzymes (Lamour et al. 2002) and substrate recalcitrance (Heaton et al. 2016), both of which affect the rate at which nutrients in the substrate become available to the organism. Both the cited studies arrive analytically at the conclusion that no fungus can reinvest more than half of its energy budget in digestive processes.

Beyond substrate composition, modellers may observe that the metabolism is bound to the constraints of several conservation laws that can be leveraged to increase realism. Stoichiometric balance or mass balance requires that chemical equations have the same amount of each type of atom on each side of the reaction. This can be exploited rather usefully even when details are unknown, as the principle also holds for the overarching chemical equation in which substrate is converted to biomass. If the chemical composition of the substrate is known, and the chemical composition of biomass is known, this is a good starting point for appreciating the amount of biomass produced per unit substrate consumed. Similarly, the metabolism must adhere to redox balance (the number of electrons is conserved across the reaction) as well as the fundamental conservation of energy. In other words, the total mass, energy, charge and oxidation state of the system should be constant as substrate compounds are metabolized. These considerations are important as they affect the flux through the various metabolic pathways, and the yield of desired products such as biomass. By applying bookkeeping principles to these quantities we can obtain valuable information about our system's behaviour without getting into the detail of particular reactions. One energy budget model considers only a fixed conversion rate between metabolized substrate and biomass produced (Heaton et al. 2016). Despite the model's simplicity, the authors demonstrate the power of conservation laws and ecological reasoning by linking optimal life-history strategies for fungal species to the transience of their natural growth substrate.

Metabolic network models increase further in detail by considering sets of metabolic pathways available to the organism as directed graphs, where chemical compounds form the nodes and reactions the edges between them. At the extreme, genome-scale metabolic reconstructions maximize the use of available information by constructing comprehensive metabolic network models. In this case, sequenced DNA is compared against enzyme annotation databases to catalogue the enzymatically catalysed reactions available to the organism. This data is then complemented with biological knowledge of the organism to construct the metabolic network model.

Analytical methods, like flux-balance analysis (FBA), can be applied to metabolic network models to make predictions about the reaction rates (metabolic flux) through each edge of the graph under specific nutrient conditions. By optimizing with regard to a goal function (such as maximizing biomass production) within the constraints of conservation laws, FBA can predict theoretical fluxes through the ensemble of metabolic pathways based on the chemical composition of the growth medium. Genome-scale metabolic models have been identified as particularly useful for various tasks in the design of synthetic microbial consortia (Du et al. 2023). In principle, meta-metabolic network models can be constructed by combining models for multiple organisms (Nyholm et al. 2020). The power of these techniques is limited by the availability of fully sequenced genomes, the incompleteness of enzyme annotation databases, and the fact that the choice of goal function that the metabolism is assumed to optimize is to some degree arbitrary, resulting in a difference between observed and predicted fluxes. Despite this, the method has industrial applications in metabolic engineering for deriving theoretical maximum yields used to inform substrate optimization and genetic modifications. Although FBA and related methods have been applied to fungi (Jouhten et al. 2012; Lu et al. 2019), the focus is on industrial processes and the technique has not been used in models of fungal morphogenesis.

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As an illustrative example from an adjacent topic, a 2D simulation of bacterial growth used FBA to demonstrate spatio-temporal variation in the distribution of metabolic modes of individuals belonging to the same colony (Bauer et al. 2017). In the simulation, the bacteria switch between metabolic strategies depending on their local environmental conditions, which in turn evolve depending on the metabolic modes of the bacteria. This loop motif results in a spatial organization with clearly defined zones of bacteria with distinct behavior. In terms of generalization, genome-scale metabolic network reconstruction is a promising technique because it relies only on strain specific genome data—which is becoming increasingly accessible—to make high resolution predictions. In an optimistic scenario, detailed metabolic models could be constructed systematically on a per species or strain basis after genome sequencing—offering great generality and reducing the need for laboratory experiments. Solving comprehensive metabolic networks, as in FBA, does, however, imply a significant increase in computational complexity. A hybridized metabolic model may achieve a compromise between resolution and efficiency. As an example of such a compromise, a descriptive approach was combined with a minimal metabolic network model by considering three rate-limiting substances (carbon, oxygen and nitrogen) along with eight metabolic pathways and their compartmentalization, to show how metabolic phenotype of a yeast varies spatio-temporally as a function of environmental conditions (La et al. 2020).

