ENHANCED DYNAMIC FLUX VARIABILITY ANALYSIS FOR IMPROVING GROWTH AND PRODUCTION RATE IN MICROBIAL STRAINS

MOHAMMAD FAHMI ARIEEF BIN KHAIRIL ANUAR

A thesis submitted in fulfilment of the requirements for the award of the degree of Master of Philosophy

School of Computing
Faculty of Engineering
Universiti Teknologi Malaysia

AUGUST 2018

“My dearest dad, mum, brother, sister and friends”

This is for all of you

ACKNOWLEDGMENT

Firstly, I would like to express my deepest gratitude and praise to God for giving me such a wonderful blessing and without His blessing, I may not be able to complete this research. In particular, I would like to express my sincere thanks to my supervisors, Dr. Azurah Binti A.Samah and my co-supervisor, Prof. Dr. Mohamad Saberi Mohamad, and Prof. Dr. Safaai Deris for their guidance, support and advice despite their busy schedule.

I also would like to express my thanks to my family for their endless care and support that have given me strength in completing this research. Besides, I am grateful to have friends that have been a great assist and always share their knowledge, experiences and suggestions to me. My special thanks to everyone in AIBIG for their helpful comments and suggestion during the weekly seminar.

ABSTRACT

Metabolic engineering is highly demanded currently for the production of various useful compounds such as succinate and lactate that are very useful in food, pharmaceutical, fossil fuels, and energy industries. Gene or reaction deletion known as knockout is one of the strategies used in *in silico* metabolic engineering to change the metabolism of the chosen microbial cells to obtain the desired phenotypes. However, the size and complexity of the metabolic network are a challenge in determining the near-optimal set of genes to be knocked out in the metabolism due to the presence of competing pathway that interrupts the high production of desired metabolite, leading to low production rate and growth rate of the required microorganisms. In addition, the inefficiency of existing algorithms in reconstructing high growth rate and production rate becomes one of the issues to be solved. Therefore, this research proposes Dynamic Flux Variability Analysis (DFVA) algorithm to identify the best knockout reaction combination to improve the production of desired metabolites in microorganisms. Based on the experimental results, DFVA shows an improvement of growth rate of succinate and lactate by 12.06% and 47.16% respectively in *E. coli* and by 4.62% and 47.98% respectively in *S. Cerevisae*. Suggested reactions to be knocked out to improve the production of succinate and lactate have been identified and validated through the biological database.

ABSTRAK

Kejuruteraan metabolik kini telah menjadi suatu permintaan yang amat tinggi untuk menghasilkan sebatian yang berguna seperti suksinat dan laktat yang sangat berguna dalam industri pemakanan, farmasi, bahan api fosil dan tenaga. Teknik penghapusan gen atau reaksi merupakan salah satu strategi yang digunapakai dalam kejuruteraan metabolik silico yang mampu mengubah metabolisma sel mikrobial yang dipilih untuk mendapatkan fenotip yang dikehendaki. Walau bagaimanapun, saiz dan kerumitan rangkaian metabolik menjadi satu cabaran dalam menentukan set reaksi yang hampir optimal untuk dihapuskan dalam metabolisme kerana adanya reaksi yang mengganggu penghasilan yang tinggi metabolit yang dikehendaki, yang membawa kepada kadar pengeluaran yang rendah dan kadar pertumbuhan mikroorganisma yang diperlukan. Di samping itu, ketidakcekapan algoritma sedia ada dalam membina semula kadar pertumbuhan dan kadar pengeluaran yang tinggi menjadi salah satu isu yang perlu diselesaikan. Oleh itu, kajian ini mencadangkan algoritma *Dynamic Flux Variability Analysis* (DFVA) untuk mengenal pasti set reaksi terbaik untuk meningkatkan pengeluaran metabolit yang dikehendaki dalam mikroorganisma. Berdasarkan keputusan kajian, DFVA menunjukkan peningkatan dari segi kadar pertumbuhan suksinat dan laktat masing-masing sebanyak 12.06% dan 47.16% dalam *E. coli* dan 4.62% dan 47.98% masing-masing di *S. Cerevisae*. Reaksi yang dicadangkan untuk disempurnakan dalam meningkatkan pengeluaran suksinat dan laktat telah dikenal pasti dan disahkan melalui pangkalan data biologi.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

