Towards the Integration of Genome Scale Models and Bioreactors for the Production of Commodity Chemicals

Jorge Lázaro, Jorge Júlvez

Grupo de I+D en Computación Distribuida (DisCo)
Instituto de Investigación en Ingeniería de Aragón (I3A)
Universidad de Zaragoza, Mariano Esquillor s/n, 50018, Zaragoza, Spain.
Tel. +34-976762707, e-mail: *717099@unizar.es*

Abstract

Most processes for the production of commodity chemicals rely on fossil fuels, and hence, are highly pollutant. A promising alternative is to develop bioprocesses that make use of genetically engineered cells. A novel modeling framework is proposed to speed up the overall design of bioprocesses and optimize their productivity.

Motivation and Goal

Very roughly, the design of a bioprocess consists of two steps: a) engineering a cell strain; and b) establishing the optimal parameters of the bioreactor in which the cells are cultured. These steps are costly in terms of time and resources, as they usually follow a trial and error approach in the wet lab. The use of computational models can greatly help accelerate the design of bioprocesses. In contrast to existing modeling approaches which focus almost exclusively on the cell metabolism, the current proposal aims to develop a model that integrates the metabolic network of the cell into a mathematical model of the bioreactor. Such an integration will enable us to perform a global optimization of the system by taking into account simultaneously both the microscopic metabolic network of the cell and the macroscopic dynamics of the bioreactor. Moreover, the overall model will account for continuous cultures which are expected to have higher performance than the commonly used batch cultures. The modeling framework will be based on Flexible Nets (FNs)[1], a novel formalism for dynamical systems.

The reminder of this contribution is organized as follows: First, constraint-based models and the basic dynamics of bioreactors are introduced. Then, after a brief introduction of FNs, an integrated model of a metabolic network and a bioreactor is discussed. Finally, as a proof of concept, we consider the production of citramalate by *E. coli*.

Constraint-based models

Metabolism is the set of chemical reactions of the cells that sustain life. Each chemical reaction has a set of *substrates* that are transformed into a set of *products*. Given that the products of one reaction are substrates of other reactions, the graphical representation of metabolic reactions leads to the so called metabolic network. Constraint-based modelling is the most popular formalism to build genome-scale models, particularly for metabolic networks. A constraint-based model can account for metabolic reactions, genetic information and flux constraints. This contribution makes use of the constraint-based model of *Escherichia coli* strain K-12 MG1655 introduced in [2]. The model is named iJO1366 and accounts for 1805 metabolites, 2583 reactions and 1367 genes.

Bioreactors

Bioreactors are devices in which cell cultures can be grown. In continuous fermentation, fresh medium is supplied continuously to the tank while used medium with toxic metabolites, consumed nutrients and cells are removed simultaneously. The dynamics of the bioreactor are determined by [3]:

$$\frac{\mathrm{d}X}{\mathrm{d}t} = (\mu - D)X \tag{1}$$

$$\frac{\mathrm{d}s_i}{\mathrm{d}t} = (c_i - s_i)D - u_i X \tag{2}$$

where X ($g_{DW}L^{-1}$) is the cell density in the tank, μ (h^{-1}) is the effective cell growth rate, D (h^{-1}) is the dilution rate, c_i (mM) is the concentration of metabolite i in the medium, s_i is its concentration in the culture and u_i is its specific uptake rate (mmol $g_{DW}^{-1}h^{-1}$).

Flexible Nets

FNs are inspired by Petri nets and aim to solve some of the modeling aspects and optimization challenges presented by biological systems. In addition to places and transitions, a key element of a FN is the *handler*, represented graphically as a dot, with which sets of linear inequalities can be associated. Such inequalities allow FNs to accommodate both uncertain stoichiometric and kinetic parameters, and hence, integrate heterogeneous models seamlessly.

