Genome-Scale Modeling of Microbial Metabolism with Temporal and Spatial Resolution

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Abstract

Most natural microbial systems have evolved to function in environments with temporal and spatial variations. A major limitation to understanding such complex systems is the lack of mathematical modeling frameworks that connect the genomes of individual species and temporal and spatial variations in the environment to system behavior. The goal of this review is to introduce the emerging field of spatiotemporal metabolic modeling based on genome-scale reconstructions of microbial metabolism. The extension of flux balance analysis to account for both temporal and spatial variations in the environment is termed *Spatiotemporal Flux Balance Analysis (SFBA)*. Following a brief overview of flux balance analysis and its established dynamic extension, the SFBA problem is introduced and recent progress is described. Three case studies are reviewed to illustrate the current state-of-the-art and possible future research directions are outlined. The author posits that SFBA is the next frontier for microbial metabolic modeling and a rapid increase in methods development and system applications is anticipated.

Introduction

Metabolic flux balance analysis (FBA)

Whole cell metabolic modeling is highly challenging due to the large-scale and interconnected nature of microbial metabolic pathways. Rigorous modeling of metabolism requires descriptions of enzyme synthesis, kinetics and regulation at the individual reaction level. With the notable exception of primary metabolic pathways in model organisms [1–3], the development of such kinetic models is currently impractical due to lack of *in vivo* data on enzyme kinetics and regulation. As a result, a less detailed modeling approach commonly termed *Flux Balance Analysis (FBA)* based purely on metabolite mass conservation and reaction stoichiometry has emerged as the dominant methodology for describing whole cell metabolism [4, 5]. A stoichiometric model can be constructed by incorporating reactions derived from an annotated genome and augmented with additional "gap filling" reactions required to complete otherwise incomplete pathways [6–9]. Due to the key role of the annotated genome in reaction identification, the resulting model is usually called a *Genome-*

scale Metabolic Reconstruction (GSM). GSMs are now available for dozens of microbial organisms [10–12], and new GSMs are published on a monthly basis.

Given the stoichiometric matrix and a specified set of available nutrient uptake rates, the goal is to solve the GSM for the unknown intracellular reaction rates (*i.e.* intracellular fluxes) and the metabolic byproduct secretion rates (*i.e.* secretion fluxes). Because the stoichiometric equations invariably contain more unknown fluxes than mass balance equations, the GSM has an infinite number of solutions and the flux distribution cannot be uniquely determined. To overcome this limitation, cell metabolism is assumed to be regulated to achieve some type of cellular objective [13, 14]. The most common objective is maximal growth, which requires the incorporation of a biomass stoichiometric equation in terms of biomass precursors (*e.g.* RNA, DNA, proteins, carbohydrates, etc.) [15]. The combination of the stoichiometric equations and the growth rate maximization objective produces a linear programming (LP) problem, which can be efficiently solved to generate predictions of the intracellular flux distribution, uptake and secretion (exchange) fluxes and the growth rate (Figure 1). FBA and other so-called constraint-based computational tools [16, 17] have been widely used to analyze the metabolism of both wild-type [18–22] and engineered [23–27] microbial strains.

Dynamic flux balance analysis (DFBA)

FBA is based on assumptions that both intracellular metabolism and the extracellular environment are time invariant. While removal of the intracellular steady-state assumption requires the incorporation of enzyme kinetics, the steady-state assumption on the extracellular environment is more easily relaxed through an extension of FBA commonly termed *Dynamic Flux Balance Analysis (DFBA)* [28, 29]. A DFBA model is formulated by combining a GSM of intracellular metabolism with kinetic expressions for the uptake rates of growth limiting nutrients and dynamic mass balance equations for cellular biomass, limiting nutrients and secreted metabolic byproducts under the assumption that cells rapidly equilibrate to environmental changes (Figure 2). While difficult to verify, this assumption has been accepted for many systems including batch biochemical reactors where nutrient concentrations varying due to cellular consumption and possibly nutrient feeding [29]. A major advantage of DFBA is that the model outputs include biomass and extracellular metabolite concentrations rather than just the growth rate and exchange fluxes as in FBA [30]. Furthermore, the extracellular concentrations as well as intracellular fluxes are predicted with temporal resolution.

