Development of a Differential Evolutionary Algorithm Application in Optimizing Microbial Metabolic System

Adel Maghsoudpour
Department of Mechanical and Aerospace Engineering, Science and Research Branch, Islamic Azad University, Tehran, Iran

Ali Ghaffari
Department of Mechanical Engineering, K. N. Toosi University of Technology, Tehran, Iran

Mohammad Teshnehlab
Department of Electrical Engineering, K. N. Toosi University of Technology, Tehran, Iran

ABSTRACT
Advancement of powerful biological technology has caused to achievement to numerous omic data that possibility of using algorithmic methods in analysis and optimizing of biological system has provided beside advancement of calculative biology. In this study, optimizing calculative instrument of microbial metabolism is extended on base of differential evolutionary algorithm with vision from bi-level optimizing functions. The outcome algorithm has been used for optimizing of succinic acid microbial production. The result shows the algorithm can reproduce scenario of metabolic engineer in less calculative time which were previously produced by other bi-level microbial optimizing methods, on the base of linear programming. Also the algorithm has adjusting parameters so that user has the capability of collation and adjustment with studying problem. In addition, it provided possibility of using non-linear goal function in optimizing on base of differential evolutionary algorithm and also possibility of finding strategy of metabolic engineer that cause to efficiency of optimizing production in microbial system.

Keywords
Differential evolutionary algorithm, Optimizing microbial metabolism, Metabolic modeling.

1. INTRODUCTION
Microorganisms are extensively used for antibiotic production, curing protein, food additions, fuel, vitamins, biotic polymers, and other biochemical substance. For economic production of desirable yield is possible by cell-molecular systems, usually need to improve microorganism metabolism of yield producer. Because they have developed generally in the kind, that accessible resources are using for maximum biomass production. Amendment of microbial metabolic is performed conventionally through classic methods of improvement strain including random and screen mutation however rational design strategy on base of genetic engineer was performed with increasing success for strain amendment in recent years, which is usually, said metabolic engineer. Meanwhile analysis methods of developed vital systems in biology system beside mass received information of biological can have caused to form techniques on base of algorithm for predicting biotic behavior of industrial strain and optimizing of metabolic goal function through recombinant of gene system. The possibility of analysis of systematic instruction and chemical intera-cellular transaction causes to light limitations and potential of microbial system in compliance of desirable metabolic goal. So that more elected and flexibility are in forward for decreasing limitation and increasing metabolic capacity of system. Calculative biology, with indicating two instruments set, provided the possibility of metabolic analysis and amendment of metabolism function of microbial test. The first instrument was mathematic and computerized model of biotic organism and the second instrument was analyzing algorithm of microbial model. [1-7] . stoichiometry models of genome scale is indicating comprehensive metabolic potential of one microorganism. In the other words genome models of mass balance and stoichiometry equation scale with companion topologic structure of metabolic net of special microbial strain use, so the largest distribution set of accessible flux potential provides for cellule. This set is including all of possible metabolic phenotypes steady state. Since so many reactions happen in cellular metabolism, the dimension of solution space ,or number of possible metabolic phenotype, is so much ,which is definite with, scale genome model [8-9]. So that because of complexity synthetic problem, analysis all possible metabolic phenotypes on base of microbial genotype is impossible [13] .Evolutionary algorithm is using from Darwinism evolutionary principles for searching evolution through mutation and reproduction and finding the absolute optimum solution. Direct relationship of evolutionary algorithms with biological evolution changes it to one natural method for selecting amendment genetical scenario for optimizing phenotype. Using of evolutionary search method has two more advantage than linear programming methods. First, these methods need less time for analysis of searching space to finding optimum solution. so that, solution of large scale problem is possible in less time, which have special importance in relationship with analysis scale genome models of microbial system, because relationship between size problem (defined with number of enzymes and deleting gene) and the corresponding researching region (model dimensions and composition of the enzyme that can delete) is synthetic connection. Consequently, the number of composition of deletion is four reactions in a model with 300 reactions more than $7.9*10^9$. Mean while accessible scale genome model have more numerous reactions. Second formulation on the base of evolutionary algorithm can provide possibility optimizing of nonlinear function that is considered on the viewpoint of economic. The amount of production to time unit is one example fore efficiency problems. One of the methods

