DEVELOPMENT OF SPATIOTEMPORAL MODEL OF GROWTH AND PROLIFERATION IN FUNGI WITH KINETICS OF INTRACELLULAR METABOLISM

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ABSTRACT

DEVELOPMENT OF SPATIOTEMPORAL MODEL OF GROWTH AND PROLIFERATION IN FUNGI WITH KINETICS OF INTRACELLULAR METABOLISM

Filamentous fungi are important hosts of industrial biotechnology. Yet, the production capacity suffers from the fungal morphology limitations. While the filamentous form is appropriate for production, in particular secreted products, pellet form is better suited for growth. By solely producing, adequate product levels cannot be obtained, as critical biomass is also needed, yet by solely growing, product yield would be low, especially for non-growth associated products. As a result, optimization of pellet or filamentous form in fungi became an important topic for biotechnological industry. *S.cerevisiae* existing in pellet or the intermediate form pseudohypha has long being studied for optimizing the suitable form for industrial fungi.

To better understand, the trade-off between growth and production a spatio-temporal model (changing with time and space) has been developed taking into account simplified central metabolism of fungi. In doing so, pseudohyphal growth in solid state fermentation by *S. cerevisiae* was mimicked. To do so, general mass balances has been set up to describe how the cells grow and proliferates over time and space using partial differential equations. The resulting PDE has been solved using method of lines, discretizing space in two dimensions. The model takes growth as a function of intracellular ergosterol levels, thereby taking into account underlying metabolism, via mevalonate and ergosterol pathways starting from acetate. The acetate production has been linked to glucose representing a highly simplified form of central metabolism. The model could be used to study to understand how pathogenic fungi proliferates, and attack other cells, how the kinetics of intracellular reactions effect the phenotype, and the trade-off between growth and production in terms of distribution of energy among different cellular processes.

ÖZET

MANTARDA HÜCREİÇİ METABOLİZMA KİNETİĞİ KULLANILARAK BÜYÜME VE YAYILIM İÇİN SPATİOTEMPORAL MODEL GELİŞTİRİLMESİ

Filamante fungiler biyoteknoloji alanında üretimler için önemli kaynaklardır. Ancak üretim kapasiteleri morfolojilerindeki limitasyonlar nedeniyle düşüktür. Özellikle salgılanan ürünler için filamente form ve üreme içinde pellet formu daha uygundur. Kritik biyokütle gerekliliği göz önünde bulundurulmadan yeterli seviyede ürün edilememektedir. Aynı şekilde sadece üreme göz önünde bulundurulduğunda büyümeye bağlı olmayan ürünler için verim sağlanamamaktadır. Sonuç olarak pellet veya filamente formun fungilerde optimizasyonu biyoteknoloji endüstrisi önemlidir. *S.cerevisiae* hem pellet hem de yalancı hif olarak adlandırılan ara forma sahiptir. Bu tür, endüstriyel fungilerin optimizasyonunun nasıl olması gerektiği konusunda araştırılmaktadır.

Büyüme ve üretim arasındaki etkileşimi daha iyi anlamak için fungal merkez metabolizmayı içeren bir spatiotemporal model (uzay ve zaman bağlı olarak değişen) bir model geliştirilmiştir. Bu yapılırken *S. cerevisiae* tarafından katı hal fermentasyonunda yalancı hiflerle büyümesi taklit edilmiştir. Bunun için kütle dengelerine bağlı olarak hücrelerin zaman ve uzaya bağlı olarak büyümeleri ve yayılmaları kısmı diferansiyel denklemler kullanılarak açıklanmıştır. Bu denklemler çizgi metodu kullanılarak uzayı iki boyuta indirgemiştir. Yapılan model büyümeyi hücre içi Ergosterol seviyelerinin bir fonksiyonu olarak kabul edip altında yatan metabolizmayı göz önünde bulundurmaktadır. Bu metabolizma asetattan başlayıp mevalonat ve Ergosterol yolaklarını içermektedir. Ayrıca asetat üretimi direkt olarak glukoza bağlatılıp merkez metabolizmanın basitleştirilmiş bir formu da yapılmıştır. Bu model farklı hücresel işlemler arasında enerji dağılımına bağlı olarak büyüme ve üretim arasındaki ilişkinin, patojenik fungilerin yayılmalarını ve saldırmalarını, ve hücre içi reaksiyon kinetiğinin fenotipe etkisininin araştırılmasında kullanılabilir.

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D	Diffusion coefficient
∂f	Partial derivative of f(x,t)
∂x	Partial derivative of space (x)
∂t	Partial derivative of time (t)
df	Ordinary derivative of function f(x)
dx	Ordinary derivative of x
f(x)	Function of x
f'(x)	Derived function
f(x+a)	Function at the point x+a
f(x,t)	Partial differential equation of function
k	Filamentation rate
S	Same substrate in following compartments
μ	Specific growth rate
u_{fil}	Biomass growth for filamentous form
u_{pel}	Biomass growth for pellet form
X	Biomass in a compartment
X128	ATP
X161	CoA
X163	Acetyl-CoA Synthetase
X171	Thiolase/Synthase
X172	HMG Reductase
X173	Mevalonate Kinase
X174	Squalene Synthase
X175	Squalene Epoxidase
X176	Lanosterol C-14 Demethylase
X177	Δ(24)-Sterol C-Methyltransferase
V	Compartmental volume
V^o	Consumption and production rate in steady-state
V1	Hypothetical consumption rate of glucose

$V1_{inhb}$	Hypothetical consumption rate of glucose inhibited by ergosterol
V2	Hypothetical consumption rate of acetate
$V_{Acetly\text{-}CoA\ Synthetase}$	AV model consumption rate of internal acetate by acetyl-CoA synthetase
$V_{Thiolase/Synthase}$	AV model consumption rate of acetyl-CoA by thiolase / synthase
$V_{Hmg\ Reductase}$	AV model consumption rate of hmg-CoA by hmg reductase
$V_{Mevalonate\ Kinase}$	AV model consumption rate of mevalonate by mevalonate kinase
$V_{Squalene\ Synthase}$	AV model consumption rate of farnesyl-PP by squalene synthase
$V_{Lanosterol\ C14\ Demethylase}$	AV model consumption rate of lanosterol by lanosterol C14 demethtylase
$V_{\Delta 24\text{-}Sterol\ C\ Methyltransferase}$	AV model consumption rate of zymosterol by Δ24-Sterol C Methyltransferase

Ac	Internal Acetate
AcCoa	Acetyl-CoA
Erg	Ergosterol-ER(Endoplasmic Reticulum)
FPP	Farnesyl-PP
Glc	Glucose
HCoa	HMG-CoA
Lan	Lanosterol
Mev	Mevalonate
Sqa	Squalene
Zym	Zymosterol

1. INTRODUCTION

1.1. FILAMENTOUS FUNGI

The process of filamentous growth in fungi is widely observed as a nutritional scavenging response. The process results in extension of microbial cell body without cellular division.The hypha is the basic unit in filamentous fungi resembling chain of elongated cells. Each cell is separated from each other by cell walls named as septa. The general form of hyphae and septum (in red arrows) was visualized in left side of side Figure1.1.. During hyphal growth, synthesis of cell wall is occurred as the part of nutritional scavenging mainly consisting of filamentation coupled with secretion of exoenzymes for substrate lysis [1-3].

The hyphal growth occurs as tip extension visualized in right side of Figure 1.1. The cells behind the tip provides material supplement in order to carry out tip extension. Compared with regular hyphal cells they are not separated from each other. Through being between the first septum and apex, cells are contained in the region named as apical compartment. The cells just behind the apical compartment are known as subapical compartment [4, 5].

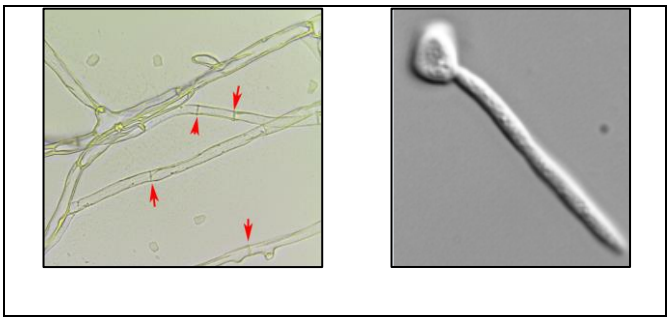

Figure 1.1. Hyphae-Septum (*Penicilium*) (left) True Hyphae (*C. albicans*) (right)

1.1.1. Fungi for Industrial Biotechnology

In industrial biotechnology, microorganisms are used as a source for metabolites and enzymes in their productions. Among microorganisms, fungi are important for producing wide variety of compounds with fermentation process. Fungi have long been used for its

fermentation capability of different food products and safety in order to develop marketable tastes. There are fungal species important for hydrolyzing industrially important compounds which are actually difficult and expensive to process with regular mechanical and approaches [2, 6-8].

1.1.1.1. Secondary Metabolites

Secondary metabolites are known as organic compounds without any direct involvement in primary metabolic processes at which they branch off to their biosynthetic pathways. They are important source product in pharmaceutical industry. Through, filamentous fungi, various secondary metabolites such as compounds with antibacterial, antifungal and antitumoral activities are being produced . For example, *Aspergillus flavus*, *Aspergillus nidulans* and *Penicilium chryosegenum* are important species for penicilin production [6, 7, 9, 10].

1.1.1.2. Organic Acids

Organic acids are biological compounds having the building block role for various chemicals. These are used in food, chemical, agricultural and pharmaceutical industries. Major types of organic acids are mainly produced by microbial approaches. For example, *Aspergillus citricus* and *Trichoderma viride* are important species for citric acid production [6, 10, 11].

1.1.1.3. Enzymes

Filamentous fungi can be used in protein industry through being able to produce high quantity of proteins. By using filamentous fungi both native and heterologous proteins can be synthesized in large quantity. For example, *Aspergillus awomori* and *Penicilium funiculosum* are important species for β-Galactosidase production [6, 10, 12].