The metabolic models found in the growth-modelling literature are generally greatly reduced, which may cause problems with generalization. Metabolic network modelling offers an alternative with an arbitrary level of detail, at the expense of computational resources. Since the relative abundance of chemical compounds varies across space, the metabolic model needs to be solved at every location and iteration, which can amount to a considerable expense. In finding the appropriate compromise between tractable and mechanistic metabolic descriptions, future modellers need to consider how increasing complexity relates to utility with regard to their model use case (Gernaey et al. 2010). Metabolic robustness may imply that simple models perform relatively well for central yields like biomass, whereas the prediction of fluxes through specialized metabolic pathways, and the subsequent anticipation of local material properties, may be highly conditional and therefore demand a higher-dimensional model. Although not included in the growth modelling literature, metabolic activity is also influenced by factors like pH and temperature (Panagou et al. 2003). Temperature is of particular interest since it can be effectively controlled in bioreactors, greatly influences growth rate (van Schie 2024), and is influenced by the heat generated by the organism.

Transport

Having arrived at a satisfactory description of local biomass synthesis, modellers must consider how this new biomass is distributed non-locally by internal translocation mechanisms. Additionally, insofar as the metabolic description relies on substrate chemistry, there must be an accounting of how compounds vary spatio-temporally within the substrate not only as a consequence of metabolic flux, but also by means of diffusive and advective mass transport. Transport of substances in the external medium is not only important with regard to the metabolism, but also potentially affects colony propagation in species exhibiting chemotropism—where hypha follow a chemical gradient, see Figure 6.

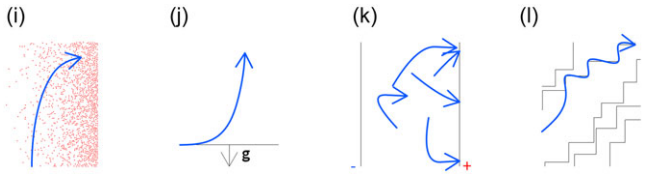

Figure 6. Some tropisms reported in fungi. **i) Chemotropism** is where the direction of hyphal extension is either attracted or repelled by a chemical gradient. In so far as secretion and depletion of substrate compounds create such gradients, chemotropism could provide signals that allow hyphae to navigate towards or away from each other, *autotropism*. **j) Gravitropism** can be observed where hypha extend away from the substrate surface as in aerial hypha, directed by the gravity vector. **k) Galvanotropism** lets hypha reorient in response to electric fields. It is perhaps not a naturally occurring phenomena, but an effect of the role of ions in the general navigation mechanism. **l) Thigmotropism** is where hypha appear to follow geometrical features like ridges or grooves in the environment and is mediated by pressure or stretch sensing. Thigmotropism may play a role where fungi navigate structured substrates like wood, and where hypha bundle together with other hypha, providing another instance of autotropism.

External transport

External mass flux comprises an advective and a diffusive component. While it is difficult to eliminate advection, it is arguably possible to construct experimental conditions where its influence is limited relative to other factors. In models that replicate growth in a Petri dish, advection is disregarded, presumably under the implicit assumption that the environment is closed and will therefore be exposed to minimal perturbations, or that the macroscopic mixing of the gas medium is sufficiently smooth compared to the model resolution that the concentration of constituent gases can be considered uniform, or that it can be captured descriptively by the diffusion term. In LSF, mixing is often integrated in the process to homogenize the substrate, resulting in the possibility to make similar model simplifications. If simplifying assumptions for advection are not possible with regard to model utility—if the organism is grown in an unsteady system or if aeration is one of the control vectors—the model must integrate computational fluid dynamics methods. Advective transport of substrate compounds is not encountered in the literature under review. However, under simplifying assumptions, the bulk propagation of hyphal tips can be modelled as advection (Edelstein-Keshet and Ermentrout 1989).