for determining metabolic phenotype ,flux that pass from all of the metabolic reaction, is using of flux balance analysis (FBA) [10, 12]. In FBA a special flux or linear composition of different flux as goal function in model optimizes through linear programming which causes to achieve one solution for flux distribution in all metabolic reaction.Evolutionary during life history, metabolic adjustment net of microorganisms is evolutionary in the way that microbial organism follows optimum using of accessible resources for maximizing growth as metabolism goal. Surveyed evident shows that microbial systems as general follow from optimum using of accessible resources for achieving to maximum rate growth as metabolism optimum goal [14-17]. So with infusion from the phenotype character of microbial systems, maximizing biomass production is usually using as optimizing goal function in FBA analysis. The models on the base of FBA analysis have extensively used in most of successful studies which its goal supporting desirable phenotype in cellular metabolism. Some instances that can mention are including: produced penicillin by *penicilium Chyrysogenum* [18], *Corynebacterium melassecola* growth on glucose, fructose [19], secreted lateral product by *E.coli* in different rate of giving oxygen[20], secreted acetic acid by *E.coli* in maximized of ATP production[21], increased yield of Amino Acid production by *Corynebacterium glutamicum*, omitting phosphorus in ware infiltration and improvement of culturing animal cells in large scale[22-27], amended succinic acid by *E.coli* [28-30] and *M. cunicproducens* [34], increased yield of lactic acid production by *E.coli* [29], improvement of yeast S.cerevisiae for full yield bioethanol production [32] and rebuilding of E.coli strain that produces lycoyin[33]. General operation in using of FBA analysis in above studying is comparable of disarray genotype and phenotype for finding scenario on base of imposed of genetical variation in microbial genome for achievement to desirable phenotype. This scenario includes extensive range of available methods in instrument boxes of metabolic engineer that is used in recombinant technology including delete gene from microbial genome, add external gene to DNA cell and exhibition and support express gene. However, in recent years, strategies of rational design on base of genetic engineer with increasing rate have substituted traditional methods of random and scrining. In many cases, improved archived yield by the technique have limited. Because these methods lean on manipulation terminals of biocyentatase main direction of wanting yield principally. Although microbial metabolism is usually changing by genetic adjustment, which limits with laws of mass and energy changing on numerous cell metabolites. This subject hardens predicting the effect of a special genetic on special behavior. In Addition because of metabolic rout and dependent adjusting process constitute a complication molecular and transaction net [35-36]. Predicting quantities and quality of one special genetic amendment is possible only by total metabolism analysis in cast of one comprehensive system. On this basis, attempts have done for orienting problem of flux carbon from central metabolism to secondary metabolisms with considering general view of metabolism [37]. However the predicting synergy variation of multiple genetical effects at the same time which exacted on central metabolism and also fixing behavior of redesign organism in different laboratory condition have been difficult [38]. Above difficulties - originated from high complicated biotic systems- extention analysis methods that can consider the effect of multiple genetic amendments on metabolic net of one microbial producing system, is unavoidable systemically. Extended algorithm by Maranas *et al* [39-40] which is named OptKnock is one of the earliest frames of rational model for suggesting gene deletion scenario that causing to increase a special metabolite. OptKnock is searching the collection of gene ,reactions, that deletions of them cause to increase flux to special product and maximize biomass production at the same time. So that deletion the introducing gene compels the microorganism to product a production from general phenotype path of microbial systems ,maximizing growth rate. In reality biologic philosophic hidden in OptKnock uses natural advantage of microbial metabolism characteristic until proceed the metabolism to optimize of favoring phenotype. Relationship between multi goals of OptKnock with developed biological goal function change it to one attractive modeling frame for metabolic engineer in computer. Bilevel linear optimizing problem with using Mixed Integer Linear Programming (MILP), formulates OptKnock. In this study with fusion of hidden biologic philosophic in OptKnock is introducing the new optimizing formulate on base of mixing metabolic flux analysis with searching development methods of disconnected space for redesigning microbial producing systems. This formulation, unlike OptKnock that uses linear programming methods in optimizing two levels, in internal surface which its goal is optimizing microbial growth uses linear programming methods, and in external surface which its goal is optimizing the production of desired metabolism uses differentials evolutionary algorithm. It must cared that analytic engine is mixed integer linear programming (MILP) in most above frames. This analytic method has two faults. The first MILP is principally slow method and the second it produce only one optimum solution ,overall optimum. While we will see the nets with thousands reaction and metabolite with companion large scale nets of adjusting trascriptomy in near future with increasing extension and development the metabolic nets. While optimizing time scale with using Mixed Integer Linear Programming(MILP) on available nets ,specially bilevel optimizing, is multiple hours and it is clear that leaning on linear programming for optimizing much more than extensive nets will enlarge the studying time scale. In this study new instrument is extended with using differential evolutionary(DE) algorithm.