Forms of filamentous fungi can provide proper Post Translation Modifications (PTMs), important for heterologous proteins requiring PTMs for proper folding, prior to their secretion [6, 13].

1.1.2. Advantages and Disadvantages of of Industrial Fermentation with Filamentous Fungi

Filamentous fungi involving industrial production processes suffers due insufficient understanding of optimization of fungal morphology, which has great importance in viability and productivity. In filamentous fungi, growth as either pellet or filamentous morphology has been recorded to cause harmful stress in either way during industrial processes due to insufficient understanding of their morphogenesis. On one hand, pellet morphology causes core region area's oxygen or nutrient limitation due to insufficient mixing. On the other hand, filamentous morphology is observed as causing higher viscosity in their biomass broth leading to decreased oxygen transfer. Therefore, different optimizations of fungal morphology for every biotechnological process, due to specific product properties, are required. For example, morphology of filamentous fungi *Aspergillus niger* can be optimized via pH adjustment and volumetric power input [6, 14].

In a successful biotechnology-based industrial process, it is expected to have the ability of producing heterologous proteins (cellulase, amyloglucosidase, lipase etc.) at high productivity, yield and titer for a process to be economically feasible. Filamentous fungi provide advantage via secretion of protein products, resulting in cheaper downstream processing. Forms of filamentous fungi can provide proper [6, 13].

Fermentation processes being applied on filamentous fungi are important for affecting their productivity. Filamentous fungi fermentation can be performed in solid-state cultivation. This method is known for providing the best productivity for filamentous fungi. The method is important via mimicking the environment of these microorganisms. At larger scales of production, the method has the benefit of applying water-insoluble feed stocks such as agricultural wastes. Yet, scale-up process is challenging due to the difficulties in adjustments of vital problems such as overall pH, temperature, substrate, oxygen level etc. [6].

1.2. PSEUDOHPYHAL GROWTH IN *SACCHAROMYCES CEREVISIAE*

Filamentous fungi grow in either pellet or filamentous form depending on environmental, physiological and genetical characteristics. In dimorphic switch, pseudohyphae is the

middle point between pellet and true hyphae form. *S.cerevisiae,* mainly observed in pellet form, can grow into pseudohyphae. As visualized in Figure 1.2, it occurs as the combination of unipolar budding and morphological changes which results in structures stretching far away from the colony into unpopulated region [5, 15, 16].

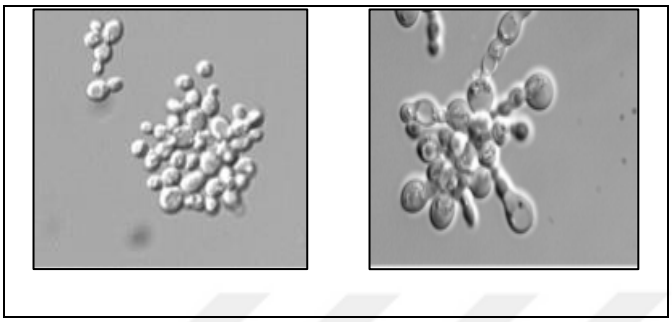

Figure 1.2. Forms of pellet (left) and pseudohpyhae (right) in *S.cerevisiae*

Pseudohyphal growth in *S.cerevisiae* is observed in both diploid and haploid strains. In diploid strains it is observed as extension of filaments on the surface of agar. In haploid strains, there is lesser extension of filaments. In both type of strains, there is invasive characteristics. In diploid strains, the filaments are more resistant to the extermination compared with haploid strains. Therefore, diploid strains provide higher invasiveness [17].

1.2.1. Biotechnological Importance of *Saccharomyces cerevisiae* in Optimizing Fungal Dimorphism

The behaviour of cell growth affects production of desired compounds and separation of soluble product separation from cellular structures. Therefore, controlled cellular morphology is a necessity for facilitated production processes [18].

Fungal dimorphism has been studied in many model species. *Saccharomyces cerevisiae* is known as one of the most common among the studies. It is known for a genetically suitable microorganism in order to carry out studies in fungal dimorphisms. The pathways and chemical processes in *S.cerevisiae* has already been identified which is important for integrating filamentous growth signals and physical manifestations occurred from these signals [18].

1.3. MECHANISMS AND DYNAMICS IN FILAMENTATION

1.3.1. Ergosterol Pathway and Fungal Filamentation

In the plasma membrane of eukaryotes, sterols are known as essential molecules for the assembly and functioning of whole structure. There are three predominant sterols found among eukaryotes named as cholesterol, phytosterols and ergosterol. In animals, cholesterol is known as main sterol compound. Cholesterol is involved with plasma membrane formation, synthesis of vitamin D, steroid hormones and bile acids. In plants, phytosterol is known as main sterol compound. It is essential for providing plant adaptation to various stress conditions (e.g. temperature and pH etc.) It has also similar properties with cholesterol which is being synthesized together with it. It is important marker for determination of fungal biomass. The three sterols have common initial steps of reaction for their biosynthesis which is the part acetyl-CoA (initial molecule) to squalene epoxide (last molecule). After this point of reaction, production of lanesterol becomes common between vertebrates and fungi in order to further produce cholesterol and Ergosterol [19, 20].

Ergosterol binds to membrane phospholipids for stabilizing membrane structure as well as regulating membrane fluidity, permeability, membrane-bound enzymes' activities and substrate transportation. Compared with cholesterol, ergosterol biosynthesis was observed as energetically inefficient. Yet, according to applied researches about terrestrial fungi, it was indicated that ergosterol is important for providing the mechanical resistance of fungal plasma membrane. Each cells having ergosterol as major sterol has been observed as containing rigid cell wall next to the plasma membrane. It is observed as providing their adaptation for protection against frequent fluctuation of humiditiy as well as its' resulting situation of oxidative stress due to lipid peroxidation [20, 21].

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For providing the intracellular balance of sterols, ergosterol is also stored in lipid droplets with steryl ester form in cytoplasm. During the fermentation process, fungal stress adaptation is essentially affected from ergosterol levels as well [19, 20].

Ergosterol is also a valuable product for pharmaceutical industry; it is used precursor for the production of vitamin D2 and steroid hormone drugs (cortisone, progesterone etc.). Besides, medical importance, produced vitamin D2 is also applied as feed additive for poultry supporting oviposition and hatching rate. Ergosterol biosynthesis pathway is a target for fungicidal drugs. Against fungal human infections, there are few drugs for treatment due to the difficulty in identification of unique targets not shared with human hosts. This situation led to the development of the inhibitors of ergosterol biosynthesis. The most common drug is fluconazole which is an azole used to inhibit the activity of lanosterol 14α-demethylase. Among the intermediate products of ergosterol biosynthesis, squalene is also a valuable product, a precursor for steroid biosynthesis. Furthermore, the molecule has similarity with β-carotene, coenzyme q10, vitamins K1, E, and D [20, 22, 23].

According to the intermediate end products the whole pathway can be divided into three modules:

1.3.1.1. Mevalonate Biosynthesis

The whole process is conserved among all eukaryotic species. First of all, two acetyl-CoA are condensed to produce acetoacetyl-CoA catalyzed by acetoacetyl-CoA thiolase (ERG10). The molecule is carried into the vacuole. The process is followed by condensation of third acetyl-CoA molecule into acetoacetyl-CoA to produce 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) catalyzed by hydroxymethylglutaryl-CoA synthase (ERG13). The finale molecule is carried into mitochondria, for reduction in to mevalonate by HMG-CoA reductases (HMG1 and HMG2). In Figure 1.3 module-1 the process was visualized [20].

1.3.1.2. Farnesyl Pyrophosphate Biosynthesis

The reaction in the whole process is carried out in vacuoles. First of all, mevalonate is phosphorylated two times by mevalonate kinase (ERG12) and Phosphomevalonate kinase (ERG8) to form mevalonate pyrophosphate (Mevalonic-5-PP). The process is followed by the decarboxylation of mevalonate pyrophosphate to produce isopentenyl pyrophosphate (isopentenyl-PP) catalyzed by Diphosphomevalonate decarboxylase (ERG19). Then, isopentenyl pyrophosphate is isomerized into dimethylallyl pyrophosphate catalyzed by Isopentenyl diphosphate isomerase (IDI1). Finally polyprenyl synthetase (ERG20) enzyme catalysis the two step reaction to produce farnesyl pyrophosphate. First of all, the enzyme is used to produce geranyl pyrophosphate. Then, the intermediate product geranyl pyrophosphate is used to produce farnesyl pyrophosphate. In Figure 1.3 module-2 the process was visualized [20].

1.3.1.3. Ergosterol Biosynthesis

The final process is the most complicated compared with the previous two modules. First of all two molecules of farnesyl pyrophosphate are used to produce squalene catalyzed by squalene synthase (ERG9). Squalene is then applied to epoxidation to produce squalene epoxide catalyzed by squalene epoxidase (ERG1). The reaction is followed by synthesis of lanesterol catalyzed by lanosterol cyclase/lanosterol synthase (ERG7). Lanesterol is demethylated by Cytochrome P450 lanosterol 14a-demethylase (ERG11) to produce 4,4-Dimethyl cholesta-8,12,24-trienol. The latter molecule is reduced into 4,4-Dimethylzymosterol by Sterol C-14 reductase (ERG24). 4,4-Dimethylzymosterol is applied to demethylation complex consisting of three different enzymes C4 sterol methyl oxidase (ERG25), sterol C-4 decarboxylase (ERG26) and C-3 ketoreductase (ERG27) to produce zymosterol. Then, zymosterol is used to produce fecosterol catalyzed by C-24 methyltransferase (ERG6). After that, fecosterol is isomerized into episterol by C-8 sterol isomerase (ERG2). Furthermore episterol is applied to desaturation by 3 different enzymes in a row which are C-5 sterol desaturase (ERG3), C-22 sterol desaturase (ERG4) and C24 (28) sterol reductase (ERG5) respectively to produce ergosterol. In Figure 1.3 module-3 the process was visualized [20].