Meanwhile, diffusion is difficult to simplify away since it describes the increase in entropy through Brownian motion. This phenomenon can be disregarded only by arguing that its effects are negligible at the spatial or temporal scale being modelled. As fungal growth takes place over the scales of days and centimeters this is not often the case. Most fungi are aerobes, which implies that oxygen is locally depleted through respiration—and unless oxygen is somehow replenished this will eventually lead to local hypoxia. Even in facultative aerobes, such as yeasts, we should expect metabolic yields to be heavily influenced by access to oxygen as the organism switches between metabolic modes—over 30 ATP are produced for each glucose molecule through aerobic respiration, compared to the measly 2 ATP produced under anaerobic conditions. The product formation of fungal pellets in bioreactors has been linked to the effect of biomass density on diffusivity (Schmideder et al. 2021). Further stressing the importance of diffusive processes, one review focuses specifically on the critical relationship between oxygenation and morphology in the context of yield optimization in bioreactors (Veiter et al. 2018).

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The scale of diffusive processes compared to fungal growth means they are modelled as continuous, even in ABM models, resulting in discrete-continuum hybridization (Boswell 2008). Typically, this requires one parameter per diffusive substrate compound variable—the diffusion rate—which must be derived experimentally. The effective diffusion rate of a gas in a substrate will depend on inter alia temperature, water content, and solubility, and may even be anisotropic depending on the geometry of the substrate structure. There are simulation approaches to arrive at an effective diffusivity (Lattice Boltzmann Method, random walkers, the Laplace equation), but these are computationally intensive and complex scenarios still require experiments to verify results. As a direct consequence of growth and decay, mycelium and substrate geometry is constantly changing and with it the transport properties of the medium. Denser mycelium implies impeded gas exchange, as do mycelium mats forming on the surface of substrates. To take this into account a simulation approach would require recalculation of the transport properties of the medium at every time step. To mitigate this problem a reduced model has been suggested (Peszynska et al. 2021).

In summary, depending on experimental conditions, external transport can sometimes be simplified. However, the metabolic import of oxygen means that gas exchange and morphogenesis are mutually dependent. In computational fluid dynamics, advection and diffusion are modelled as the first and second partial derivatives in space, respectively, which may pose challenges in numerical implementation.

Translocation (internal transport)

Fungal growth takes place at the hyphal tip region, and propagation may proceed over nutrient-free domains, as, for example, in the aerial hyphae and fruiting bodies that extend into gas medium. This is only possible with the displacement of foraged resources through the hyphal network, which must be accounted for in any growth model that takes interest in biomass distribution in response to heterogeneous environmental conditions. A discussion of the transport mechanisms of filamentous fungi can be found in Fricker et al. 2017. Four modes are considered: active vesicle transport by molecular motor proteins, cytoplasmic streaming induced by drag of aforementioned vesicles, hydraulic currents caused by exudation of water or volume expansion (as in tip growth), and diffusion within the cytosol, see Figure 4. The prevalence of these modes of transport will depend on species, localization within the mycelium, and environmental conditions. Their relative importance is likely to depend on the chemical compound of interest. Not all compounds will be transported in vesicles, and notably, vesicular compartmentalization and cytoskeletal tethering may exclude the other modes of transport.

In models with discrete microstructure, internal transport is most commonly represented as one-dimensional diffusion along hyphal segments. Where diffusion is used as a simplification of other transport mechanisms, this approach runs a risk of representing as bidirectional a mode of transport which is actually unidirectional. Active transport facilitated by molecular motors has been described with a lattice gas model (Shinde et al. 2020), and can also be represented as advection. Another discrete simplification at the level of individual hypha is the tanks-in-series method (Balmant et al. 2015), which poses tip extension as the combined result of both vesicular transport of cell wall material, and cytoplasmic diffusion of nutrients through a series of hyphal compartments.