2. METHODS

2.1. Differential evolutionary (DE) optimization algorithm

Differential evolutionary(DE) algorithm is optimizing microbial frame on base of bilevel optimizing and using DE algorithm. Before introducing the way of analysis function need to survey the rational researching methods on base of extended evolutionary strategy. Differential evolutionary algorithms is a partly new optimizing techniques, which enumerate one easy strategy for using in continues and discontinues space. Also is fast and permanent in solution of numeral optimizing problem. This algorithm is a parallel searching method that uses NP vector of population with following *parameter D.*

$$X^{G}_{i,\ i=1,2,\ldots,NP}$$

G is symbol of population for each generation. NP do not change during optimizing, primary population vector was selected randomly and covers all parameter space. Therefore, it is hypothesized that a steady likelihood distribution is indefeasible for all random decision. DE produce vectors of new parameter with adding symphonic differences between two vectors of population to third vector, which we say to this operation mutation. Then, until forming test vector, mutated vector parameters composite with one determined vector ,goal

vector. Composition of parameters usually named crossover or recombination. If test vector impute less amount to expense function, test vector will substitute to goal vector in next generation. Each vector of population is considering as goal function until providing competitive of NP population in one generation.

2.1.1.Mutation operation

For each goal function $X_{i,G,1}$ i=1,2,…,NP, one mutated vector is produced similar following formula.

$$X_{r1,G+1} + F.(X_{r2,G} - X_{r3,G})$$

that random index $r_1, r_2, r_3 \in \{1,2,\ldots,NP\}$ is Integer number and F>0. Integer numbers r_1, r_2, r_3 are selected as way that is different from performing index . Therefore NP must be larger than or equal to 4 for possibility of this claus. F∈[0,2] is a real number and constant factor which control extentive of differentials variable. Figure .1 shows one example of two dimensions those showing different vectors, which is ruling in production of mutated vectors.

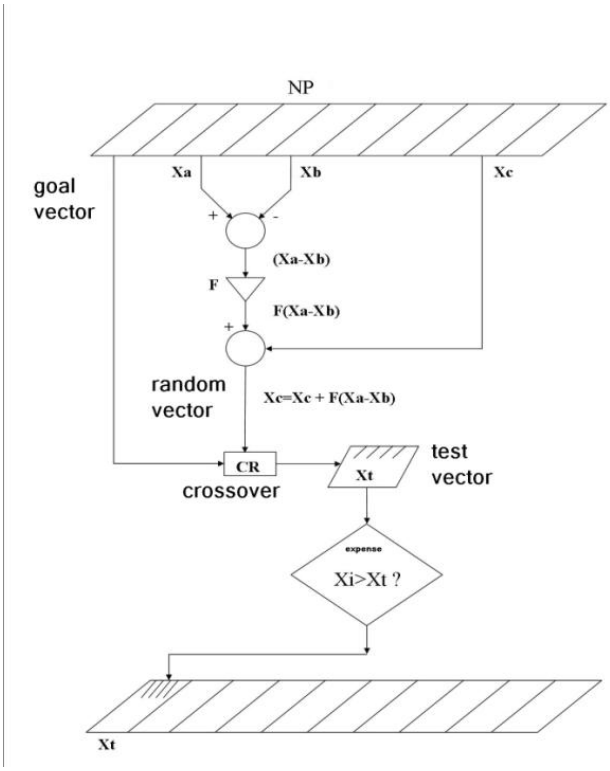

Figure 1: showing off graphic of differentials evolutionary algorithm function

In present modeling, constituted population is including vectors of Individuals (chromosomes) which each of them is comprising sum of deleting genome from microbial organism. Each deleting gene displays in vector of population through its comparison index which adjusting with its presence ordinal number in scale genome model. Mutation operations accomplish with using of change creation in genome composition of each chromosome vectors of population ,the same as above formula.