BiGG	-	Biochemical Genetic and Genome
COBRA	-	Constraint Based Reconstruction Analysis
DBFBA	-	Differential Bees and Flux Balance Analysis
DFBA	-	Dynamic Flux Balance Analysis
DFVA	-	Dynamic Flux Variability Analysis
DOA	-	Dynamic Optimization Approach
E.coli	-	Escherichia coli
FBA	-	Flux Balance Analysis
FVA	-	Flux Variability Analysis
GA	-	Genetic Analysis
KEGG	-	Kyoto Encyclopedia of Genes and Genome
KO		KEGG Ortholog
LP	-	Linear Programming
MFC	-	Microbial Cell Factory
MILP	-	Mixed Integer Linear Programming
MOMA	-	Minimization of Metabolic Adjustment
NLP	-	Non Linear Programming
ODE	-	Ordinary Differential Equation
QP	-	Quadratic Programming
ROOM	-	Regulatory On/Off Minimization
S.cerevisae	-	Saccharomyces cerevisae
SBML	-	System Biology Markup Languange
SGD		Saccharomysces Genome Database
SOA	-	Static Optimization Approach

LIST OF SYMBOLS

gDCW	-	gram dry cell weight
h	-	hour
mmol	-	millimole
P	-	Probability
S	-	stoichiometric matrix
$\mathcal{S}_N$	-	Standard deviation
v	-	Flux vector
X	-	the population of the colony size
Xn	-	individuals in the population of the colony size
Z	-	objective function

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Overview

This chapter introduces the concept of succinate and lactate production in *Escherichia Coli (E.coli)* and *Saccharomyces cerevisiae (S.cerevisiae)*. Prior knowledge on this matter is important because this research mainly focused on optimizing the production of succinate and lactate from *E.coli and S.cerrevisiae.* Furthermore, this chapter also explains basic knowledge behind the production of succinate and lactate, as well as other related information that will give us adequate knowledge in order to achieve the goal and objectives of this research. In addition, the aim, objectives, scope are also included to give a clear view of this research.

1.2 Introduction

Metabolic engineering has shown a big impact and nowadays is getting more popular. Metabolic engineering has been used to study and manipulate the biological microbial cell metabolism by many researchers in this area. An example of strategy

that has been introduced by metabolic engineering method is by suggesting any genes or reactions from its complex metabolic network to be removed from its own pathway. (Azhar *et.al*, 2016). This technique has shown many improvement in genome-scale metabolic network model in addressing high yield of by-product secretion and cell growth rate.

To conduct gene knockout experiment in wet laboratories is very time consuming and costly because it deals with numerous number of genes and various microorganism strain. Some of the information of the genes within these microbial strain such as the function are generally known. However, the problem arise when the knockout requires the combination of these genes in order to obtain the optimal design. For that reason, the modelling approaches are required to predict the best set of candidate genes from the genome scale model to ensure feasible solution that can be obtained in shorter time and avoid the intuitive experiments (Mahalik et al.,2014).

The current technology in metabolic engineering has gained more attention as it is able to improve any desired metabolites strain that can be used by other process to become a valuable products to be marketed in industries (Nielsen & Keasling.,2016). With such results, there are more improvements of the models and algorithms by using computational approaches are popular in these recent years. However, the current production rate achieved by the researchers is still low than its theoretical maximum value due to the lack of effective computational method developed to modify the metabolic model of the microorganisms. Even the current modification of biological network of an organism has become a successful technique, constructing a mutant strain of genome model is a big challenge to increase the production of the desired metabolites beyond its wild type limit.

Metabolic network are very big in size, thus this may lead to the increasing of the computational time needed to compute all the networks. Thus, some of the computationally pre-process steps are required, which this may matches with the biological theory to have more suitable, less complex and compatible data. Other than that, the models need to undergo an optimization process in order to prevent the solution from being trapped in local optima where it may cause a premature convergence. The potential to increase the production of targeted metabolites is obscure, and relates to the lack of genome models. On the other hand, the presence of competing pathway of non-desirable product may affect the desired metabolite production.

The main reason of using current advance computational approach in latest research is because it is powerful and very helpful to save costs and time in manipulating phenotype to enhance the desired strain model compared to traditional laboratory procedures. The goal of metabolic engineering is to develop an effective methods which can improve the metabolic environment in order to produce desired metabolites in microorganism for industrial purpose, (Gupta et al., 2016). Industries nowadays use microbial strains in order to produce a large amount of biochemical products, antibiotics, drug targets, therapeutic proteins, food ingredients, vitamins, fuels, and other useful chemicals.

1.2.1 Gene Knockout Strategy

Any genes in the organisms that has been genetically knockout, missing or deleted completely from the organism was an explanation of gene knockout technique. Some part of the gene from the sequenced gene characteristics can be learned by researchers by using this technique.

The term *knockout* refers to knocking out a gene by generating a new mutant for specific microorganism. In this technique, there are doubled, tripled and quadrupled knockout which indicates the knockout genes is two, three and four. The gene knockout technique is used to test the isolated gene functions. Thus, knockout technique does not happen when repeated reaction or function of multiple genes appeared. Today this technique has widely used for commercial use for specific genes function.

