Journal of Theoretical Biology 345 (2014) 12–21

Contents lists available at ScienceDirect

Journal of Theoretical Biology

journal homepage: www.elsevier.com/locate/yjtbi

Incorporation of flexible objectives and time-linked simulation with flux balance analysis ☆

Elsa W. Birch [a], Madeleine Udell [b], Markus W. Covert [c,*]

[a] Chemical Engineering, Stanford University, Stanford, CA 94305, USA
[b] Computational and Mathematical Engineering, Stanford University, Stanford, CA 94305, USA
[c] Bioengineering, Stanford University, Stanford, CA 94305, USA

HIGHLIGHTS

- The rigid flux balance analysis (FBA) biomass reaction hinders whole-cell modeling.
- New flexible FBA can produce subsets of biomass reactants.
- Time-linked FBA removes the reactant-to-byproduct long-time assumption.
- Our new methods avoid low-copy enzyme metabolic artifacts for whole-cell modeling.

ARTICLE INFO

Article history:
Received 30 August 2013
Received in revised form
25 November 2013
Accepted 4 December 2013
Available online 17 December 2013

Keywords:
Metabolism
Integrated modeling

ABSTRACT

We present two modifications of the flux balance analysis (FBA) metabolic modeling framework which relax implicit assumptions of the biomass reaction. Our flexible flux balance analysis (flexFBA) objective removes the fixed proportion between reactants, and can therefore produce a subset of biomass reactants. Our time-linked flux balance analysis (tFBA) simulation removes the fixed proportion between reactants and byproducts, and can therefore describe transitions between metabolic steady states. Used together, flexFBA and tFBA model a time scale shorter than the regulatory and growth steady state encoded by the biomass reaction. This combined short-time FBA method is intended for integrated modeling applications to enable detailed and dynamic depictions of microbial physiology such as whole-cell modeling. For example, when modeling *Escherichia coli*, it avoids artifacts caused by low-copy-number enzymes in single-cell models with kinetic bounds. Even outside integrated modeling contexts, the detailed predictions of flexFBA and tFBA complement existing FBA techniques. We show detailed metabolite production of *in silico* knockouts used to identify when correct essentiality predictions are made for the wrong reason.

1. Introduction

Quantitative metabolic models are important tools for understanding and engineering the behavior of microorganisms. Flux balance analysis (FBA) is a powerful technique to simulate large metabolic networks for which kinetic parameters are unavailable. FBA simulations capture microorganism growth, nutritional resource consumption, and waste-product secretion rates (Varma and Palsson, 1993; Mahadevan et al., 2002). In addition FBA can generate knockout essentiality predictions which can be treated as hypotheses to explore an organism's metabolic capability (Covert et al., 2001; Edwards and Palsson, 2000).

Classical implementations of FBA quantify microbial growth using a rigid biomass reaction which represents all the processes of cell replication as a single proportion of the reactants required and byproducts returned. It is used to quantify microbial growth even when another objective is used to refine flux predictions or evaluate perturbations (Raman and Chandra, 2009; Schuetz et al., 2007; Segrè et al., 2002).

The biomass reaction can produce only balanced growth or complete inactivity as predictions. For many applications the assumptions underlying this all-or-nothing behavior have been valid and the results have been useful. However, current *in silico* biology incorporates FBA in integrated models which combine mathematical models of different types to interact over a simulation (Birch et al., 2012, Gonçalves et al.). For these applications — most notably whole-cell models (Karr et al., 2012) — the rigid biomass reaction is a limitation.

* Corresponding author.
E-mail address: mcovert@stanford.edu (M.W. Covert).

0022-5193/$ - see front matter © 2013 The Authors. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jtbi.2013.12.009

To enable whole-cell modeling, we require a more nuanced alternative to the biomass reaction so that FBA can produce metabolites in non-wild-type and non-steady-state proportions.

In this work, we relax two implicit assumptions of the biomass reaction to construct new FBA methods. The first assumption is of balanced population average growth, encoded by the biomass reaction's fixed proportion of reactants. The second assumption is of steady state growth, encoded by the biomass reaction's fixed proportion of byproducts to reactants. Relaxing the reactant and byproduct assumptions results in the flexible FBA (flexFBA) and time-linked FBA (tFBA) approaches, respectively.

Together, the balanced and steady-state growth assumptions inherent to biomass reaction in FBA make the method applicable to a timescale longer than regulatory and cell process interactions. By combining the flexFBA and tFBA methods which relax these assumptions, we obtain a short-time FBA appropriate to use in whole-cell models. This short-time scale is consistent with whole-cell models which evaluate the metabolic model on timescales shorter than the regulatory and process interactions they explicitly represent.

1.1. Biomass reaction and assumptions

The biomass reaction is ubiquitous in microbial FBA because it lends great predictive power to the under-constrained metabolic network. It has a succinct mathematical form and is composed of straightforward parameter values. In addition to quantifying growth, the biomass reaction flux is often used as an optimization objective and in this case may be called the 'biomass objective' (Feist and Palsson, 2010). Much literature evaluates the ability of various FBA objectives to mimic observed growth, gene essentiality, or flux states (Schuetz et al., 2007; Harcombe et al., 2013; Burgard and Maranas, 2003; Reed, 2012; Zomorrodi et al., 2012), often in comparison to 'biomass objective' performance. In contrast, here we discuss simulation regimes in which the biomass reaction does not adequately model the range of metabolic network function, and is no longer relevant as a quantification of growth.