A DFBA model is comprised of ordinary differential equations (ODEs) describing the extracellular environment and a LP describing intracellular metabolism. As compared to FBA, a notable disadvantage of DFBA is the computational complexity of this hybrid ODE/LP system. Initial attempts to solve DFBA models were based on sequential strategies in which the LP was repeatedly solved according to a prespecified time step and the ODEs were integrated between time steps using the most current LP solution [28, 31]. Due to the unpredictable accuracy and stability of these sequential methods, more current solution techniques are typically based on simultaneous strategies in which the LP is embedded with the ODE solution [32, 33]. DFBA has emerged as a standard tool for the analysis [34–39]

and optimization [32, 40–42] of individual species as well as microbial communities [43–45] in dynamic environments. While some DFBA models can be effectively solved by a straightforward combination of ODE and LP solvers, more advanced techniques may be required due to complications associated with the LP unpredictably becoming infeasible or producing alternative optima characterized by non-unique exchange fluxes. Sophisticated simulation methods that explicitly address and effectively overcome these complications are now available [46, 47].

Spatiotemporal Flux Balance Analysis (SFBA)

While DFBA accounts for the effects of extracellular dynamics on intracellular metabolism, the method is based on the assumption that the extracellular environment is well mixed and spatially homogeneous. Many engineered microbial systems such as biochemical reactors are carefully designed to achieve spatial homogeneity through liquid mixing [48]. However, most naturally occurring microbial systems exist in spatially heterogeneous environments that also exhibit time variations. The presence of spatial heterogeneity plays an essential role in the evolution and function of natural microbial species [49–52]. Perhaps the most common and important example of spatially heterogeneous environments is that established due to biofilm formation and development (Figure 3a) [53–56]. Concentration gradients in key nutrients due to limited diffusion establish unique metabolic niches within the biofilm that produce spatial variations in biomass density in the case of single species biofilms [57] and additionally spatial partitioning of species in the case of biofilm consortia [53]. The development of metabolic models that capture such spatial and temporal variations is important to analyze and manipulate complex microbial systems.

Model formulation

Mathematical models that account for both spatial and temporal variations are commonly termed spatiotemporal models [58, 59]. Therefore, we refer to the extension of DFBA to include spatial heterogeneity in the environment as *Spatiotemporal Flux Balance Analysis (SFBA)*. The author believes that SFBA is the next frontier for microbial metabolic modeling and that the nascent activities to date will soon be followed by a rapid increase in methods development and system applications.

A SFBA model is formulated by replacing the time-varying ODEs in DFBA with partial differential equations (PDEs) expressed in terms of time and some spatial coordinate(s) as independent variables [60]. The PDEs represent extracellular mass balance equations for biomass, metabolite and possibly other chemical species concentrations and account for the transport mechanisms that induce the spatial variations, which commonly include metabolite diffusion and liquid/gas phase convection. Boundary conditions must be imposed at the boundaries of the spatial domain to ensure that the PDEs are well posed. As in DFBA, the GSM and the extracellular mass balance equations are linked through nutrient uptake kinetics.

Some systems such as microbial biofilms involve mixed boundary conditions when nutrients enter and/or byproducts exit the biofilm at different locations [60, 61]. Furthermore, the spatial domain may shrink and/or grow with time due to cellular growth and/or death [62].

For the sake of illustration, consider a single species biofilm (Figure 3b) with a fixed thickness L in which a single growth limiting nutrient is available at the bottom of the biofilm ($z = 0$) and a single synthesized byproduct exits the top of the biofilm ($z = L$). The PDEs describing biofilm diffusional processes can be written as follows assuming that spatial variations occur only in the axial direction z of the biofilm,

$$\frac{\partial X(z,t)}{\partial t} = \mu X \qquad \frac{\partial X(0,t)}{\partial z} = 0 \qquad \frac{\partial X(L,t)}{\partial z} = 0 \qquad X(z,0) = X_I$$

$$\frac{\partial S(z,t)}{\partial t} = v_S X + D_S \frac{\partial^2 S}{\partial z^2} \qquad S(0,t) = S_0 \qquad \frac{\partial S(L,t)}{\partial z} = 0 \qquad S(z,0) = S_I$$

$$\frac{\partial P(z,t)}{\partial t} = v_P X + D_P \frac{\partial^2 P}{\partial z^2} \qquad \frac{\partial P(0,t)}{\partial z} = 0 \qquad P(z,t) = 0 \qquad P(z,0) = P_I \qquad (1)$$