Regardless of modelling approach, material must reach the growing tip at a rate greater than or equal to the growth rate (a higher rate implies a build-up of material at the tip which must somehow be resolved—perhaps by branching). What internal transport speeds this requires depends on where the material is sourced. With slow tip elongation rates, diffusion may be sufficient to fuel growth (Fricker et al. 2017). In contrast, the filamentous fungi *Neurospora crassa* grows rapidly and its reliance on molecular motor proteins is evidenced by their exceptional speed (Steinberg and Schliwa 1996) and impaired growth in their absence (Seidel et al. 2013). Internal transport is linked to the organism's life-history strategy. As a general consideration, active transport is potentially both inefficient and slow compared to passive modes of transport. Translocation speeds far in excess of molecular motors can be achieved passively, at least theoretically, by simply managing local membrane permeability to water (Heaton et al. 2010). Mushroom gills and evidence of evaporative cooling (Husher et al. 1999) speak to the employment of hydraulics to transport nutrients against gravity through a nutrient free domain to the reproductive fruiting bodies. Under this scheme, vesicular transport and diffusion would be required only supplementally. The branching structure of mycelium implies local currents would be distributed as an increasing function of the number of downstream hyphal tips (Heaton et al. 2012), possibly providing local cues for cord-formation (Heaton et al. 2010). Hydraulic transport also has the advantage of preferentially, promptly and proportionately distributing resources (potentially bidirectionally) to growth regions without the need for a signalling system.

Translocation mechanisms in filamentous fungi are not well understood and hence described in little detail in the modelling literature. When included, translocation is usually described as a diffusive process, which may be because solvers for unsteady advection are more demanding to implement algorithmically. For morphogenesis, internal transport processes are essential as they determine how foraged materials are redistributed in the growing organism. A first step may be to determine, for each organism under study, if any mode of transport dominates the others, as this will greatly influence the model dynamics and may allow simplifying assumptions. An experimental method for characterising fungal species by active or diffusive transport modes for various compounds can be found in Olsson 1995.

Microstructure

As previously alluded to, internal transport mechanisms depend non-linearly on the mycelial microstructure. In all modes of internal transport, hyphal length, diameter and bifurcations play a non-trivial part in determining the spatio-temporal distribution of resources, calling for the modelling of microstructural dynamics such as anastomosis (see Figure 5), branching and cord-formation to describe network topology. Moreover, it is also necessary to consider factors that influence the directionality of hypha, see Figure 6. In the modelling literature, thigmotropism—where hyphae follow surface features—and chemotropism are somewhat represented, but more types of tropism have been described (Brand and Gow 2009). Mycelium microstructure may also influence transport mechanisms externally by altering geometry, as in gas exchange through porous substrates. In terms of design targets and material properties, microstructure is likely to influence the mechanical responses of a cultivated material (Jones et al. 2018), as does, on an even smaller scale, the local molecular structure of the cell wall (Rigobello and Ayres 2023). All of this stands in support of

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explicit microstructural representations. This is where the dilemma of incomplete information becomes particularly acute. Explicit microstructural modelling may grant modellers data to feed into non-linear transport descriptions and mechanical simulations, but as long as microstructural dynamics are described non-mechanistically their predictive value is limited.

Continuous representations may, in this sense, be more true to the state of available knowledge. Depending on the case, PDEs may also be able to capture significant non-linear relationships with mean-field reasoning. In so far as microstructural properties can be predicted, they can, just as biomass, be described as density distributions. For example, a simple argument holds that mycelium expanding radially at constant density and hyphal diameter must have a constant net branch formation rate, hence net branch formation is directly proportional to biomass density and follows the same spatio-temporal distribution. Similarly we can calculate the average number of hyphae through a grid element and arrive at local hydraulic conductance in a continuous regime. In the specific case of the Fungateria project, the model species *Schizophyllum commune* rarely exhibits anastomosis under laboratory conditions, so the dynamics of internal transport can be simplified by disallowing lateral translocation. With similar and more sophisticated lines of reasoning it is often possible to find continuous equivalents of discrete microstructural dynamics, the question being whether or not all the non-linear effects relevant in view of our modelling scope can be captured.