2.1.2.Crossover

Crossover operations are performed for increasing diversity vectors of mutated parameters. Therefore population vector

$$V_{i,G+1} = (U_{1i,G+1}, U_{2i,G+1}, \ldots, U_{Di,G+1})$$

organize which on that:

$$U_{ji} = \begin{cases} V_{ji,G+1} & \text{if } (randb(i)) < CR \text{ or } j=rnbr(i) \\ X_{ji,G} & \text{if } (randb(i)) > CR \text{ and } j \# rnbr(i) \\ & i = 1,2,\ldots,D \end{cases} \quad \textbf{(1)}$$

in above equation randb(j) is evaluation of produced number from steady producer of random number between 0,1. CR is recombinant constant that determined by user. rnbr(i) ∈ (1,2,….D) is andis ,which have selected from space of parameter D randomly –that guarantee $U_{i,G+1}$ is getting at least one parameter from $V_{i,G+1}$. Figure.1 shows example of integration mechanism for one r-dimensions vector. In present modeling, recombinant operation means substituting chromosome that contains deleting mutation genes from genome instead of previous chromosome or retention chromosome before mutation in population the same as equation (1) condition.

2.1.3.Selecting

for decision about whether test vector $U_{i,G+1}$ can be a member of generation G+1 or not, this vector comparison with goal vector $X_{i,G}$ for amount of expense function , if test vector $U_{i,G+1}$,from the cost function viewpoint, is suitable than goal vector $X_{i,G}$, so $U_{i,G+1}$ is equaled with $X_{i,G+1}$, otherwise remains $X_{i,G}$. In formulations, selecting operations means to fix integration mutated chromosome in population of gene vectors which deletion them cause to improving metabolic goal function. With performing selecting operation organizes new generation containing mutated chromosome or remain chromosome from previous generation that can enter to new evaluating circuit and calculating of mutating and recombinant. This circuit continues until finding the chromosomes (containing deleting genes) that it causes to achieve to suitable phenotype.

2.2. Preparation the model

Evolutionary algorithm avoids from complete and exhausting search of solution space. Therefore, in their designing, suitable formulating is essential for avoiding from trapping in local optimizing point. Hence, metabolic model ready at fist, so the reactions, which their net disconnected with other reactions, do not passed any flux, (The reactions that called dead end) and the reactions, which deleting them cause to stop growth bacterium, is deleting from searching space of evolutionary algorithm. This preparation causes to decrease search space of algorithm considerably and decreases the number of optimum practical solutions. Next step is reading model that including adjust internal oxygen in aerobic conditions or zero internal flux oxygen to model for anaerobic conditions of growth microbe. Used model in present model is model iJR904 that is used in most successful studies for

analysis metabolic net of *E.coli*[45]. This model is including 904 genes and 1075 metabolic reactions and also comprising adjusting information of bacterium genome of *Escherichia coli*.