By constraining together all process reactant requirements and byproduct returns the biomass reaction combines the two subtly different assumptions that deal with the (1) reactant-to-reactant and (2) byproduct-to-reactant groups.

Reactant-to-reactant fixed proportion in the biomass reaction assumes population average balanced growth: homogeneity between cells and within cells over time. This assumption is contained in the biomass reaction's negative coefficients. As a consequence, the biomass reaction scales the fractional fulfillment of all process reactants to whichever one is most limited. Homogeneity between cells arises from the biomass reaction because its coefficients are bulk cell composition values. For single cells and short timescales this homogeneity conflicts with biological reality. Bulk phenotypes are given by an average and neglect variance in the underlying population (Taniguchi et al., 2010; Lidstrom and Konopka, 2010). Strict temporal homogeneity of metabolite production ratios is unreasonable because the transcriptional and translational regulatory mechanisms which could enforce it operate on timescales longer than the typical FBA time step (1 s to a few minutes Covert and Palsson, 2002; Covert et al., 2008). Furthermore, regulatory interactions may not exist between all metabolites included in the biomass reaction to enforce their proportional production. Experimental observations reveal that even essential metabolites can be produced in non-wild-type proportions (Jackowski and Rock, 1981; Goldstein et al., 1959; Goss et al., 1964; Ohashi et al., 2008). Additionally, all metabolites included in the biomass reaction are essential for model growth. If the biomass reaction includes process reactants which are non-essential for cell replication, then false-essential predictions will result (Feist et al., 2007). Previously, the inflexible ratio and essentiality of the biomass reaction have been addressed via alternate biomass reaction definitions (Feist et al., 2007; Nookaew et al., 2008) or reactions allowing similar metabolites to substitute for one another (Heavner et al., 2012); though these approaches are not practical for the entire scale or all pathways of metabolism.

Byproduct-to-reactant fixed proportion in the biomass reaction assumes steady state metabolic function. This assumption is contained in biomass reaction's positive coefficients. The principle example is the return of spent energy carrier ADP set proportional to the amount of ATP produced within a time step. Proportional byproducts to reactants means the ADP return is immediately matched to the capacity of metabolism to recharge it to ATP, rather than being consistent with the previous time step's metabolic conditions. Relating the reactant and byproduct quantities is reasonable, but a long-time assumption is implied within a single evaluation of the FBA optimization. Perturbations or changes in available media resources therefore result in immediate transition to a new steady state in the single time step at which they are applied. This type of transition is unrealistic for short time step evaluations or if the energy carrier supply or turnover is limited.

2. Methods

Mathematically the biomass reaction consists of coefficients m_i of metabolites M_i appended as a single reaction column to the stoichiometric matrix $\mathbf{S}$ as in the equation,

$$\left[\begin{array}{c|c} \mathbf{S} & \begin{matrix} m_1 \\ m_2 \\ \vdots \\ m_n \end{matrix} \end{array}\right] \begin{bmatrix} v \\ v_{\text{bio}} \end{bmatrix} = \frac{\mathrm{d}[M]}{\mathrm{d}t} = 0, \tag{1}$$

where values $m_i < 0$ represent consumption of process reactants, and $m_i > 0$ represent byproduct return. A majority of the n total m_i values are zero because the associated metabolites do not participate in cell processes beyond metabolism. Coefficient magnitudes for anabolic products are given by relative quantities found in bulk biomass (Feist and Palsson, 2010); coefficient magnitudes in the case of catabolic energy carriers are given by requirements for macromolecule synthesis or calculated from bulk yields (Feist and Palsson, 2010). Units of m_i are usually chosen so that the flux through the biomass reaction, the last element of flux vector v denoted v_{bio}, can be directly interpreted as a microbial growth rate (Varma and Palsson, 1993). Metabolite accumulation $\mathrm{d}[M]/\mathrm{d}t$ is set to zero (Varma and Palsson, 1993), applying the steady state assumption to metabolic network intermediates on the timescale of evaluation Δt, typically 1 s or longer. Using the biomass reaction flux as the maximization objective, the optimization problem is

$$\begin{array}{ll} \text{maximize} & v_{\text{bio}} \\ \text{subject to} & \mathbf{S}'v' = 0 \\ & v_{\text{l}} \le v \le v_{\text{u}}, \end{array} \tag{2}$$

where $\mathbf{S}' = [\mathbf{S}|m]$ and v' is v_{bio} appended to the end of v as in Eq. (1).

Because the coefficients m_i are quantities required for some basis amount of cell mass, we find it convenient to think of the biomass reaction flux v_{bio} as the fractional fulfillment of that requirement per time. The classical FBA biomass reaction therefore requires the fractional fulfillment of all the metabolite requirements to be the same.

2.1. Designing a biomass reaction alternative

While we sought to relax the biomass reaction's assumptions, we wanted to simultaneously preserve its behavior in the wild-type and long-time limits. We developed flexFBA to produce all possible process reactants without inhibition from distant/unrelated blocked