Ultimately, the merits of continuous or discrete representation comes down to the granularity of the result we seek and the model use case. For the Fungateria project, modelling is aimed towards ELM design. For this purpose, the scale of interest does not require explicit microstructure, as long as the model provides sufficient information to predict variation in material properties.

Discussion

Review limitations

The review of the state-of-the-art has taken into consideration mycelium modelling at the macroscopic scale of functional materials, attempting to link morphogenesis with the most relevant processes at the microscale. The scope being thus limited, we have, wherever possible, attempted to point the interested reader towards useful sources of more in-depth accounts of particular topics. That being said, it would be an oversight not to mention some important topics that have not found much space in this review. Firstly, as focus has been on modelling how morphogenesis can be influenced by environmental conditions, the practical considerations of how the environmental conditions can be controlled have been left largely untouched. A complementary review could focus on bioreactor design and control strategies as they relate to morphological control vectors. Secondly, an important aspect of morphogenesis is the differentiation of hyphae into specialized tissues. The reason for the sparse mention of this topic in this review is its rare occurence in the modelling literature. We can speculate that this has to do with the fact that it is a complex topic that needs to be disentangled together with cell regulatory mechanisms. Another reason may be that the current state of modelling is rather agnostic to different species of fungi, and models could potentially adapt to variation in behavior between tissue types by adjusting sets of model parameters just as the model would be fitted to other species. Further research in mapping out the signals responsible for differentiation would be of great value for both genetic approaches to bioengineering with mycelium and approaches pertaining to modulating cultivation conditions. Lastly, tropisms are mentioned throughout the review and figure in many of the models, but usually in a generic sense where the model is agnostic to the origin and nature of the gradient that biases the hyphal propagation. Here we would also encourage continued research efforts so that tropism is linked to observed fungal behavior and becomes more than a theoretical component in our models.

Continuing along this line, we have synthesized criteria for developing a modelling framework based on the particular needs of ELM design, as outlined below.

Research directions

It is telling that, of the models considered in this review, few consider a 3D domain, extend beyond the dimensions of a Petri dish, consider variations in environment variables, or offer completeness in terms of describing in similar level of detail the dynamics across the four categories: metabolism, transport, microstructural dynamics, and propagation. The existing models are predominantly constructed from the perspective of mycological experiments under laboratory conditions, aiming to understand and describe specific aspects of fungal behavior. None of the models aim to describe a process of cultivating mycelium-based materials, let alone control such a process. Modelling for biological research and modelling with the ambition of bioengineering and design pose very different challenges. From the perspective that one of the goals of ELM research is to develop differentiated materials, there is more to expect from our modelling endeavours. We propose the following areas for future ELM modelling research:

- Strategies to address biological diversity and improve model generality,
- Large-scale holistic modelling of biological systems in heterogeneous 3D domains,
- Integration of dynamic metabolic models that account for variation in substrate composition,
- Integration of differentiation and tropism,
- Integration of simulated bioreactor environments,
- Adoption of high throughput numerical techniques.

These topics are expanded upon in turn in the following.