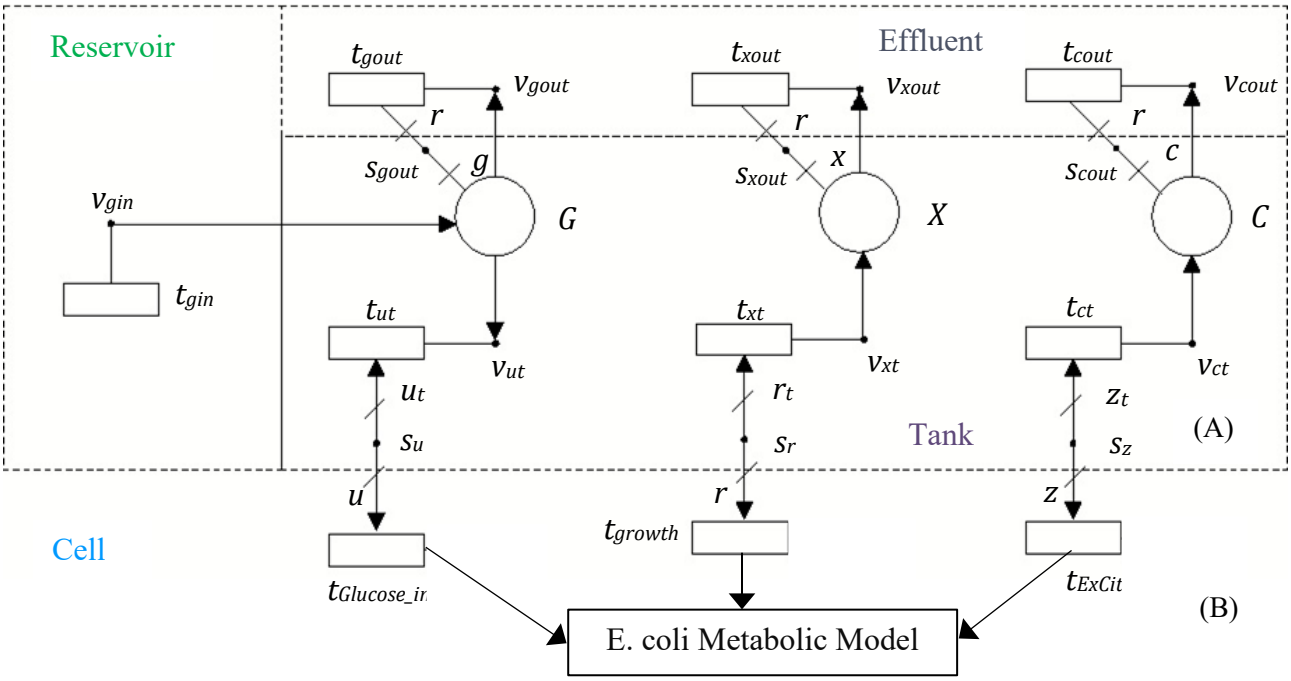

Figura 1. Flexible Net integrating the bioreactor dynamics and the metabolic network of *E. coli*. The net has four different compartments delimited by dashed lines: reservoir, effluent, tank and cell. Subnet (A) corresponds with the bioreactor macroscopic variables, subnet (B) corresponds with the intracellular genome-scale constraint-model of the metabolism of *E.coli*. Places *G, X* and *C* represent glucose, cell density and citramalate in the tank.

Model integration

The FN in Figure 1 combines the dynamics of the bioreactor defined by Eqs. (1) and (2) and the fluxes through the reactions of the model iJO1366 of *E. coli*.

The state of the tank is represented by the following variables: nutrient concentration (glucose), biomass (cell density) and product concentration (citramalate). These variables are modeled by the *places* G (units: mM), X (units: 10^6 g_{DW} L^{-1}) and C (units: mM), respectively. The dynamics of X is ruled by the Eq. (1) and G and C are ruled by the Eq. (2). The elements taking part in the interface and connection between the cell and the tank compartments are the intensity handlers s_u, s_r and s_z. These intensity handlers are the elements that allow the model associate the macroscopic variables of the bioreactor with the exchange fluxes of the cell in such a way that each macroscopic flux equals X times the exchange flux of a cell.

Citramalate production

Methyl methacrylate (MMA) is a volatile synthetic chemical used for the production of solvents, adhesives, sealants or leather [4]. The alternative for the non-sustainable MMA production is based on the capability of *Methanocaldococcus janaschii* to generate citramalate, an MMA precursor.

M. janaschii owns the *CimA* gene that codifies the citramalate synthase enzyme, responsible for the 3(R)-citramalate synthesis. We have designed a model based on FNs of a genetically modified *E. coli* capable of producing citramalate. The model was implemented in Python and the production of citramalate under different media.

Conclusions

A suitable model for the *E. coli* metabolic network including a bioreactor to simulate the nutrient supply and the removal of the metabolites has been successfully designed using Flexible Nets. The predictions of the simulations were consistent with the values of citramalate production provided by collaborators in the University of Nottingham.

References

1. Jorge J, Oliver SG. A Unifying Modelling Formalism for the Integration of Stoichiometric and Kinetic Models. 2019;(December).
2. Orth JD, Fleming RMT, Palsson BØ. Reconstruction and Use of Microbial Metabolic Networks : the Core Escherichia coli Metabolic Model as an Educational Guide. 2013;(2).
3. Fernandez-de-cossio-diaz J, Leon K, Mulet R. Characterizing steady states of genome-scale metabolic networks in continuous cell cultures. 2020; Available from: http://dx.doi.org/10.1371/journal.pcbi.1005835
4. Directorate EH, Canada H, Organisation IL. Concise International Chemical Assessment Document 4 METHYL METHACRYLATE. 1998;