Here $X(z, t)$, $S(z, t)$ and $P(z, t)$ represent the biomass, substrate and byproduct concentrations at location z and time t. The growth rate μ, substrate uptake rate v_S and byproduct synthesis rate v_P are obtained by solution of the GSM. The substrate and byproduct diffuse through the biofilm with efficient diffusion coefficients D_S and D_P, respectively, while the biomass is assumed to be non-motile. The second column lists the boundary conditions at the bottom of the biofilm, which are based on the assumptions that substrate is available at a concentration S_0 and the biomass and byproduct do not flux across this boundary. The boundary conditions at the top of the biofilm listed in the third column are based on the assumptions that the biomass and substrate do not flux across this boundary while the byproduct removal rate is sufficiently high that the boundary concentration is zero. Finally, the initial conditions in the fourth column are based on the assumption that the biofilm is spatially uniform at $t = 0$.

Model solution

A SFBA model is comprised of PDEs describing the extracellular environment and LPs describing intracellular metabolism [60]. Pure species systems have a single LP [63], while multispecies systems require solution of a LP for each species [64]. Because no computational methods exist to directly solve such hybrid PDE/LP models, the PDE transport behavior must be approximated in some manner to generate a solvable model. To date, three alternative methods have been proposed for implementing this approximation: (M1) table lookups of computed FBA solutions combined with integration of the PDEs on a coarse spatial grid [61, 63, 65]; (M2) real-time FBA solution combined with lattice-based descriptions of metabolite transport [62, 64]; and (M3) spatial discretization of the PDEs followed by time integration of the resulting ODE/LP system (Figure 4) [60, 66]. These methods differ according to whether the LP solutions are precomputed (M1) or generated in real-time (M2, M3) and whether the PDEs are discretized directly (M1, M3) or approximated indirectly (M2). While exhaustive studies of these methods have not yet been performed, the most appropriate method is very likely to be problem dependent.

Representative studies

To illustrate the current state-of-the-art, three representative studies that represent the breadth of the spatiotemporal modeling methods described above are reviewed. In the first study [61], method M1 was used to study electricity generation in a microbial fuel cell where the anaerobic bacterium *Geobacter sulfurreducens* formed a biofilm on the anode. A

metabolic model was developed to analyze the biofilm that oxidized acetate (the electron donor) to carbon dioxide with electron transfer to Fe(III) (the electron acceptor) mimicking the anode. Cellular growth was assumed to create an axial velocity that drove biofilm expansion and along with acetate diffusion created spatial heterogeneities across the biofilm. The steady-state assumption was invoked such that time was eliminated as an independent variable and the transport equations only involved spatial derivatives in the axial direction, thereby simplifying the spatiotemporal model to a time-invariant spatial model. The spatial model consisted of a published *G. sulfurreducens* GSM [67], Michaelis-Menten uptake kinetics for acetate and Nerst kinetics for Fe(III) reduction, and reaction-convection-diffusion type equations for the fractions of active, respiring and inert biomass, acetate concentration, current density, local potential and axial velocity.

The *G. sulfurreducens* GSM was solved off-line at different values of the acetate uptake and Fe(III) reduction rates to generate a 20×20 lookup table of LP solutions. The boundary value ODEs representing the reaction-diffusion-convection equations were solved using a continuation method [68] with the LP lookup table queried as needed to resolve intracellular metabolism at different points in the biofilm. Unfortunately, details concerning the continuation solution method and the spatial grid used are not reported. The spatial metabolic model was solved for varying extracellular conditions (*i.e.* the presence/absence of NH_4) and maintenance energy demands to investigate their effects on biofilm thickness and electrical current production. Simulations combined with validation experiments provided key predictions into biofilm and fuel cell behavior including that: (1) limited acetate diffusion through the biofilm induced low local acetate concentrations that restricted biomass formation and current generation; and (2) respiring cells that did not grow but produce current located in acetate limited regions had a substantial impact on fuel cell performance.

In the second study [64], a spatiotemporal modeling framework called Computation of Microbial Ecosystems in Time and Space (COMETS) was developed to investigate the emergent behavior of two- and three-member synthetic communities. A two-dimensional lattice was defined, with each box within the lattice representing a distinct spatial location. GSMs of the participating species were used to solve an independent DFBA problem for each box assuming that each species had the potential to consume available carbon sources according to the same uptake kinetics. A sequential solution strategy was used where the species LPs were solved to generate local growth rates and fluxes, and then the extracellular ODEs were integrated over a fixed time step with these constant LP solutions to generate local biomass and metabolite concentrations. Before resolving the LPs to start the next iteration, two-dimensional diffusion equations were solved over the same time step to allow species cell mass and metabolites to diffuse between boxes.