Strategies for improving generality

The utility of a model depends on how well it is able to predict experimental outcomes and the size of the problem space it applies to. Demanding more in terms of accuracy also implies more rigorous validation. The currently prevailing practice, where the output of a model with fitted parameters is compared against experimental results, may suffice to demonstrate specific principles in experiments with a limited focus. For practical applications we require that predictive power applies also outside the experimental conditions, which generally precludes fitting and requires that all model parameters are possible to measure experimentally. Sensitivity analysis can alleviate some of the apprehension associated with fitting, but even so, it is problematic in terms of experimental economy that comprehensive models necessitate large experimental sets, that are likely to require replication for every strain and every significant update to the model. One remedy could be to increasingly found models on information that can be

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acquired without experimentation: this could include genome sequencing, life-history strategies, fungal trait databases and biological knowledge about ecological context and competitive landscape. Such knowledge could potentially allow models to make informed guesses about important parameters based on standardized strain classification. Another strategy is to describe dynamics in terms of parameters pertaining to more fundamental phenomena. For example, in some cases modellers may be able to break down high level biological mechanisms into lower level physical mechanisms that are more likely to generalize across fungal strains. Finally, as a complement to strategies for reducing experimental load, a valuable contribution would be to develop experimental protocols that can efficiently determine essential modelling parameters for an arbitrary strain.

Large-scale holistic modelling

A model is only as reliable as its least accurate component. The models represented in the literature vary in scope and are therefore selective in the dynamics they include. To increase the predictive ability of models it is necessary to include a larger span of dynamics, integrating metabolism, transport, microstructural dynamics and propagation within a single framework. For greater utility and realism such a framework should accommodate 3D simulation domains where conditions are not assumed to be uniform and constant throughout. This is essential both in view of achieving more controlled mycelium materials and for spatial modulation of material properties. If our model is not sensitive to local differences in the evolving system, we cannot hope to understand and control spatial variation.

Fully 3D simulations pose additional challenges for modellers both in the form of visualization and implementation. 3D visualization is not straightforward as depth makes it challenging to simultaneously display all data. Here, we could take inspiration from medical imaging techniques that commonly use volumetric ray-casting to produce images reminiscent of X-rays, or resort to 2D-slicing.

Integration of dynamic metabolic models

The metabolism does not receive much attention in the mycelium morphogenesis literature, a reason perhaps being that metabolic modelling is a field in its own right with important implications for industrial fermentation processes and pharmaceuticals, amongst others. For morphological modelling, the challenge is to find tractable ways to make use of techniques already developed. Accurate metabolic predictions are valuable in growth-medium optimization, and can be leveraged for spatial modulation of material properties simply by manipulating initial substrate conditions. To inform about spatio-temporal variation in metabolic behavior, which may also provide cues for differentiation, these models need to be solved locally throughout the simulation domain. With the thousands of metabolic reactions available to fungi, this can pose a computational challenge and necessitate clever simplifications. For comprehensive metabolic models it is possible that pre-trained machine learning models could provide prompt estimates without compromising detail. That being said, not all metabolic pathways are equally important, and low-dimensional models can get far. However, depending on the strain and the application it may be desirable to increase the resolution of metabolic models in terms of substrate specificity. If we, for example, are modelling a strain of wood rot fungus growing on wood substrate, the distribution and form of the different types of lignocellulosic carbon sources and minerals can significantly influence the growth rate and total resource utilization (Rigobello 2023 p.68). Continuing this line of reasoning, resolution could also be improved with regard to enzymatic activity, regulatory mechanisms and metabolic modes. Although glossed over in this review, metabolic activity is likely to exhibit feedback loops by locally altering hydration, pH and temperature.

Integration of hyphal differentiation and tropism

The focus of the state-of-the-art has been in describing the dynamics involved in morphogenesis as if the mycelium was a homogeneous organism. In reality, hypha differentiate to assume specialized behavior according to context, resulting in regions of varying material properties. Formation of tissue types is of course of great interest in morphogenesis, but the signals leading to differentiation are not well charted and vary significantly between strains. It is likely that the formation of specialized structures involves regulatory mechanisms that belong to the problem space of systems biology. As such, this may be an area of future research not so much for modellers as for mycologists, but advances would nevertheless be invaluable for morphological models. Similarly, many models include bias of random walk propagation by external signals, but the literature on tropic responses in fungi naturally suffers from large variation between strains. Protocols to efficiently test strains for tropic responses would be valuable for improving generality of models and offering vectors of control. For example, galvanotropism could potentially offer dynamic, non-invasive and detailed regulation of hyphal orientation.