3. RESULTS AND DISCUSSION

Primary attempt for production of microbial strain with proper yield of organic acid product leant to partly directed methods of deleting competitive pathway and deleting deterrent feedback in biosynthetic pathway [42]. These operations do not bring about increase predicting yield Because of difficulties in readapting adjusting step through mutating methods. At first recombinant technology had focused on terminal pathway of amino acid biosynthesis. This technique primarily 1) strengthen express enzymes that controlling rate 2) enzyme introducing that can provide bypass for adjusting step of biosynthesis path and 3) emphasis on increasing action of primary enzyme in terminal pathway [41-43-44] . limited impression of above method - base on rational analysis of biosynthesis pathway- showed that the strategy of trivial view on metabolism cannot force developed net and permanent metabolic of microbial system to distance from permanent position that originated from evolutionary pressure to favorite phenotype. Most of prominent unsuccessful of above strategy originate from profiting microorganism from saved information in its evolutionary history that able to activate express some of silent genes of metabolic condition retrieval during multi generation before than mutating and neutral the effect of imposed variation. As a result, necessity achievement to overall view from cell metabolism and perception from inner-cell interaction system are unavoidable. Since biotic systems are the systems with high complicating rate, the best solution for achieving to on comprehensive view from their vital mechanism is rebuilding metabolic models and extending analytic instrument for analysis system of this models. Therefore, two strategies modeling were used on base of theories and extended methods in biology system as follow. The first modeling is on base of kinetic data and the second on base of stoichiometry data. In present study genome-scale model *E.coli* iJR904 was used for analyzing of succinic acid synthesis. Also on base of hidden biologic theory in formulation of OptKnock algorithm, new evolutionary algorithm was extended for exploring microbial genome system for finding genes set that deletion them provides achievement to favorite phenotype from favorite phenotype path of microorganism that means growth maximizing. Table .1 shows the result of differentials evolutionary algorithm performance in optimizing microbial system. With performance, this analytic frame on metabolic model *E.coli* determined a set of reaction (genes) which deletion them from the genome bacterium cause to increase succinic acid produced by bacterium. Primary succinic acid flux produced by the bacterium was zero nearly. Performance of differentials evolutionary algorithm cause to find a set of gene, which deletion them from genome increases maximized biomass production dependent with succinic acid flux as way that organism forces to product a minimum succinic acid flux for growth. Here it is essential to care the point that on base of done experiment, deletion none of single gene from *E.coli* bacterium genome is not significant effect on increasing yield of succinic acid production. However, when the set of this genes with each other deleted from bacterium genome , we see improve in organic acid yield numerously. This process, which is named synergy, indicating that the effect of imposed disarray sum on system is more than the effects of each single disarray on system. According to this synergic characteristic is finding a set of deleting reaction, which got is impossible practically by deletion of consecutive reaction and evaluating their synergic effect in strengthen the phenotype.

Because of high complication vital microbial system and numerous searching statuses without gaining from modeling instrument and extended mathematic analysis in systemic biology, Quantity and quality of internal variations that creates the synergic effect –which in our discussion coincide with kind and number of deleting gene of genome, doesn't recognize. In other sentences answer to this question that how many or what groups of genes are in DNA microbe, - which deletion them cause to optimum yield of biologic product such as succinic acid -is possible only through extension and using analytic instrument. Here needs to anslytic frames that able to use high rat rational algorithm analysis the enormous hal and suggest sum of optimum solution. Differentials evolutionary algorithm is the reply to this need as is shown. It is clear with taking such operation progress rate is increasing with severity in redesigning produced microbial systems for new biological product or improving economical biotechnology process. Search space of metabolic engineer in finding a set of validate scenario for adding exogenous reaction to microbial metabolic net through recombinant technology is more extended. While rational designs in recombinant techniques lean to strengthen express enzymes mainly that controll rat or delete limiting feedback of rate in head biosynthesis path of favorite product. Calculation Analytic methods directed by methods show with increasing one or some seeming –non- related reactions with head path can effectively lead carbon flux to producing reaction. This case itself provides opportunity and challenge in improvement of microbial producing system.

One of the opportunities is possibility finding some reaction through thousands recognized metabolic reaction in different microorganism that if adding their corresponding gene to the microbial system of genome, biologic productive yield able to increase even more than available industrial microorganism that is used in biological industry. Nevertheless, change that come forward is how recognize the set of reactions and gene through numerous of biological information.

Differentials evolutionary algorithm can use to encounter this challenge. Differentials evolutionary algorithm can use for reaction set of rational search which add them to metabolic net simultaneous can increase favorite productive yield through the analysis gene express, Also This optimizing frame can use for determining reactions that their decreasing and increasing setting can complete optimization one special metabolite product through optimum growth.