COMETS was used to investigate engineered metabolite cross-feeding in a two-species synthetic community consisting of *Salmonella enterica* and *Escherichia coli*. The spatiotemporal model was able to reproduce experiments showing the two species robustly co-existed and converged to a population fraction of 79% *E. coli* regardless of the initial fractions. Similar results were obtained for a considerably more complex three-species synthetic community consisting of *S. enterica*, *E. coli* and *Methylobacterium extorquens*.

The two-species system was used to investigate the impact of spatial structure. The model correctly predicted that community growth would diminish as the two species were inoculated further apart from each other due to diffusional limitations of the cross-fed metabolites. Moreover, the model reproduced experiments showing community growth would be enhanced by placement of a second colony of engineered *S. enterica* between the *S. enterica* and *E. coli* populations but that placement of a wild-type *S. enterica* colony which did not require the cross-fed metabolite from *E. coli* would reduce community growth.

In the third study [66], method M3 was used to predict synthesis gas (CO, H_2) conversion to ethanol by the anaerobic bacterium *Clostridium ljungdahlii* in a vertical bubble column reactor. Synthesis gas and liquid media feed streams were introduced into the bottom of the column and flowed up the column with different velocities, producing large spatial gradients due to cellular growth and gas depletion. The metabolic byproducts ethanol and acetate were recovered in the liquid phase stream exiting the top of the column. The spatiotemporal metabolic model consisted of a published *C. ljungdahlii* GSM [69], Michaelis-Menten uptake kinetics for CO and H_2, and reaction-convection type PDEs for *C. ljungdahlii* biomass, liquid phase CO, H_2, ethanol and acetate, and gas phase CO and H_2. The PDEs were discretized with 100 spatial node points, and lexicographic optimization [47] with 6 LPs at each node point was used to ensure unique exchange fluxes. The large discretized model consisting of 900 ODEs in time and 600 LPs was efficiently solved within MATLAB (MathWorks, Natick, Massachusetts, USA) using the DFBAlab tool (Figure 4) [46].

Dynamic simulations performed for a wide range of column operating conditions and nutrient uptake parameters generated predictions about column behavior and bottlenecks to ethanol production consistent with available experiments including: (1) typical CO rich syngas will produce substantial acetate due to H_2 depletion in the upper part of the column, suggesting that H_2 augmentation of the syngas feed may be beneficial; (2) efficient gas-liquid mass transfer is critical to achieve high ethanol production and high conversions, demonstrating the need for continued development of advanced bubble column designs that achieve very high gas-liquid mass transfer rates; and (3) enhanced H_2 uptake rates substantially increase the ethanol titer and the ethanol/acetate ratio, suggesting that *C. ljungdahlii* engineering efforts should focus on increasing H_2 uptake rates.

Conclusions

Spatially heterogeneous environments are the rule rather than the exception for naturally occurring microbial systems. Our continued ability to fundamentally understand and rationally manipulate microbial systems will depend on the development of predictive modeling frameworks that connect the genetic capabilities of individual species and the spatiotemporal characteristics of growth environments to system function. Over the past five years, spatiotemporal metabolic modeling techniques that leverage the increasing availability of genome-scale reconstructions have been developed and evaluated. Despite their limitations with regard to intracellular organization, dynamics and regulation, these *Spatiotemporal Flux Balance Analysis (SFBA)* methods hold great promise for analysis of natural microbial systems as well as the design of engineered systems that exploit the

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evolved capabilities of microbes to optimally function in highly dynamic and spatially heterogeneous environments [70, 71]. Possible research directions for the nascent SFBA field are myriad and include: (1) continued identification and study of microbial systems such as the human microbiome [72, 73] and lignocellulosic degrading communities [74, 75] that would benefit from spatiotemporal analysis; (2) incorporation of higher fidelity intracellular models that extend beyond just reaction stoichiometry [76, 77]; (3) development of alternative methods for formulating SFBA models that are more amenable to efficient numerical solution; (4) development and testing of general purpose software such as DFBAlab [46] for the solution of the large-scale differential equation and linear program systems that result from SFBA models; and (5) experimental testing of SFBA model predictions through the collection of omics data with both temporal and spatial resolution [78–80]. Based on the overarching importance of the problem and the initial successes reported this review, SFBA can be expected to become the next major frontier for microbial metabolic modeling.