Integration of simulated bioreactor environments

It is natural that existing modelling literature focuses on the dynamics of the mycelium, but the more accurately the organism can be modelled with regard to its environment, the more important it becomes to model the evolution of that environment. Many models already accommodate diffusion of chemicals through the medium, which is essential for chemotrophy, gas exchange and respiration, but no model considers how gas exchange is affected by the kind of porous substrates used in SSF, by advective flow, by metabolic production of water and by the mycelial biomass itself. However, more specialized research exists to address these issues. In the context of mycelium-based ELM design, the bioreactor environments typically used for cultivation are of particular interest. While bioreactors are valuable in keeping environmental parameters like temperature and carbon dioxide concentration fixed, they also offer the possibility of interventions in the cultivation process. Modelling the bioreactor environment, perhaps with computational fluid dynamics, would assist in harnessing the potential of reactors and reactor design.

High throughput numerical techniques

In framing models as tools not only for research purposes, but also for bioengineering and design, tractability becomes increasingly important. Design is typically an iterative process characterized by cycles of speculation, evaluation and feedback that eventually converge on one or many acceptable solutions. The more time a simulation takes and the less flexible it is, the lower its utility in a

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design process. With the potential modelling advances outlined in this section, models would become increasingly comprehensive, with a corresponding increase in demand for computational resources—only going from 2D to 3D represents an exponential increase in time complexity. Clearly, these considerations emphasize the value of computational efficiency, but should not be seen as a legitimate ground for reductionism.

Sometimes, algorithmic ingenuity can achieve equivalent results with greatly increased performance. For example, in combining a discrete lattice with a continuous random walk vector, calculations could be significantly sped up while effectively retaining the smooth hyphal trajectories of lattice-free ABM models (Du and Perré 2020). Other times, computational complexity can be reduced by converting detailed dynamics to continuum representations, as in fitting a PDE model to a previously developed ABM (Du et al. 2019).

When simplifications are not enough to guarantee simulation promptness, or would compromise fidelity, modellers may find themselves compelled to make use of hardware acceleration. General-purpose computing on the graphics processing unit is very suitable for the kind of calculations involved in both ABM and PDE models, where simple calculations are repeated many times and can easily be parallelized. To avoid losing accessibility and accountability in doing so, researchers might consider using open and platform agnostic frameworks: code written in OpenCL will make use of both CPU and GPU resources where available, and the recent WebGPU API facilitates computation within a browser environment, making resources easy to disseminate.

Conclusion

Returning to the research question posed in this journal: to build with materials with inherent agency, it is useful to first adopt the mode of developmental biologists, to map out the signals that shape the system's evolution. Then we can act as control theorists, to harness its functional potential, and, finally, as process engineers, to tame and refine it. The Good Regulator Theorem states that control of a dynamic process requires a predictive model of at least the same level of complexity (Conant and Ashby 1970). For the fabrication of ELMs, involving the functionalization of biological agents, mathematical modelling is an essential exercise for achieving a degree of process control and to allow goal-oriented design. As morphology is inextricably linked to both metabolism (via transport phenomena) and mechanical performance (via topology) this paper has reviewed the existing literature pertaining to these areas in the context of the modelling of morphogenesis in fungi, to the best of our capacities.

The biological mechanisms underpinning morphogenesis must be regarded in concert with the evolution of the environment in which the organism is embedded. In so far as the ELM cultivation environment is allowed to vary spatially and temporally, modelling of the dynamic environmental conditions is likely to require a PDE representation. Depending on model granularity, either the PDE system can be extended to include the dynamics of the organisms, or, where explicit microstructural detail is necessary, the model can be complemented with a an agent-based representation of individual hyphae.