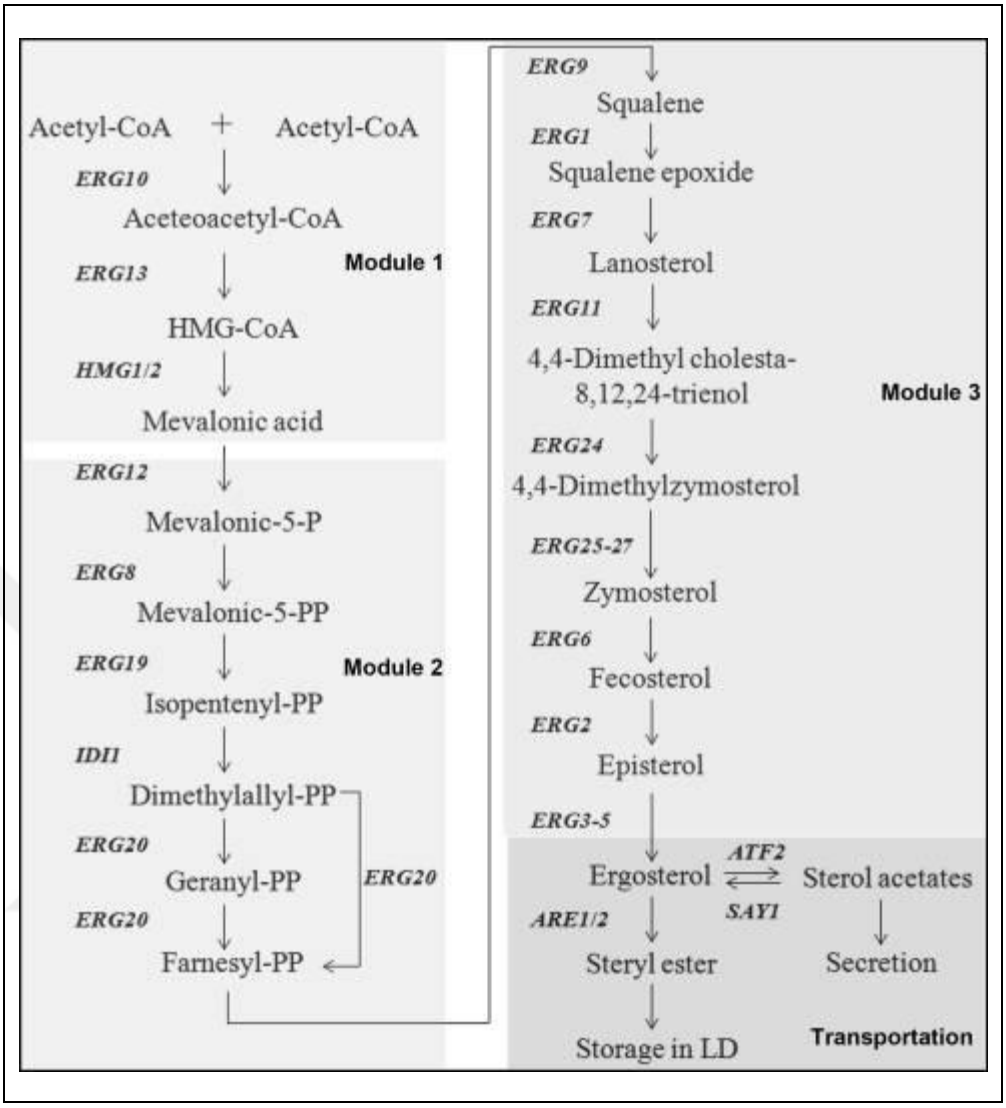


Figure 1.3. Coupled pathways of mevalonate, farnesyl-PP, ergosterol including storage-transportation processes of ergosterol [20]

1.4. KINETIC MODELLING IN INDUSTRIAL BIOTECHNOLOGY

In industrial biotechnology, cellular systems are being used in order to convert substrates into (value-added) products. Understanding the total functioning mechanism in cellular structures is one of the main challenges in biotechnology. The complexity is due to high number of interactions among these biomolecules. Mathematical modelling is one of the approaches to unravel the complexity of such systems [24].

Dynamic systems can be modeled considering rates of biochemical reactions in the system. These types of mathematical models, typically involving ordinary differential equations with time as the independent variable, are known as kinetic models [25]. They are used to represent the mechanisms of biological phenomena. Here, the dependent variables typically represent the state of system, e.g. metabolite concentration at a given time. For example, it is assumed that there is a bioreactor containing substrate and enzyme produces desired product. The substrate and product concentrations change as the time passes. Yet, the enzyme concentration remains constant [26].

1.4.1. Spatiotemporal Modelling of Hyphal Growth

Spatiotemporal models are used to describe process dynamics in both space and time, e.g. the hyphal growth. Such models would be valuable in visualizing the morphological differences [27].

Models are constructred starting from mass-balances. The chemical reaction network of these models are solved in the form of ordinary differential equations if it occurs in well-stirred volumes. Yet, in cellular structures, the environment is non-homogenous. Therefore, it changes depending on time and space. In this case, application of partial differential equations for the developed mass-balance equations is required (Equation 1.1) [28].

$$f\left(\nabla x, \frac{\partial x}{\partial t}, x, t, \gamma\right) = 0 \tag{1.1}$$

Where, x is a state (dependent) variable (e.g. concentration of a metabolite or biomass level), ∇x is change of the state variable over space ($\partial x/\partial$x,$\partial x/\partial$y and $\partial x/\partial$z), $\partial x/\partial t$ is the time-derivative of the x, and γ is the set of parameters including kinetic, physical (e.g. diffusion, proliferation) parameters.

To simulate the system, the resulting PDE need to be discretized. In thesis, method of lines is used to discretize the space and the resulting ODE (over time) has been solved [27].

1.5. LATTICE-BASED MODELLING

Among the studies of hpyhal growth modelling, development of discrete model are investigated under two sub groups named as lattice-based and lattice-free models [29].

In a lattice based model, formed hyphal units are limited as only to be able to exist in grid points which were interpreted as square lattice units in the thesis. In a lattice-free model there is no predetermined grid points. Therefore, growth is allowed to become boundless as it occurs [29, 30].

In Figure 1.4, simulations for both modelling approaches were provided. In the figure left, there is given an example of hyphal growth in heterogeneous environment including squared-obstacles. In the figure right, hyphal growth was visualized with a number of trophism consisting of positive and negative were provided in the model for affecting the whole growth [29, 31].

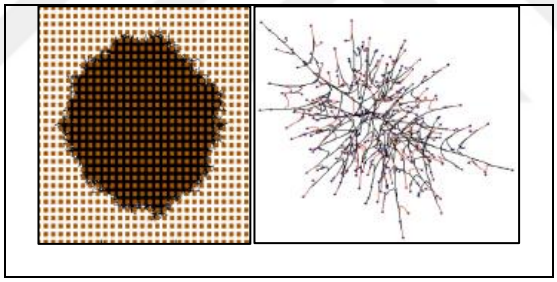


Figure 1.4. Comparison of lattice-based (left) and lattice-free models (right)

The main differences between both approaches are listed below:

- Lattice-based modelling is limited in the geometry in the growth process.
- Lattice-free modelling is computationally expensive

Among the studies of hyphal growth modelling, lattice-based approach has been generally applied. The facilitated model construction and computationally inexpensive simulations through regular geometric shapes are the reasons for its preferation. In this thesis, discretization of spatiotemporal model was done as lattice-based [29, 30].

1.6. SENSITIVITY ANALYSIS OF KINETIC MODELS

In development of metabolic models, the parameters are either calculated by using statistical techniques or estimated from the experimental data [32]. The sensitivities of paramater values for good models are expected not to be too large or too small. Too large sensitivities indicates large changes in the predictions of model that cannot be properly confirmed. Too small sensitivities make parameters of interest generate same (or very similar) predictions leading to problems in reliance of model. As for best model, small variance is being searched among candidate models [32, 33].

In case of kinetic models, metabolic control analysis is carried out for sensitivity analysis. It provides a quantitative approach for visualizing the relationship among connected biochemical reations under steady-state as well as an understanding of properties of these reactions [34]. In metabolic control analysis the overall aim is defining the bottleneck enzyme (rate-limiting step) in given metabolic pathway. For the case of fermentation processes, bottleneck enzymes are known as important as these are targets for increased productivity [35, 36].

1.7. AIM OF STUDY

The industrial fungi have pellet like growth and production capability of filamentous fungi. In order to maintain further this condition, understanding the dynamics of fungal growth and proliferation processes are important. In this area, there is not any available spatiotemporal models taking consideration of the effect of metabolism. In this study, development of spatiotemporal model of fungal growth under the effect of ergosterol pathway metabolism in *S. cerevisiae* was aimed.

2. MATERIALS AND METHODS

2.1. MATERIALS

The constructed model describes growth in filamentous and pellet forms, where growth in pellet form is considered as a function of ergosterol. The model is developed for simulation over time and space representing growth and proliferation during e.g. solid-state fermentation or fermentation on immobilized supports such as agar plates.

2.1.1. Ergosterol Pathway Model and Biomass Growth

The developed model starts from general Mass Balance Equation in a control volume, referred here as compartment:

$$\text{In} + \text{Generation} - \text{Out} - \text{Consumption} = \text{Accumulation} \quad (2.1)$$

Biomass grew at a given compartment and proliferates additionally to neighboring compartments. Metabolites, however, did not diffuse among compartments yet their levels change within one compartments. Therefore, the mass balance can be separately conceived for Biomass and Metabolites. **For Biomass**, the In/Out terms would represent the proliferation from/to adjoint compartments while generation term would represent the growth within a compartment. **For Metabolites**, the In/Out terms, representing the rates of metabolite inflow or outflow into/from a compartment and generation/consumption terms represent the production or consumption rates with corresponding biochemical reaction.

