Computers and Chemical Engineering 33 (2009) 1438–1450

Contents lists available at ScienceDirect

Computers and Chemical Engineering

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

Multi-objective optimisation of metabolic productivity and thermodynamic performance

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ARTICLE INFO

Article history:
Received 1 February 2008
Received in revised form 11 March 2009
Accepted 15 March 2009
Available online 27 March 2009

Keywords:
Metabolism optimisation
Gibbs free energy change
Thermodynamic analysis
Genetic algorithm
Multi-objective optimisation

ABSTRACT

Novel multi-objective optimisation methodologies, including a two-step sequential optimisation approach and multi-objective optimisation approaches using non-dominated sorting genetic algorithms (NSGAs) and MATLAB based linear programming integrated with genetic algorithms have been developed for the first time to engineer the cellular metabolic productivity and process performance simultaneously. The simultaneous optimisation of cellular metabolic productivity and thermodynamic performance deduces a unique set of enzyme catalysed pathways and flux distributions for a given metabolic product of importance. It has been demonstrated that the energy generating pathways associated to drive a desired productivity are prioritised effectively by multi-objective optimisation approach. A case study on the pentose phosphate pathway (PPP) and glycolysis of *in silico Escherichia coli* has been used to illustrate the effectiveness of the methodologies.

1. Introduction

The chemical components in a living cell participate in many different reactions catalyzed by different sets of enzymes. The sum of such reactions is referred as a cell's metabolic system (Light, 1968). Each metabolic pathway can thus be seen as a series of enzyme catalyzed reactions, by which a cell can precisely manoeuvre the chemical transformation of its substrate, via a sequence of intermediate substances, to produce, ultimately, biomass and an end product from the pathways (Cohen, 1991). Such pathways are central to cellular biochemical activities, which channel substrate metabolites into the production of energy, building blocks for biosynthesis, energy reserves, eliminating waste products, and for recycling and reducing equivalents (Nolan, Fenley, & Lee, 2006). The rational decomposition of biochemical networks into sub-structures has emerged as a useful approach to study the design of these complex systems (Yoon, Si, Nolan, & Lee, 2007). In this light, to quantify intracellular reaction steps or pathways, and infer the objectives of cellular metabolic systems, rational optimisation strategies need to be developed for manipulating cell properties, hence productivity. Moreover, robust optimisation strategies by considering more than one objective and decision variables simultaneously can be adopted for directing cellular functionalities towards engineered products and systems.

In recent years, several theoretical approaches have been developed to assign metabolic priorities through engineered cells, including structural (topological) pathway analysis (Liao, Hou, & Chao, 1996; Mavrovouniotis, 1990; Schilling, Letscher, & Palsson, 2000; Schuster, Fell, & Dandekar, 2000; Seo, Lee, Park, Fan, & Shafie, 2001; Simpson, Follstad, & Stephanopoulos, 1999), metabolic flux analysis (MFA) (Stephanopoulos, Aristidou, & Nielsen, 1998), metabolic control analysis (Fell, 1996; Heinrich & Rapoport, 1974; Kacser & Burns, 1973) and dynamic simulation (Tomita et al., 1999). A representative modelling framework for metabolic analysis is the flux balance analysis (FBA), which is a constrained optimisation approach based on linear programming (LP) (Bonarius, Schmid, & Tramper, 1997; Edwards & Palsson, 1998; Varma & Palsson, 1994). The basic idea behind these modelling approaches is the prediction of pathways that are related to experimental observation or to predict productivity. All these approaches are designed to deal with metabolic systems with a single optimisation objective. However, many metabolic engineering problems require simultaneous optimisation of a number of objectives that may be competing and non-commensurate. A better understanding of the trade-offs among these objectives will be valuable in identifying corresponding cellular metabolic functionalities. The presence of more than one objective in studying metabolic systems is very common and has been appreciated by researchers recently. Burgard, Pharkya, and Maranas (2003) have proposed an optimisation-based framework,

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doi:10.1016/j.compchemeng.2009.03.008

Nomenclature

c^0	standard state concentration (1 M) (mol/l)
G	extensive Gibbs free energy of a system (kJ)
G'	extensive transformed Gibbs free energy of a system (kJ)
ΔG	Gibbs free energy change of reaction (kJ)
ΔG^0	standard Gibbs free energy change of reaction (kJ)
$\Delta_f G_i^0$	standard formation Gibbs free energy of species i at specified T, P, and I (kJ/mol)
$\Delta_f G'^0$ (react)	standard formation Gibbs free energy of reactant i at specified T, P, pH and I (kJ/mol)
$\Delta_f G'_{e\in EM}$	standard formation Gibbs free energy of external metabolites e at specified T, P, pH and I (kJ/mol)
$\Delta G'^0_{p\in P}$	standard Gibbs free energy change of pathway p at specified T, P, pH and I (kJ/mol)
I	ionic strength (mol/l)
N_i(H)	number of H atoms in species I
pH	$-\log([H^+]/c^0)$
P	pressure (bar)
R	gas constant (8.31451 J K^{-1} mol^{-1}) (J K^{-1} mol^{-1})
T	temperature (K)
z_i	charge of ion I
μ_i^0	standard chemical potential of species i at specified T, P, and I (kJ/mol)
$r_{reacting_e}$	reacting rate of external metabolites e ($e \in UPT$) (% of molar glucose uptake rate)
K	equilibrium constant of reaction
$B^{opt}(p)$	optimal mass flux distribution of pathway p (% of molar glucose uptake rate)
G_{tot}	total Gibbs free energy change of the system (kJ/100 mol of glucose)
G_{tot}^{opt}	minimum Gibbs free energy change of the metabolic system (kJ/100 mol of glucose)
pH′	optimal corresponding cellular condition of pH
I'	optimal corresponding cellular condition of ionic strength (mol/l)