Acknowledgments

Abbreviations

COMETS	computation of microbial ecosystems in time and space
DFBA	dynamic flux balance analysis
DFBAlab	dynamic flux balance analysis laboratory
FBA	flux balance analysis
GSM	genome-scale metabolic reconstruction
LP	linear program
ODE	ordinary differential equation
PDE	partial differential equation
SFBA	spatiotemporal flux balance analysis

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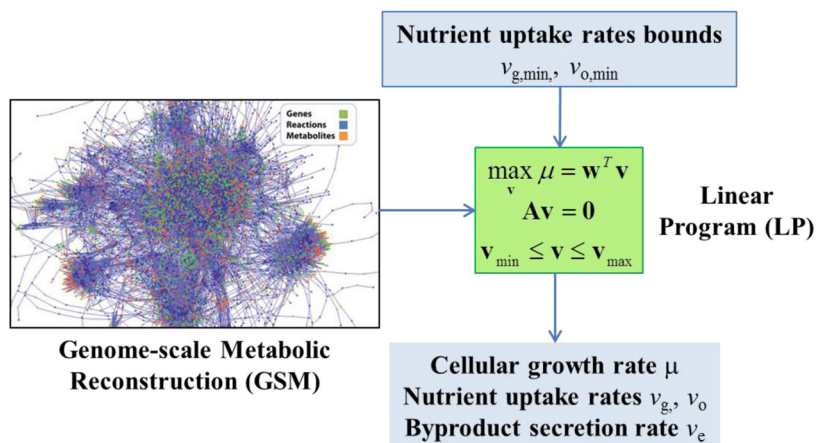
Figure 1. Genome-scale flux balance analysis

A GSM is used to formulate a LP to compute the unknown fluxes $\mathbf{v}$ from knowledge of the stoichiometric matrix $\mathbf{A}$, biomass composition weights $\mathbf{w}$, flux bounds $\mathbf{v}_{min}$ and $\mathbf{v}_{max}$, and specified bounds on the nutrient uptake rates. This specific example shows glucose (g) and oxygen (o) as the growth limiting nutrients and ethanol (e) as the primary metabolic byproduct. From specified transport bounds on the two nutrient ($v_{g,\,min}$, $v_{o,\,min}$), the cellular growth rate (μ), the actual nutrient uptake rate (v_g, v_o), and the ethanol secretion rate (v_e) are computed.
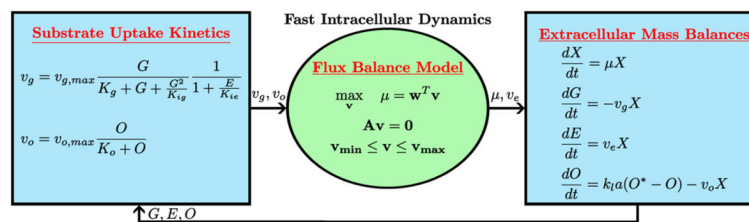
Figure 2. Genome-scale dynamic flux balance analysis