2.1.2. Toy Model with Hypothetical Reactions

Initially, a hypothetical model representing central carbon metabolism from glucose to acetate as well as sterol production from acetate to ergosterol was set up with hypothetical kinetic. The biomass growth was maintained to be a function of ergosterol. Consumption

rates of glucose, acetate and ergosterol were given as V1 (e.g. glycolysis), V2 (e.g. sterol production) and μ (growth) respectively.

$$\mathrm{Glucose} \xrightarrow{V1} \mathrm{Acetate} \xrightarrow{V2} \mathrm{Ergosterol} \xrightarrow{\mu} \mathrm{Biomass} \tag{2.2}$$

Hypothetical Biochemical Rate Equations are:

$$V1 = \frac{0.02 \cdot \mathrm{Glucose}}{\mathrm{Glucose} + 1} \tag{2.3}$$

$$V2 = \frac{0.008 \cdot \mathrm{Acetate}}{\mathrm{Acetate} + 4} \tag{2.4}$$

$$\mu = \frac{0.001 \cdot \mathrm{Ergosterol}}{\mathrm{Ergosterol} + 2} \tag{2.5}$$

For each reaction, the rates were defined as biomass-specific rates. The Mass Balances at a selected compartment (focusing on 2D surface, denoted with subscript i, j) become then:

$$\frac{d\mathrm{Glucose}_{ij}}{dt} = X_{ij} \cdot \left(-V1_{ij}\right) \tag{2.6}$$

$$\frac{d\mathrm{Acetate}_{ij}}{dt} = X_{ij} \cdot \left(V1_{ij} - V2_{ij}\right) \tag{2.7}$$

$$\frac{d\mathrm{Ergosterol}_{ij}}{dt} = X_{ij} \cdot \left(V2_{ij} - \mu_{ij}\right) \tag{2.8}$$

$$\frac{dX_{ij}}{dt} = X_{ij} \cdot \mu_{ij} + \sum k \nabla X_{ij} \tag{2.9}$$

In equation 2.9, the first term represented the growth within a compartment for pellet form, and second term represents the proliferation to other compartments. The proliferation was

assumed to be a function of biomass gradient over space multiplied by an arbitrarily defined proliferation coefficient $(k\nabla X_{ij})$. Numerically this was calculated using the difference between neighboring compartments.

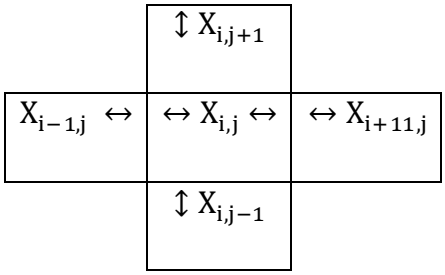


Figure 1.5. Representation of biomass(X) proliferation in neighbouring compartments

Equations 2.6 to 2.9 describes a spatio-termporal model of growth and proliferation incorporating metabolism into account. As such, a toy model that consumes Glucose and produce biomass via Ergosterol was developed.

2.1.3. Incorporating Ergosterol Pathway Model

To improve the model, a previously published biochemical model of Ergosterol pathway was integrated to the model backbone. The growth was maintained to be a function of Ergosterol.

Glucose and Ergosterol consumption rates were changed for new pathway incorporation:

$$V1' = \frac{2 \cdot \text{Glucose}}{\text{Glucose} + 1} \tag{2.10}$$

$$\mu = \frac{0.01 \cdot \text{Ergosterol}}{\text{Ergosterol} + 0.05} \tag{2.11}$$

In the following part, information over the applied equtions of consumption rates and visualization of ergosterol pathway of publishers were provided. In the pathway, applied part for model development was also marked [37].

Table 2.1 Consumption rate equations in Alvarez-Vasquez (AV) model

Metabolites	**Consumption Rate**
Internal Acetate	$V_{\text{Acetyl-CoA Synthetase}} = (0.009 \cdot X128^{0.2}) \cdot (X161^{0.2}) \cdot Ac \cdot X163$
Acetyl-CoA	$V_{\text{Thiolase/Synthase}} = (1.226 \cdot (AcCoa^{0.5}) \cdot X171$
HMG-CoA	$V_{\text{Hmg Reducastase}} = (0.105 \cdot 10^5 \ (HCoa) \cdot X172)$
Mevalonate	$V_{\text{Mevalonate Kinase}} = 0.97 \cdot 10^5 \cdot (Mev) \cdot X173$
Farnesyl-PP	$V_{\text{Squalene Synthase}} = 0.235 \cdot 10^5 \cdot (Fpp^{0.8}) \cdot X174$
Squalene	$V_{\text{Squalene Epoxidase}} = 0.74 \cdot 10^5 \cdot (Sqa^{0.8}) \cdot X175$
Lanosterol	$V_{\text{Lanosterol C14 Demetyhlase}} = 0.87 \cdot 10^8 \cdot (Lan^{0.8}) \cdot X176$
Zymosterol	$V_{\Delta 24-\text{Sterol C Methyltransferase}} = 0.124 \cdot 10^5 \cdot (Zym^{0.8}) \cdot X177$
Ergosterol-ER(μ)	$\mu = 0.01 \cdot Erg/(Erg + 0.05)$

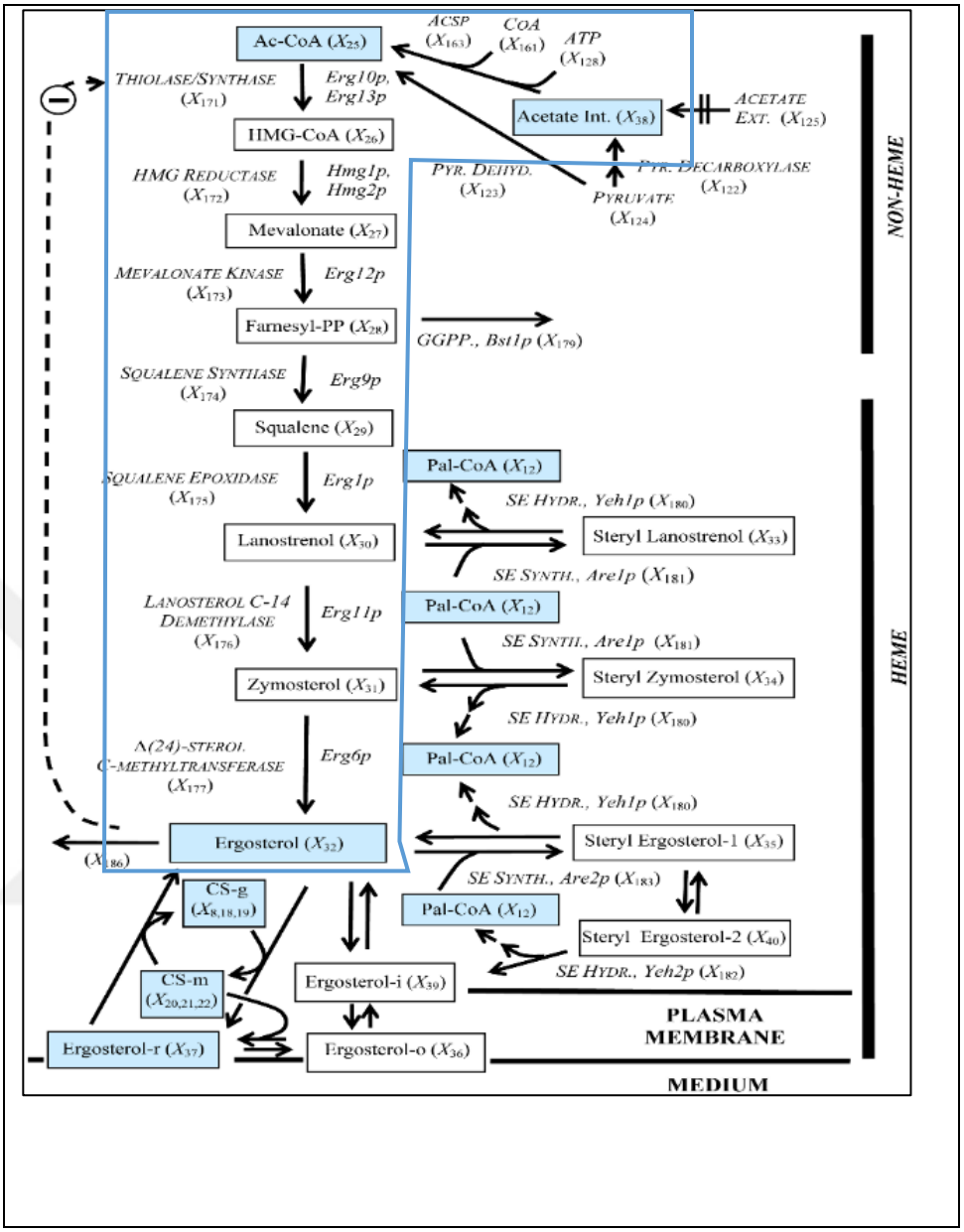


Figure 2.1. AV ergosterol pathway model [37]

2.2. METHODS

2.2.1. Solving PDE Form of Integrated Hyphal Growth and Metabolism via Approximation by Method of Lines

To solve the resulting system of PDE, method of lines was used which approximates PDEs into system of ODE's discretizing space, explicitely taking compartments into account

[38]. In all simulations, only two dimensions (x and y) were considered. With this approach the system was assumed as consisting of rows (i) and columns (j) forming square lattice units representing compartments that exchange biomass with each other. Proliferation was represented with a hypothetical value representing constant filamentation rate for the dispersion of hyphal structure. The compartments contained biomass both in pellet and filamentous form.

Two scenarios were simulated hpyhal and pellet growth:

(i). Formation of hyphal and pellet biomass at the sides:

- Biomass distributions at the all corner locations.
- Biomass distributions from all sides excluding their corner locations.