4. CONCLUTION

Differential evolutionary algorithm could produce the same results as OptKnock and other mentioned algorithm in much less time. Scale time of optimizing solution on base of metabolic net decreased from hours OptKnock to minutes by using differential evolutionary algorithm. In other hand, this significant decrease provided possibility to create higher accurate rate than achieve to overall optimization, with letting to searching algorithm in net in wider range of time. In addition, evolutionary algorithm has control variable unlike linear programming which let algorithm reconciles on base of sort problem. Therefore, with controlling evolutionary algorithm such as population number, mutating rate can find optimum adjustment for achieving to overall optimum point in the least time. This means that from all advantages OptKnock can profit in much less time than their needing time is.Additions, as said, linear programming introduce only one

overall optimum point in the end of time. Moreover, debrief from equal optimum points or under-optimum ,which in the viewpoint of engineer, they can also be valuable scenario for laboratory evolution, dependent to use linear cut method which in operation phase accompany with extra calculation and trouble. While on base of user's request, differential evolutionary algorithm can report all equal optimum point or under optimum point until desired optimum level in the end of searching. Therefore, instead of one scenario gain multiple designed scenarios that can cause to create on library in laboratory(web-lab) containing screening designs .

5. ACKNOWLEDGMENTS

The author would like to express his appreciation to the Department of Mechanical and Aerospace Engineering Science and Research Branch, Islamic Azad University, Tehran, Iran for financial support.

Quadratic Reaction Knock-outs	Enzyme	Succinic acid Production Rate (mmol/hr)
Set 1		**9.0466**
CoA + Pyruvate => Acetyl-CoA + Formate	Pyruvate formate lyase	
D-Lactate + NAD <=> Hydrogen + NADH + Pyruvate	D lactate dehydrogenase	
CoA + NAD + Pyruvate <=> Acetyl-CoA + CO2 + NADH	Pyruvate dehydrogenase	
D-Glucose + Phosphoenolpyruvate => Glucose-6-phosphate + Pyruvate	D glucose transport via PEPPyr PTS	
Set 2		**9.0511**
CoA + Pyruvate => Acetyl-CoA + Formate	Pyruvate formate lyase	
D-Lactate + NAD <=> Hydrogen + NADH + Pyruvate	D lactate dehydrogenase	
CoA + NAD + Pyruvate <=> Acetyl-CoA + CO2 + NADH	Pyruvate dehydrogenase	
2 Hydrogen(ex) + NADH + NADP => 2 Hydrogen(in) + NAD + NADPH	NAD P transhydrogenase	
Set 3		**8.0967**
CoA + Pyruvate => Acetyl-CoA + Formate	Pyruvate formate lyase	
D-Lactate + NAD <=> Hydrogen + NADH + Pyruvate	D lactate dehydrogenase	
CoA + NAD + Pyruvate <=> Acetyl-CoA + CO2 + NADH	Pyruvate dehydrogenase	
2-Deoxy-D-ribose-1-phospha <=> 2-Deoxy-D-ribose-5-phosphate	Phosphopentomutase 2 deoxyribose	
Set 4		**8.0641**
CoA + Pyruvate => Acetyl-CoA + Formate	Pyruvate formate lyase	
D-Lactate + NAD <=> Hydrogen + NADH + Pyruvate	D lactate dehydrogenase	
CoA + NAD + Pyruvate <=> Acetyl-CoA + CO2 + NADH	Pyruvate dehydrogenase	
Acetate + ATP => Acetyl-phosphate + ADP	Acetate kinase	
Set 5		**9.3268**
ADP + Hydrogen + Pyruvate => ATP + Pyruvate	Pyruvate kinase	
Acetate + ATP => Acetyl-phosphate + ADP	Acetate kinase	
Acetyl-CoA + Phosphate <=> Acetyl-phosphate + CoA	Phosphotransacetylase	
D-Glucose + Phosphoenolpyruvate => Glucose-6-phosphate + Pyruvate	D glucose transport via PEPPyr PTS	

Table 1: the performance result of differential evolutionary analytic frame, for increasing produced succinic flux, number of deleting gene took four. The first, the second, and the third column shows deleted reactons , reactions name, final siccinic acid flux, respectively. The primary succinic acid had been zero.