Within the Fungateria project, a PDE approach has been deemed more conducive to the goals and scale of the ELM development, and the dynamical system can be expressed within a standard reaction-diffusion-advection framework as outlined:

$$\dot{U}(x,y,z,t) = \begin{bmatrix} \overbrace{\dot{u}_i = \mathcal{R}_i(U)}^{\text{reaction}} & + & \overbrace{\nabla \cdot \mathcal{J}_i}^{\text{transport}} & + & \overbrace{\mathcal{F}_i}^{\text{forcing}} \\ \dot{u}_j = \cdots & + & \cdots & + & \cdots \\ \cdots & & & & \\ \dot{u}_n = \underbrace{\gamma_n(\underbrace{\mathcal{M}(U)}_{\text{metabolism}})}_{\text{yield}} & + & \nabla \cdot (\underbrace{d_n \nabla u_n}_{\text{diffusion}} \underbrace{- v_n u_n}_{\text{advection}}) & + & \underbrace{\mathcal{S}_n}_{\text{source}} - \underbrace{\delta_n}_{\text{decay}} \end{bmatrix}$$

Here U is the state vector, and $\dot{u}$ are the update functions for each variable it comprises. Complemented by a similar expression describing how the solution behaves at the boundaries, a set of functions describing the initial distributions and a list of parameter values, this tabular notation provides a succinct and unequivocal mathematical description of a generic morphogenesis model. As models become more complex, reproducibility and comparison becomes less straightforward. In this regard it would be beneficial for future modellers to converge on more systematic ways of expressing theoretical models.

The benefits of building a modelling approach on a PDE foundation is that the mathematics is explicit and consistent with other commonly simulated physical systems, with several implications:

- The link between assumptions and mathematical description is clear, facilitating systematic hypothesis testing.
- Comparability between models.
- Open to apply rigorous mathematical analysis (optimization, linear stability analysis, phase portraits, etc.).
- Separation between mathematical model and numerical implementation decouples the evaluation of a model's theoretical merits from its performance in terms of computational complexity and algorithmic finesse.
- Separation also recognizes that numerical method is a field of research in its own right. Implementation does not necessarily need to be solved by theorists and experimenters. A generic high-throughput PDE solver geared towards biological motifs could open the field by allowing efficient implementation of a wide range of models without requiring programming expertise from modellers.

The comparability and consistency of a PDE framework means that extension of a model is straightforward. The generic framework presented above can easily be adapted to include any number of environmental variables, and is modular in the sense that inclusion and exclusion of dynamics is as simple as adding or removing a term from the equations. In Fungateria, accounting for a bacterial agent in addition to the fungal agent means only the addition of another biomass variable with appropriate metabolic and transport terms. Similarly, vectors of control, such as temperature or airflow, require only a corresponding variable and boundary conditions representing the mode of intervention. Numerical solvers explicitly account for inherent uncertainty, which makes evaluation of results more stringent. Since a continuous representation scales with the model resolution, it offers the option of trading accuracy for efficiency. This is useful when iteration quantity or promptness is more important than quality or when computational capacity is limited, and means that a PDE model can be scaled arbitrarily both spatially and temporally just by modulating the resolution and precision of the solver. By contrast, discrete models, such as ABMs, cannot increase granularity adaptively in a straightforward manner since their

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fundamental representational unit (the agent) is irreducible—a larger simulation domain will in most cases require a larger number of agents, so that simulation size implies unavoidable increase in computational complexity.

Fungal biology is a field with large knowledge gaps. As such we should expect continuous updates to the theory underlying our models. This makes a modular and reusable modelling framework very favourable. With incomplete theory, another advantage of flexible models with a clear link between assumptions and model formulation is that the model can be used as a tool for rapidly screening and analysing various hypotheses. In this scenario, the model is not limited to being a codification of extant knowledge, but becomes a vessel for exploration.

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Ethics statement. This research study adheres to the highest ethical standards in its design, implementation and reporting. The nature of the investigation involves no direct or indirect harm to human or animal subjects. The research strictly follows established protocols and guidelines for responsible conduct and integrity in research.

Connections references

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