(ii). Formation of hyphal and pellet biomass emerging from inner regions of sides.

In the developed model, hyphal biomass growth under the activity of Ergosterol pathway metabolites was simulated. In order to assume solid-state fermentation process, square lattice units representing the "compartments" were assumed to be inside petri plate.

2.2.2. Effect of Inhibition in Pathway Model

End-product feedback inhibition is ubiquitous in metabolism. In AV pathway model, ergosterol inhibits consumption of acetyl-CoA. To mimic this in the Toy Model, inhibition of ergosterol for glucose consumption was additionally applied and the model is simulated in both inhibited and non-inhibited forms.

$$V1_{inhb,ij} = \frac{0.02 \cdot Glucose_{ij}}{Glucose_{ij} + \left(1 + \frac{Erg_{ij}}{0.3}\right)} \tag{2.12}$$

Furthermore, same inhibition was also applied in AV Model for consistency:

$$V1_{inhb,ij} = \frac{2 \cdot Glucose_{ij}}{Glucose_{ij} + \left(1 + \frac{Erg_{ij}}{0.3}\right)} \tag{2.13}$$

$$\left.\frac{\mathrm{dGlucose_{ij}}}{\mathrm{dt}}\right|_{\mathrm{inhb}} = \mathrm{X_{ij}} \cdot (-\mathrm{V1_{inhb,ij}}) \tag{2.14}$$

2.2.3. Effect of Filamentation Rate during Hyphal Growth

With various filamentation rate, biomass of pellet and filamentous form was expected to be distributed to certain regions. To simulate this, the filamentation rates were changed. For the clarification in results, different filamentation rates were applied to the models. For toy model filamentation rates $(\mathrm{k} = 0.001, 0.0025, 0.005, 0.01)$ were applied. For AV pathway model, filamentation rates $(\mathrm{k} = 0.001, 0.005, 0.025, 0.080)$ were applied. Each model were simulated under the competitive inhibition activity of ergosterol over glucose.

3. RESULTS

3.1. BASE CASE SCENARIOS OF MODELS

First of all, simulations without any inhibition were executed to visualize the events. In Figure 3.1. and Figure 3.2. simulations representing the whole events of consumption and production processes were visualized for toy model and AV model respectively. Depending on the rates of visualizing changes in each model, different timespans were applied.

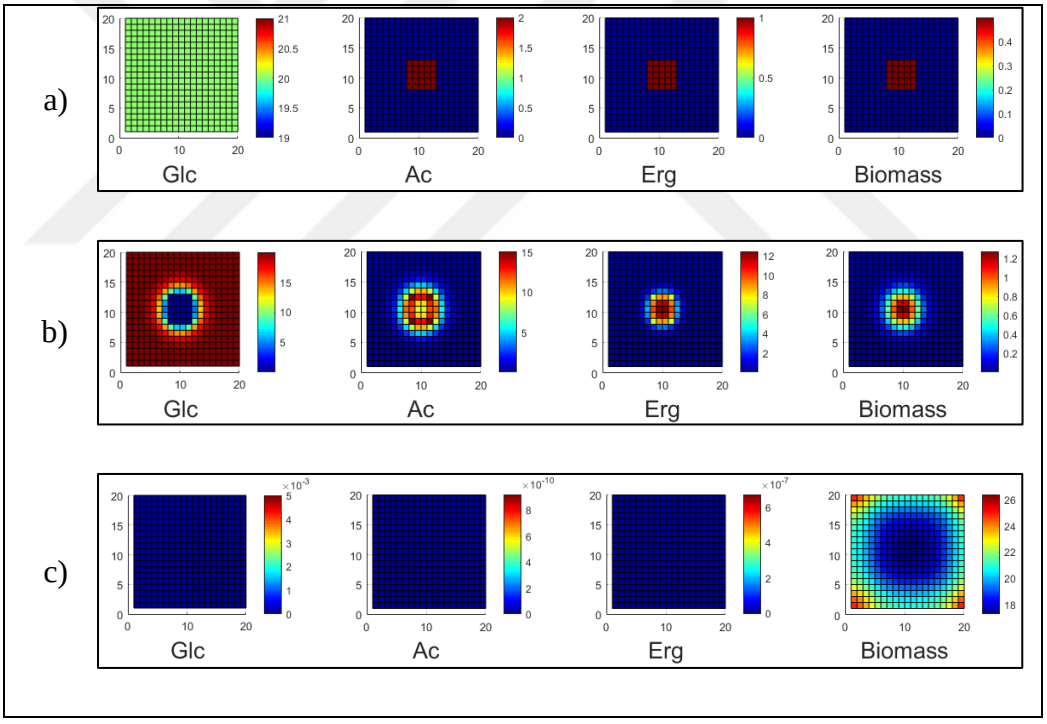


Figure 3.1. Base case scenario simulations of toy model (a-time=0, b-time=3000, c-time=15000)

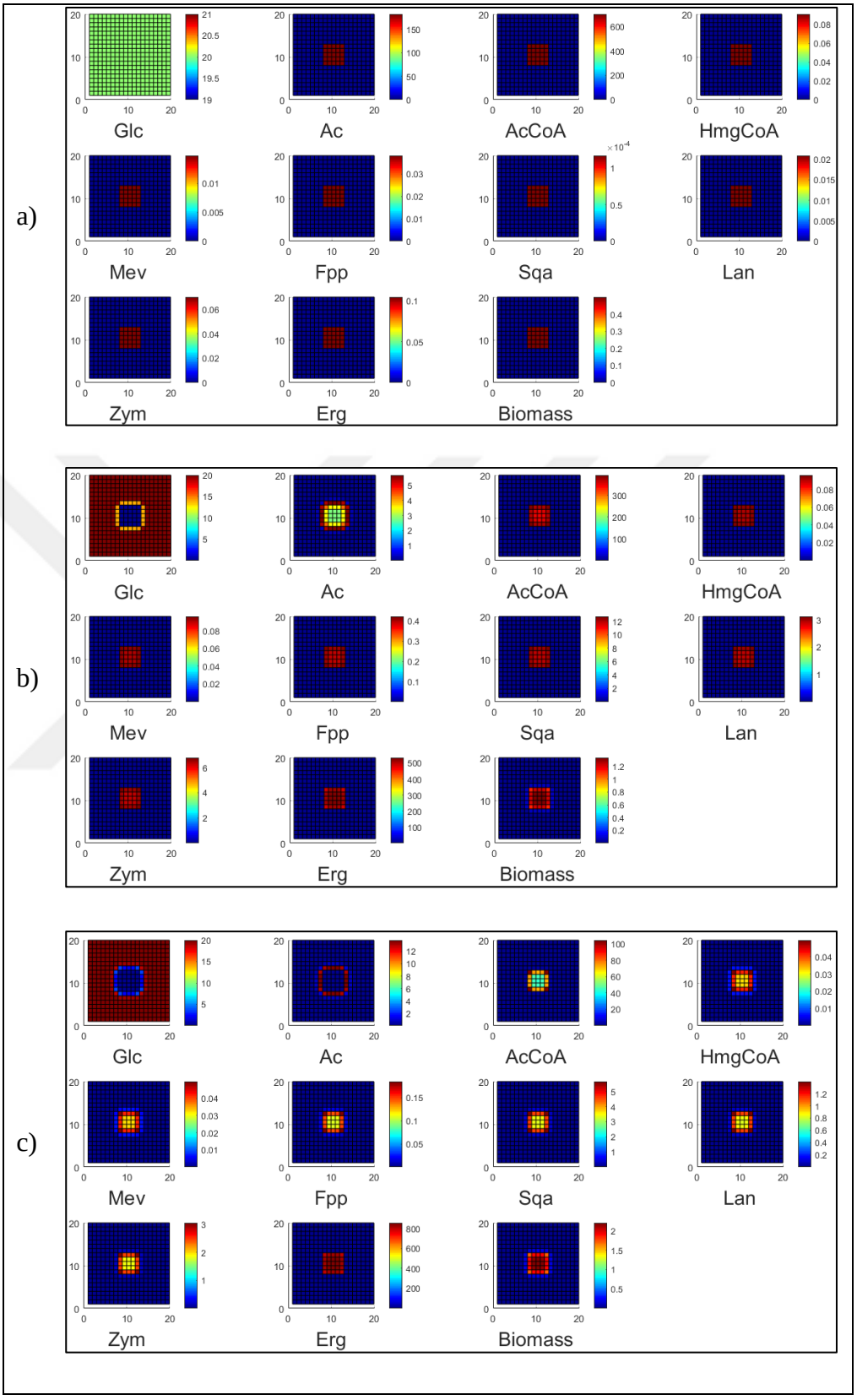


Figure 3.2. Base case scenario simulations of AV model (a-time=0, b-time=100, c-time=150)

3.2. EFFECT OF INHIBITION IN PATHWAY MODEL

Furthermore, effect of competitive inhibition of ergosterol over the consumption of glucose was simulated for visualizing any changes. In Figure 3.3. and Figure 3.4. simulations were compared under base case scenario and inhibition for toy model and AV model respectively.