FBA is performed with a GSM to predict the growth rate, nutrient uptake rates and byproduct secretion rates under the assumption that the intracellular dynamics are fast compared to the extracellular dynamics. The FBA fluxes serve as inputs to the ODEs for the extracellular environment, which are integrated to yield time resolved predictions of the biomass, nutrient and byproduct concentrations. These concentrations serve as inputs to the nutrient uptake kinetics, which are used to compute transport bounds on the nutrient uptake rates for the FBA problem. This specific example shows glucose (g) and oxygen (o) as the growth limiting nutrients and ethanol (e) as the primary metabolic byproduct where X, G, E and O are the biomass, glucose, ethanol and oxygen concentrations, respectively.
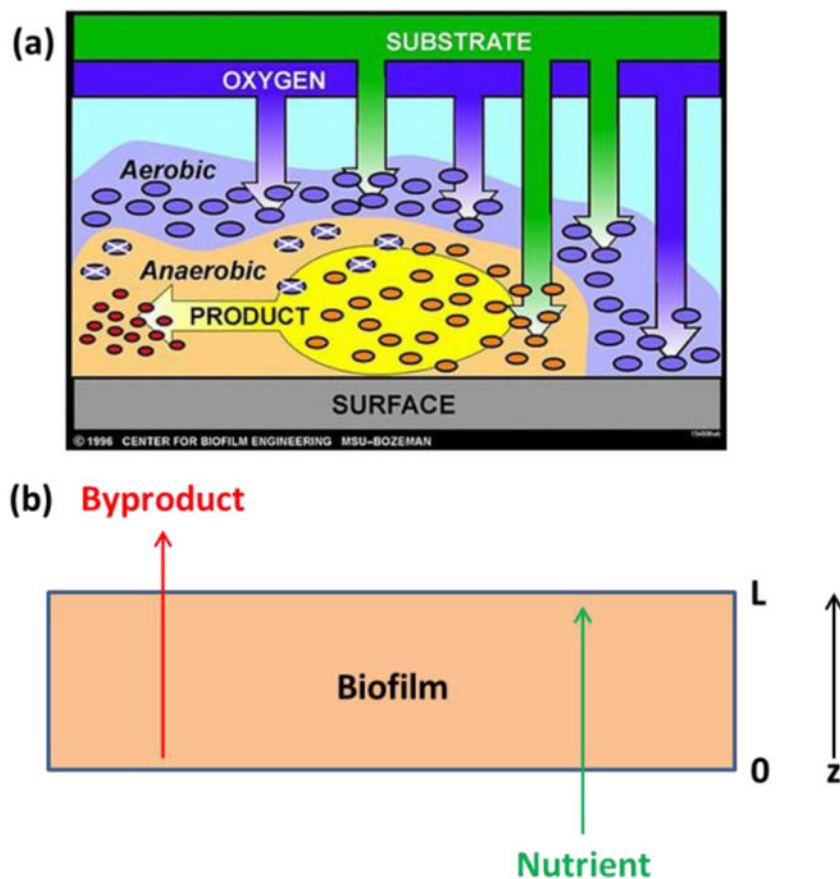
Figure 3. Spatial heterogeneities in microbial biofilms

(a) Limited diffusion of a carbon containing nutrient (substrate) and oxygen can create metabolic niches with the biofilm that lead to aerobic and anaerobic regions with differential cell growth and product synthesis (image courtesy of the MSU Center for Biofilm Engineering). (b) A single species biofilm with thickness L in which a single growth limiting nutrient is available at the bottom of the biofilm and a single synthesized byproduct exits the top of the biofilm.
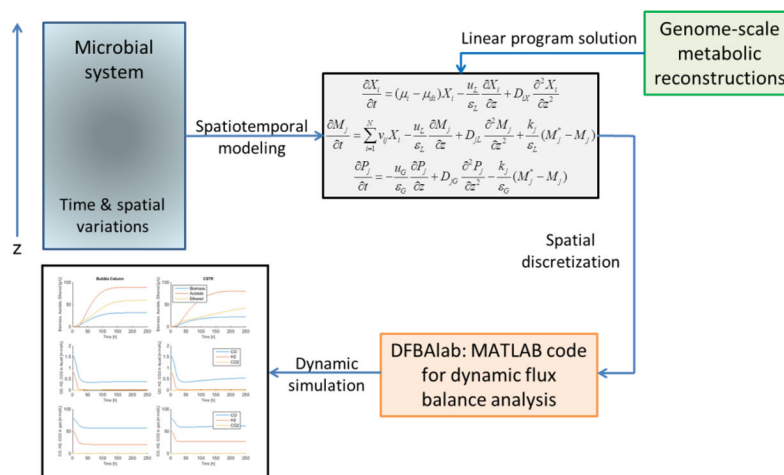
Figure 4. Genome-scale spatiotemporal flux balance analysis [60]

A microbial system with temporal and spatial variations is described by a spatiotemporal model that accounts for relevant transport processes within the system. PDEs are written with respect to the species concentration (X_i), liquid-phase metabolite concentrations (M_j) and gas-phase metabolite concentrations (P_j) assuming that spatial variations are limited to a single direction z. FBA is performed with GSMs of the participating species to predict the growth rates, nutrient uptake rates and byproduct secretion rates. The PDEs are spatially discretized to yield a large-set of ODEs with embedded LPs that are solved with the MATLAB code DFBAlab [46] to generate time and spatially resolved predictions.