Sets, variables and parameters

IM	{1, 2, …, X/internal metabolites}
EM	{1, 2, …, Y/external metabolites and cofactors}
R	{1, 2, …, N/reactions (or reaction steps) in a metabolic system}
P	{1, 2, …, M/pathways in a metabolic system}
OBJ	{e/objective product under optimisation, $OBJ \subset EM$}
UPT	{e/external metabolite from external metabolite measurement, $UPT \subset EM$}

Variables and parameters

$\bar{V}$	property vector of metabolic flux in individual reactions j, $\forall j \in R$
$\bar{B}$	property vector of metabolic flux in elementary pathways p, $\forall p \in P$
$\bar{B}^{opt}$	property vector of optimal metabolic flux for elementary pathways p, $\forall p \in P$
$\bar{S}$	stoichiometric matrix for internal metabolite i ($i \in IM$) in reaction j ($j \in R$)
$\bar{A}$	stoichiometric matrix for reaction j ($j \in R$) in elementary pathway p ($p \in P$)
$\bar{U}$	stoichiometric matrix for external metabolites e ($e \in EM$) in pathway p ($p \in P$)
$\overline{VE}$	property vector of metabolic flux for external metabolites e,$\forall e \in EM$
$\overline{GE}$	property vector of standard formation Gibbs free energy of external metabolites e ($e \in EM$) at specified pH and ionic strength
$\overline{GP}$	property vector of standard Gibbs free energy changes for elementary pathways p ($p \in P$)
$\overline{r^{reacting}}$	property vector of the reacting rates of given steady external metabolites e ($e \in UPT$)
$\overline{OBJECT}$	property vector of the productivity of desired external metabolite e ($e \in OBJ$)

called ObjKnock, for deducing genetic manipulations that lead to overproduction. This framework is aimed at balancing conflicting engineering and cellular objectives. This bi-objective optimisation problem is transformed into a single objective problem based on the strong duality theorem (Burgard et al., 2003). With this theorem, the optimality of the primal problem is regarded as a constraint to the dual problem giving rise to two nested optimisation problems.

Thermodynamic insights into metabolic reaction networks or pathways, such as the relationship between the driving force for growth in terms of Gibbs energy dissipation and biomass yield, are useful in estimating the key parameters in biotechnological cultures and thus to address reaction viability of bioprocesses (Von Stockar & van der Wielen, 2005). Similarly, Beard, Liang, and Qian (2002) have also emphasised the use energy balance analysis, the theory and methodology for enforcing the laws of thermodynamics, for eliminating thermodynamically infeasible results associated with FBA. Optimal metabolic fluxes based on mass balance does not ensure feasibility of metabolic pathways in the system. In thermodynamic terms, the difference in Gibbs free energy sets the driving force for any system undergoing changes. Thus, thermodynamic analysis based on the Gibbs free energy change is applied to elucidate the spontaneity and existence of driving force for the occurrence of metabolic pathways responsible for a desired product. Moreover, a pathway for which the free energy change is large and negative has an equilibrium that favours the side of products. Therefore, the thermodynamic tools can be instrumental to the selection of feasible pathways and identifying optimal cellular environment for metabolic systems. The minimisation of Gibbs free energy change and the maximisation of productivity of desired metabolites need to work simultaneously for an overall optimal selection of pathways and set of enzymes responsible for these pathways.

Our initial effort tackled the metabolic product engineering problem sequentially based on linear programming in general algebraic modelling system (GAMS) (Xu, Smith, & Sadhukhan, 2008). However, development of a more effective multi-objective optimisation approach is becoming necessary and valuable. In this work, we have introduced a newly developed multi-objective optimisation approach, non-dominated sorting genetic algorithms (NSGAs), by Deb (2001) to simultaneously optimise metabolic productivity and thermodynamic performance, as an integrated and engineered problem, for the first time.

Genetic algorithms (GA) illustrated in Fig. A1 and Appendix A are useful optimisation tools to address many industrial engineering activities or real-life problems. However, application of GA to optimise cellular productivity has been limited so far. Morbiducci, Tura, and Grigioni (2005) and Zhang and Yao (2007) have used genetic algorithm for mathematical modelling of glucose metabolism and simulation of flux distribution for central metabolism of *Saccharomyces cerevisiae*. In their studies, GA were used by virtue of their conceptual simplicity, the ease of programming entailed, and no requirement for a fixing initial value of model parameters, but restricted to solve single objective optimisation problems. Never-