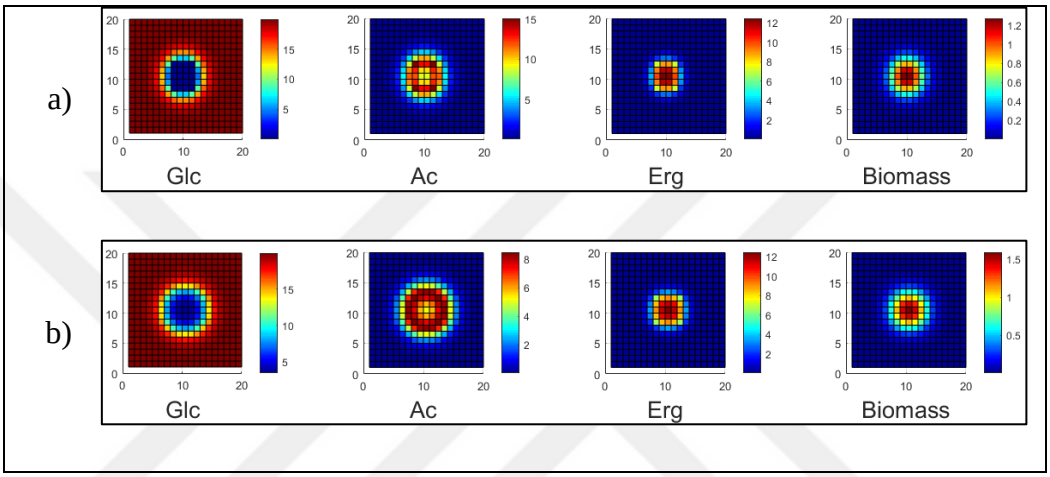
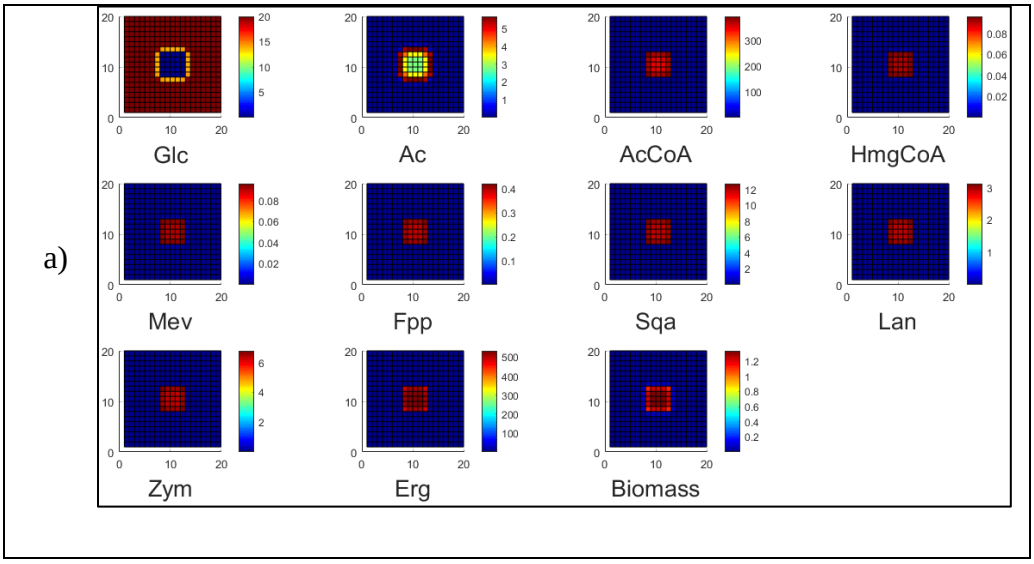


Figure 3.3. Simulations of toy model without (a) / with(b) inhibition



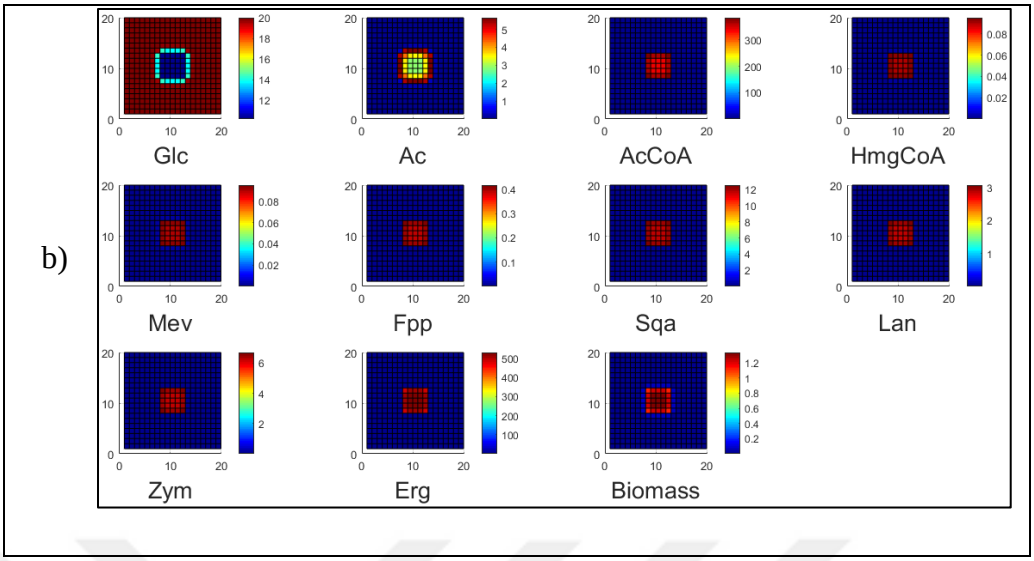


Figure 3.4. Simulations of AV model without (a) / with(b) inhibition

Lastly, to observe the effect of competitive inhibition, a single point in space (grid point denoted in Figure 3.5. and Figure 3.6.) is simulated over time for toy model (Figure 3.5.) and the AV model (Figure 3.6.).

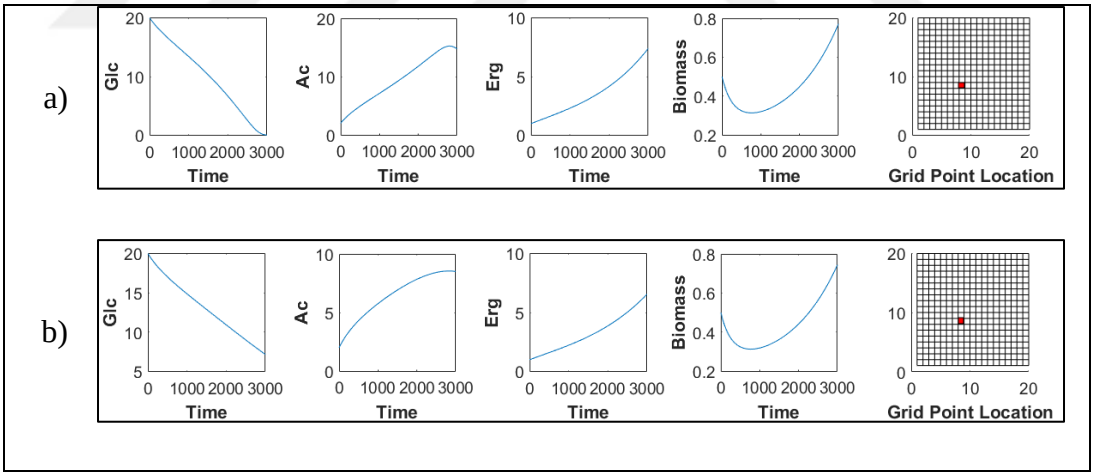


Figure 3.5. Effect of ergosterol inhibition on glucose uptake, toy model (without (a) \ with (b) inhibition)

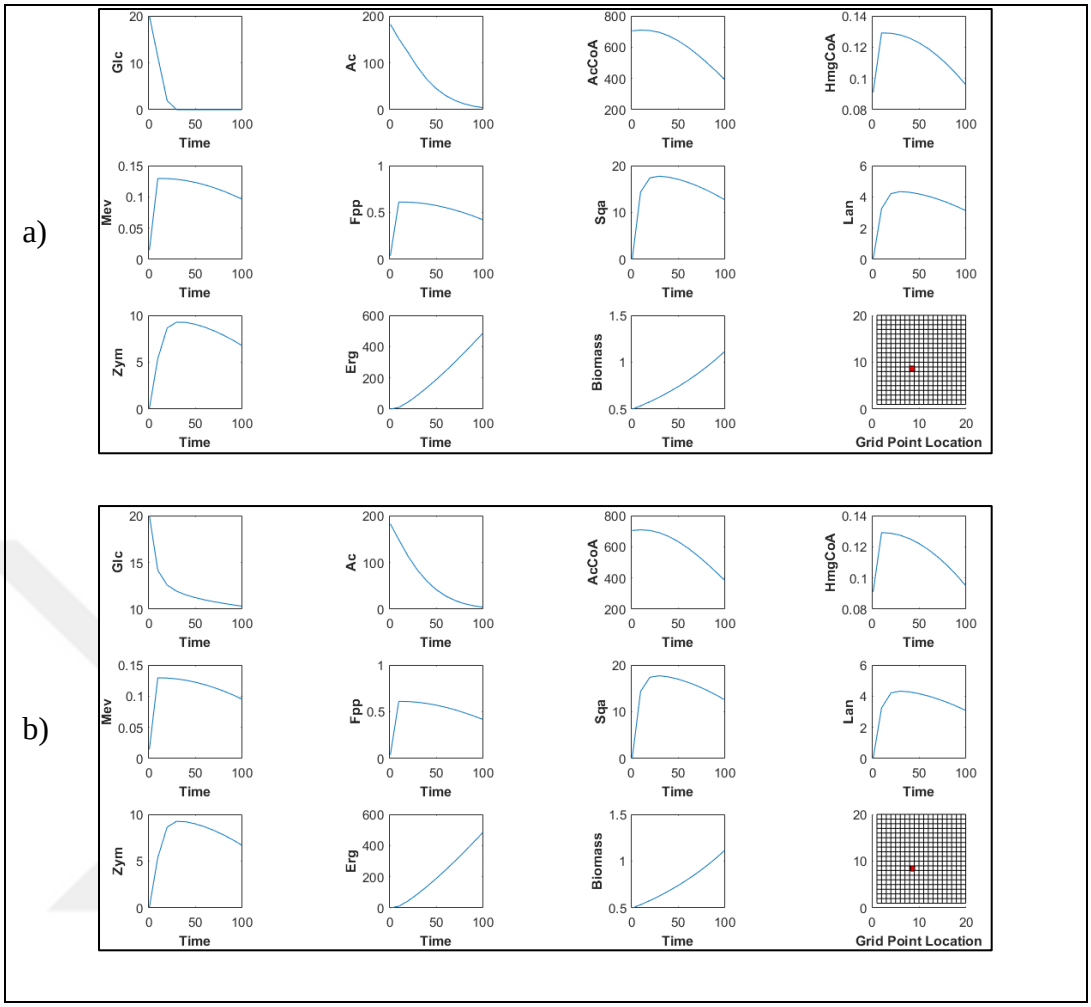


Figure 3.6. Effect of ergosterol inhibition on glucose uptake, AV model (without (a) \ with (b) inhibition)

3.3. EFFECT OF FILAMENTATION RATE DURING HYPHAL GROWTH

After that, effect of change in proliferation was simulated in models. In Figure 3.7. and Figure 3.8. simulations were prepared under different filamentation rate values for competitively inhibited toy model and AV model respectively.