6. REFERENCES

[1] Kacser H, Burns JA. 1973. The control of flux. Symp Soc Exp Biol 27: 65–104.

[2] Heinrich R, Rapoport TA. 1974. A linear steady-state treatment of enzymatic chains. Eur J Biochem 42:89–95.

[3] Hatzimanikatis V, Emmerling M, Sauer U, Bailey, JE. 1998. Application of mathematical tools for metabolic design of microbial ethanol production. Biotechnol Bioeng 58:154–161.

[4] Savageau MA. 1969a. Biochemical systems analysis. I. Some mathematical properties of the arte law for the component enzymatic reactions. J Theor Biol 25:365–369.

[5] Voit EO. 1992. Optimization of integrated biochemical systems. Biotechnol Bioeng 40:572–582.

[6] Regan L, Bogle IDL, Dunnill P. 1993. Simulation and optimization of metabolic pathways. Comput Chem Eng 17:627–637.

[7] Torres NV, Voit EO, Gonzales-Alcon C. 1996. Optimization of nonlinear biotechnological processes with linear programming: application to citric acid production by *Aspergillus niger*. Biotechnol Bioeng 49: 247–258.

[8] Price ND, Papin JA, Schilling CH, Palsson BO: Genome-scale microbial in silico models: the constraints-based approach. *Trends in Biotechnology* 2003, 21:162-169.

[9] Patil KR, Akesson M, Nielsen J: Use of genome-scale microbial models for metabolic engineering. *Current Opinion in Biotechnology* 2004, 15:64-69.

[10] Schilling CH, Letscher D, Palsson BO: Theory for the systemic definition of metabolic pathways and their use in interpreting metabolic function from a pathway-oriented perspective.

[11] Schuster S, Fell DA, Dandekar T: A general definition of metabolic pathways useful for systematic organization and analysis of complex metabolic networks.

[12] Kauffman KJ, Prakash P, Edwards JS: Advances in flux balance analysis. *Curr Opin Biotechnol* 2003, 14:491-496.

[13] Fell DA, Small JR: Fat synthesis in adipose tissue. An examination of stoichiometric constraints. *Biochem J* 1986, 238:781-786.

[14] Ibarra RU, Edwards JS, Palsson BO: Escherichia coli K-12 undergoes adaptive evolution to achieve in silico predicted optimal growth.

[15] Edwards JS, Ibarra RU, Palsson BO: In silico predictions of Escherichia coli metabolic capabilities are consistent with experimental data.

[16] Burgard AP, Maranas CD: Optimization-based framework for inferring and testing hypothesized metabolic objective functions.

[17] Famili I, Forster J, Nielsen J, Palsson BO: Saccharomyces cerevisiae phenotypes can be predicted by using constraint-based analysis of a genome-scale reconstructed metabolic network. *PNAS* 2003, 100:13134-13139.

[18] Henriksen CM, Christensen LH, Nielsen J, Villadsen J. 1996. Growth energetics and metabolic fluxes in continuous cultures of *Penicillium chrysogenum*. J Biotechnol 45:149–164.

[19] Pons A, Dussap C, Pequignot C, et al. 1996. Metabolic flux distribution in *Cornybacterium melassecola* ATCC 17965 for various carbon sources. Biotechnol Bioeng 51:177–189.

[20] Varma A, Boesch BW, Palsson BO. 1993a. Stoichiometric interpretation of *Escherichia coli* glucose catabolism under various oxygenation rates. Appl Environ Microb 59:2465–2473.

[21] Delgado J, Liao JC. 1997. Inverse flux analysis for reduction of acetate excretion in *Escherichia coli*. Biotechnol Progr 13:361–367.

[22] Xie L, Wang D. 1994a. Applications if improved stoichiometric model in medium design and fed-batch cultivation of animal cells in bioreactor. Cytotechnology 15:17–29.

[23] Xie L, Wang D. 1994b. Stoichiometric analysis of animal cell growth and its application in medium design. Biotechnol Bioeng 43:1164–1174.