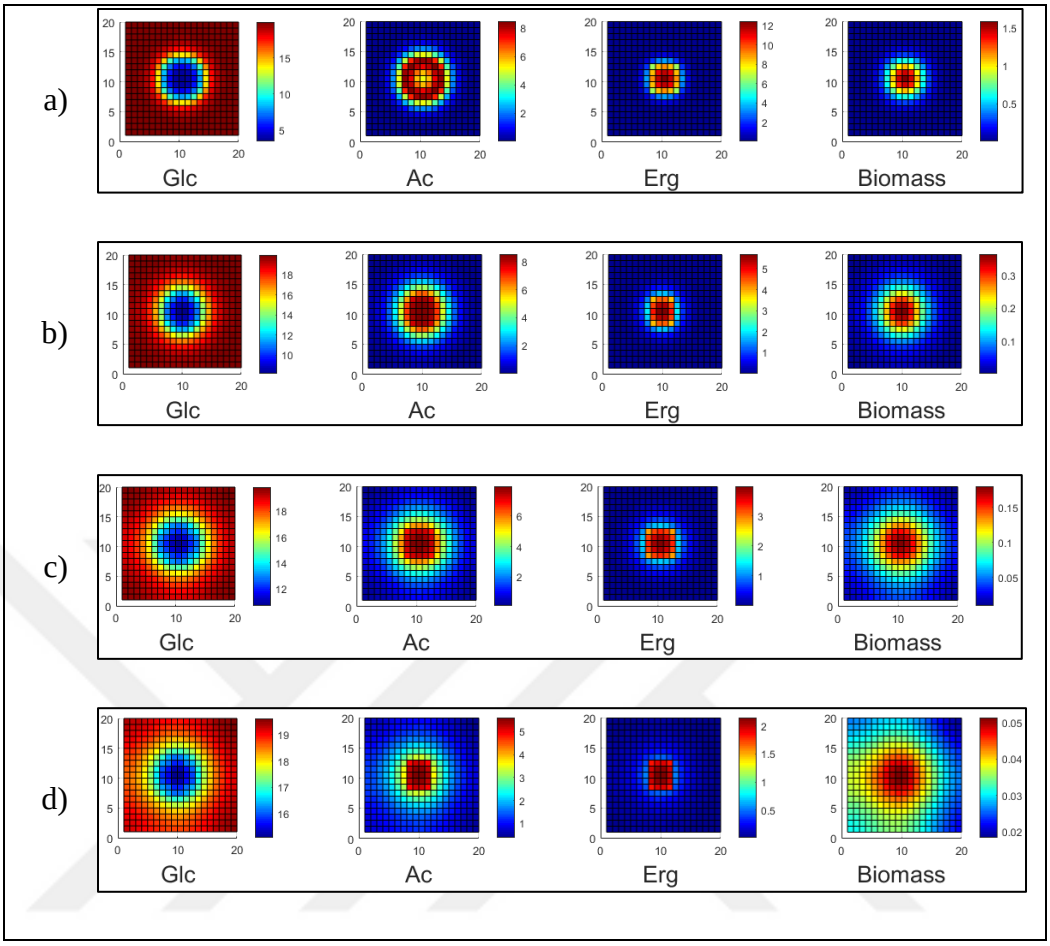
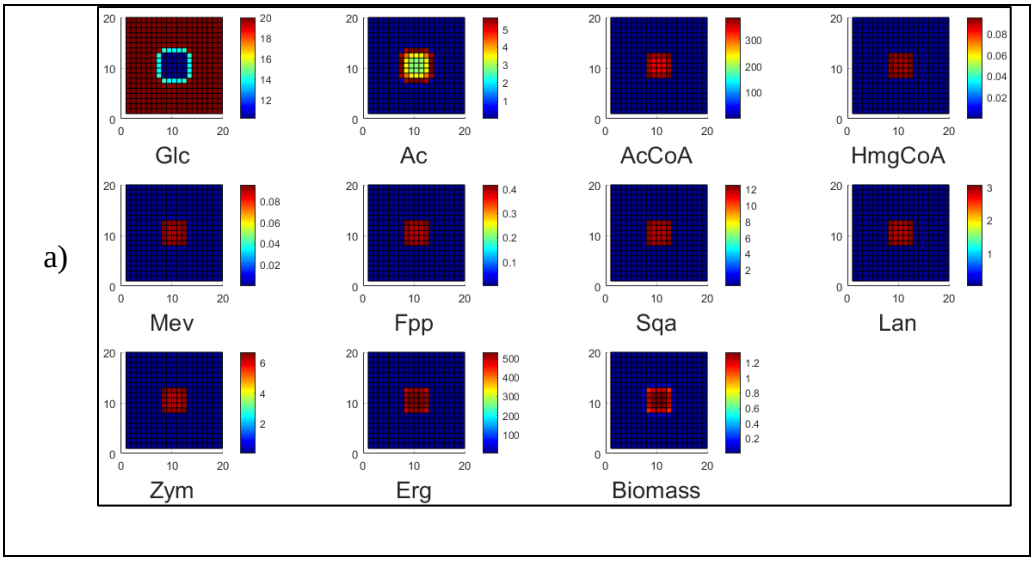


Figure 3.7. Simulations of toy model under different filamentation rates (0.001 for a, 0.0025 for b, 0.005 for c and 0.01 for d)



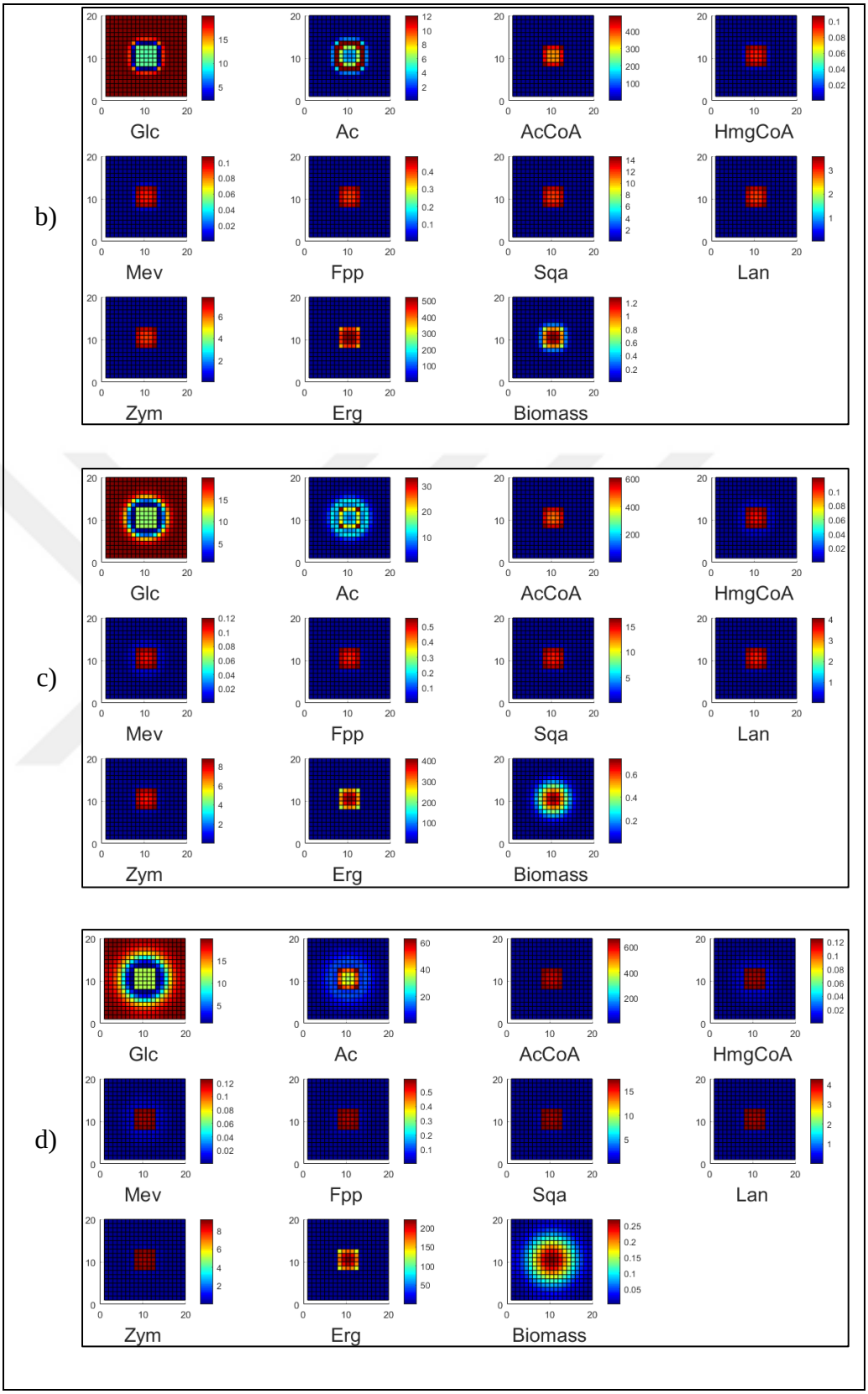


Figure 3.8. Simulations of AV model under different filamentation rates (0.001 for a, 0.005 for b, 0.025 for c and 0.080 for d)

4. DISCUSSION

This study presents a base-case scenario for simulating growth, production and proliferation over non-stirred (e.g. solid-state) fermentations and can be used for e.g. scaling-up such a highly complex system. Therefore, development of simple mathematical model is important for gaining insights for understanding. As such, the model provides a backbone in studies for further mathematical models incorporating incrementally metabolism or interactions among cells (via e.g. quorum sensing).

Previously, Nopharatana et al. developed a fungal growth on solid surfaces (polycarbonated membrane). In this study and the work of Nopharatana et al., penetration to the underground region was neglected. In both research glucose was added into the system during filamentation process. In their research, glucose was directly bound to the filamentation while in thesis, glucose was metabolized into ergosterol, thereby taking metabolism into account. The pathway presumably affected hpyhal growth and ergosterol consumption was adjusted as having the main effect for growth process. Besides, competitive inhibition of glucose consumption by ergosterol concentration was also applied. Therefore, the model developed in this thesis could be interpreted as having more detailed approach for bringing in glucose to hyphal growth in case of pathway information. In the work of Nopharatana et al, fungal biomass growth consisted of hyphal tip extension and branching processes, while only hyphal tip growth was used without any branching in this thesis. The spatial differences in biomass affected to the concentration of metabolites [39].

4.1. BASE CASE SCENARIOS OF MODELS

The simulations of toy model and AV model were executed without any inhibition activities.

In Figure 3.1., toy model simulations were provided. In toy model, increase in time initially resulted with consumption of glucose leading to increase in acetate, ergosterol and biomass. Further simulating in longer timespan, led to the total consumption of all metabolites. During the simulations positive effect of ergosterol over biomass dispersion was observed through integrating change in the metabolite to the growth equations.

In Figure 3.2., AV model simulations were provided. In AV model, increase in time resulted with consumption of all metabolites leading to accumulation in ergosterol and increase in biomass value. In the final timespan, acetate was observed as accumulating while consumption was observed for the previous timespan values. From the toy model, biomass growth was adapted into AV model, thus change in ergosterol level was positively affected biomass dispersion.

4.2. EFFECT OF INHIBITION IN PATHWAY MODEL

In each model, competitive inhibition of glucose consumption by ergosterol concentration was applied. The method was an example of product inhibition which was chosen through being a common occasion for many biological pathways.

In Figure 3.3., simulations of toy model were provided. In toy model, inhibition effect was clearly observed in all metabolites. According to the scale of values, consumption of glucose and production of ergosterol were decreased while acetate production was increased under inhibition.

In Figure 3.4., simulations of AV model were provided. In AV model, changes were observed only in glucose as being more conserved under inhibition. For the rest of metabolites and biomass the change was occurred at the rounding sides of concentration values, but they were too faint to be clearly observed.

For each model, grid points were compared as between inoculum and outer region, two points in inoculum and middle points of inoculum with/without inhibition.

In Figure 3.5., effect of inhibition on glucose uptake was visualized in toy model. In base case scenario, glucose was completely consumed while its consumption reached to steady-state under inhibition. Besides, acetate, ergosterol and biomass productions were more compared with inhibited toy model.

In Figure 3.6., effect of inhibition on glucose uptake was visualized in AV model. In base case scenario, glucose was completely consumed while its consumption reached to steady-state under inhibition. Compared with toy model, the rest of metabolites and biomass production was not observed as changing under inhibition.

4.3. EFFECT OF FILAMENTATION RATE DURING HYPHAL GROWTH

With the application of differentiating filamentation rates, different biomass patterns were observed.

In Figure 3.7. and Figure 3.8., toy model and AV model were simulated under different filamentation rates. In both models, with the increase in filamentation rate, consumption and production of metabolites were observed as much more distributed to the hypothetical area.

The increasing presence of ergosterol over the hypothetical area led to the biomass growth in covering region. The increase in filamentation rate affected the dispersion of metabolite leading to more dispersion in biomass.

4.4. LIMITATIONS IN THESIS MODEL

AV model equations were too stiff to be applied in the development of spatiotemporal model. Therefore, we had to change the variables of the applied equations. With this reason, the results may not be accurate.

Biomass growth was provided in lattice modelling which was beneficial for simplification of growth process through discretization. Yet, predefined geometry of hyphae formation may lead to decrease in accuracy of calculations. For better predictions in hyphal growth, a discrete model should be lattice free [29].

5. CONCLUSION

In this thesis, we developed a spatiotemporal model describing growth and proliferation in petri plate mimicking solid-state fermentation. In Base Case Scenario, the simulations were similar to the experimental events that under consumption of metabolites leading to fungal biomass proliferation to the covering region where the nutrients are available. In this model, effect of ergosterol in biomass growth was visualized. In model development, biomass production was consisted of equations providing growth and proliferation. Biomass growth was executed through integrating ergosterol consumption. In biomass proliferation, biomass value was dispersed to the hypothetical area with the effect of a value representing filamentation rate. Thus, the fungal growth simulation was similar to the biomass production in filamentous and pellet forms depending on the effect of metabolism and surrounding nutrients. Yet, the inhibition event was not properly integrated into the spatiotemporal model. The competitive inhibition of ergosterol over glucose consumption was properly visualized in toy model. Yet, in AV model, only glucose consumption was visually affected. For future studies, application of inhibition could be improved for visualizing more accurate outcomes.

6. FUTURE WORK

The problem of growth form in fungi has important impact over the production of desired substances, utilization of growth medium and proper separation of biomass from these desired products. For the future studies of fungal morphology optimization, thesis model could be an useful starting point [18].

Ergosterol pathway includes industrially important metabolites. Squalene is an intermediate metabolite in ergosterol pathway having widespread applications In human studies, it was found out as important in development of skin care, anticancer, drug delivery, detoxifying and disinfectant agents. It has been mainly extracted from shark liver oil,but due to ethical issues limitations have been occuring. In this situation, thesis model could be used for development of microbial strains for squalene production [21].

Thesis model can also be used for studying membrane integrity in fungal fermentation strains. Ergosterol is sterol compound found in plasma membrane. Ergosterol has been found out as providing protecting against environmental strees in large fermentation events. This situation is mainly observed as ethanol tolerance. For example, in wine fermentation studies, it was observed that *S. cerevisiae* was exposed high concentration of ethanol. In this case, ergosterol was observed as maintaining the plasma membrane integrity by affecting the permeability to the ethanol. According to another study based on modelling biomembranes, *S. cerevisiae* increases the ergosterol and unsaturated lipids in plasma membrane in order to maintain the optimal thickness for protection against ethanol toxicity. Therefore, thesis model could be used for the development of membrane integrity models [40, 41].

Experimental growth by using *A. niger* was also carried out. In Figure 4.1, inocula at the central and side regions of petri plate were applied and cell growth and proliferation was followed for 4 days. During the growth process, distinct shapes emergerging from the inocula regions were observed. Depending on the environmental conditions cellular relationships could result with various shapes of growths. This behaviour of cells could be explained by using computer based growth models.

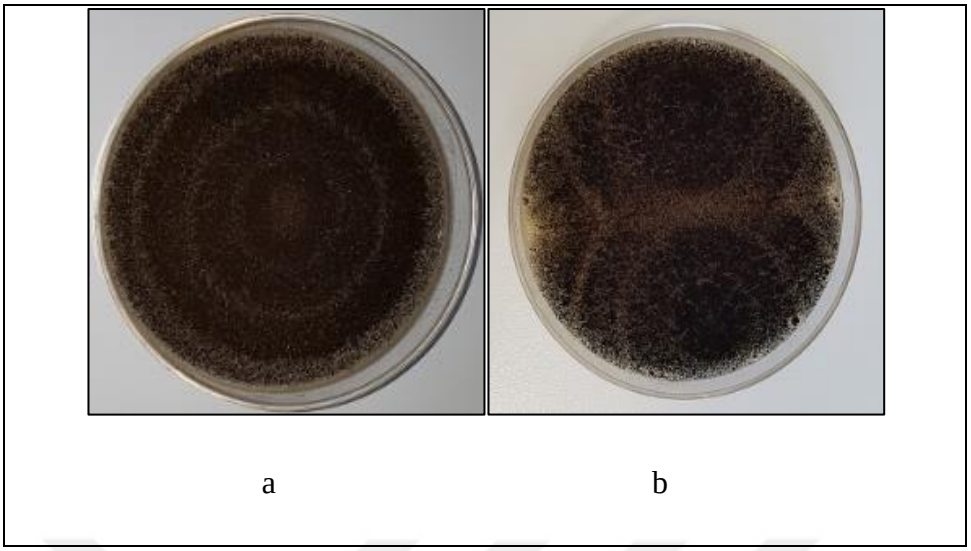


Figure 4.1. *A. niger* growth in petri plate at central region (a) and at side regions (b)

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APPENDIX A: DESCRIPTIONS OF EQUATIONS

Equation (1.1) General form of partial differential equation

Equation (2.1) General form of Mass-Balance

Equation (2.2) Hypothetical pathway model

Equation (2.3) Hypothetical glucose consumption rate in the model

Equation (2.4) Hypothetical acetate consumption rate in the model

Equation (2.5) Hypothetical ergosterol consumption rate in the model

Equation (2.6) Hypothetical glucose Mass-Balance in the model

Equation (2.7) Hypothetical acetate Mass-Balance in the model

Equation (2.8) Hypothetical ergosterol Mass-Balance in the model

Equation (2.9) Hypothetical equation of pellet growth and proliferation

Equation (2.10) Changed glucose consumption rate for AV model

Equation (2.11) Changed ergosterol consumption rate for AV model

Equation (2.12) Hypothetical glucose consumption rate with inhibition by ergosterol in the model

Equation (2.13) Changed glucose consumption rate with inhibition by ergosterol for AV model

Equation (2.14) Hypothetical glucose Mass-Balance with inhibition by ergosterol in the model