[24] Xie L,Wang D. 1996a. Material balance studies on animal cell metabolism using stoichiometrically based reaction network. Biotechnol Bioeng 52:579–590.

[25] Xie L,Wang D. 1996b. Energy metabolism and ATP balance in animal cell cultivation using a stoichiometrically based reaction network. Biotechnol Bioeng 52:591–601.

[26] Xie L, Wang D. 1996c. High cell density and high monoclonal antibody production through medium design and rational control in a bioreactor. Biotechnol Bioeng 51:725–729.

[27] Xie L, Wang D. 1997. Integrated approaches to the design of media and feeding strategies for fed-batch cultures of animal cells. Trends Biotechnol 15:109–113.

[28] In silico metabolic pathway analysis and design: succinic acid production by metabolically engineered Escherichia coli as an example. Genome Inform. 2002;13:214-23.

[29] In silico design and adaptive evolution of Escherichia coli for production of lactic acid. Biotechnol Bioeng. 2005 Sep 5;91(5):643-8.

[30] Genome-scale in silico aided metabolic analysis and flux comparisons of Escherichia coli to improve succinate production. Appl Microbiol Biotechnol. 2006 Dec;73(4):887-94. Epub 2006 Aug 23.

[31] Modeling Lactococcus lactis using a genome-scale flux model. BMC Microbiol. 2005 Jun 27;5:39.

[32] In silico aided metabolic engineering of Saccharomyces cerevisiae for improved bioethanol production. Metab Eng. 2006 Mar;8(2):102-11. Epub 2005 Nov 10.

[33] Construction of lycopene-overproducing E. coli strains by combining systematic and combinatorial gene knockout targets. Nat Biotechnol. 2005 May;23(5):612-6. Epub 2005 Apr 10.

[34] Genome-scale analysis of Mannheimia succiniciproducens metabolism. Biotechnol Bioeng. 2007 Jul 1;97(4):657-71.

[35] Patil KR, Nielsen J: Uncovering transcriptional regulation of metabolism by using metabolic network topology. *PNAS* 2005, 102:2685-2689.

[36] Ideker T, Thorsson V, Ranish JA, Christmas R, Buhler J, Eng JK, Bumgarner R, Goodlett DR, Aebersold R, Hood L: Integrated genomic and proteomic analyses of a systematically perturbed metabolic network.

[37] Flores N, Xiao J, Berry A, Bolivar F, Valle F. 1996. Pathway engineering for the production of aromatic compounds in Escherichia coli. Nat Biotechnol 14:620–623.

[38] Chandran SS, Yi J, Draths KM, Von Daeniken R, Weber W, Frost JW. 2003. Phosphoenolpyruvate availability and the biosynthesis of shikimic acid. Biotechnol Progr 19:808– 814.

[39] Burgard AP, Pharkya P, Maranas CD: Optknock: A bilevel programming framework for identifying gene knockout strategies for microbial strain optimization. *Biotechnol Bioeng* 2003, 84:647-657.

[40] Pharkya P, Burgard AP, Maranas CD: OptStrain: a computational framework for redesign of microbial production systems. *Genome Res* 2004, 14:2367-2376.

[41] Stephanopoulos G, Vallino JJ. 1991. Network rigidity and metabolic engineering in metabolite overproduction. Science 252:1675– 1681.

[42] Ikeda M. 2003. Amino acid production processes. Adv Biochem Eng Biotechnol 79:1– 35.

[43] Nathan D. Price[1], Jennifer L. Reed[1] & Bernhard Ø. Palsson, Genome-scale models of microbial cells: evaluating the consequences of constraints. *Nature Reviews Microbiology* 2, 886-897.

[44] Varma A, Palsson BO: Stoichiometric flux balance models quantitatively predict growth and metabolic by-product secretion in wild-type Escherichia coli W3110. Appl Environ Microbiol. 1994 Oct;60(10):3724-31.

[45] Reed JL, Vo TD, Schilling CH, Palsson BO. An expanded genome-scale model of Escherichia coli K-12 (iJR904 GSM/GPR). Genome Biol. 2003;4(9):R54. Epub 2003 Aug 28.