1.2.2 Overview of Microbial Cell Factory for Metabolites Productions

Microbial cell factory can be defined as microorganism that is assumed as a factory that contains of many compartments that can produce certain product. In metabolic and genetic engineering field, these microorganism has been utilized not only to be used in the production of antibiotics, vitamins, enzymes and other useful chemicals, but this helps some of the problems such as energy resources (Azhar et al., 2016).

Traditional production is very limited by using the native organism that only produced naturally of the desired products and most of the organism are less productive for industrial fermentation. With the drastically increasing of genomic information resources, metabolic engineering techniques and advanced tools have made it possible to construct microbial cell factories using non-native producer organism which may help the organism to go beyond its capabilities than the native producer.

Biofuels are one of the well-known resources to produce energy. One of the methods that produce ethanol is biomass fermentation. Biomass fermentation requires a large size of plantation area to plant corn or sugarcane in order to produce starch and sugar. Besides, rice bran is also one of the great potential material in ethanol production (Michel *et al.,* 2016). Unfortunately, the process of fermentation brought up pollution issues such as deforestation for the plantation of sugarcanes or

corns. Thus, in order to avoid most of the problems to produce ethanol, metabolic engineering on microorganism was introduced to produce ethanol by using microbe. This is due to a large number of microbes is easy to be cultivated in the laboratory and it is cheap.

Recent studies show that *E. coli* is very suitable for producing bioethanol since it is a gram-negative bacterium and has the ability to convert sugar into ethanol in high yield. Lactate has been used in a large area of processed food, cosmetics, oral and health care products and industrial applications. Besides, lactate is produced commercially by using several microorganisms (John *et al.*, 2007), such as Lactobacillus strains. In addition, simple nutritional and host production under aerobic and anaerobic conditions are one of the *E. coli* advantageous characteristics. Moreover, *E. coli* development as a host production of lactate enabled physiology of microbe with large knowledge and high established protocols for genetic manipulation (Chang *et al.*, 1999). On the other hand, under anaerobic condition, lactate, acetate, ethanol, succinate and formarate in *E. coli* are the yields of glucose mixed acid fermentation. Thus, *E. coli* is highly recommended since it is high established and an enormous knowledge explored for the physiology of *E. coli* enables it to use for genetic manipulation. In addition, gene knockout technique which made inactivation to the non-functional gene had being applied on the microorganism to boost the *E. coli* production of ethanol and lactate (Chang *et al.*, 1999).

The present computational study can identify detailed systems biology and able to simulate metabolisms of bacteria and other microorganisms about their capability in producing metabolites such as H2 and L-Phe in the mutant strain. This refers to the extensive search for reaction or gene to be added or deleted in order to increase the desired production. The intention of this research is to examine how metabolites production in microorganism can be increased using a systematic *in silico* simulation of metabolic engineering strategy, for instance, constraint-based modeling algorithms. Therefore, to elucidate interesting features of these microorganisms and identify engineering targets to achieve enhanced physiological properties of the strain, metabolic engineering which applies modeling simulation and optimization with the involvement of reaction deletion strategy is applied.

1.3 Background of Problem

The current technology in metabolic engineering strategies in enhancing cell factories become a big issue in this era. However, there are still some limitations of the current method that has not been solved yet (Azhar et al., 2017). The challenges and problems faced in metabolic engineering are predominantly caused by the complexity of the metabolic network. The complexity problems affected the production of our desired metabolites where there are many competing reactions that present. Hence in order to achieve a desired metabolic product using the genetic modification method become more difficult (Rosano and Ceccarelli, 2014). According to (Jantama et al., 2015), the production of succinate and lactate from wild-type cell is currently low. This shows that the lack of effective genome models which can do prediction and simulation of low production yields is very important aspect in order to achieve the highest fitness of objective function.

However, previous existing algorithm that had been constructed still have limitations and weaknesses. For example, Minimization of Metabolic Adjustment (MOMA) (Ye et al., 2015), and Flux Balance Analysis (FBA) (Chapman et al., 2015), have limitations in terms of flux distribution strategy. FBA gives unrealistic flux distribution value (Chapman et al., 2015) and unreliable in predicting the flux value by-product. Hence, MOMA on the other side is very weak in predicting the final steady state of growth. MOMA also is unable to represent the true metabolite state of the organisms (Ye et al., 2015). In the simplest way, the best combination can be found by evaluating all possible combination using exhaustive search. Although it guarantees to find the near optimal solution, it is very time consuming and impractical even for moderate size of solution space (Arieef *et al.*,2017).

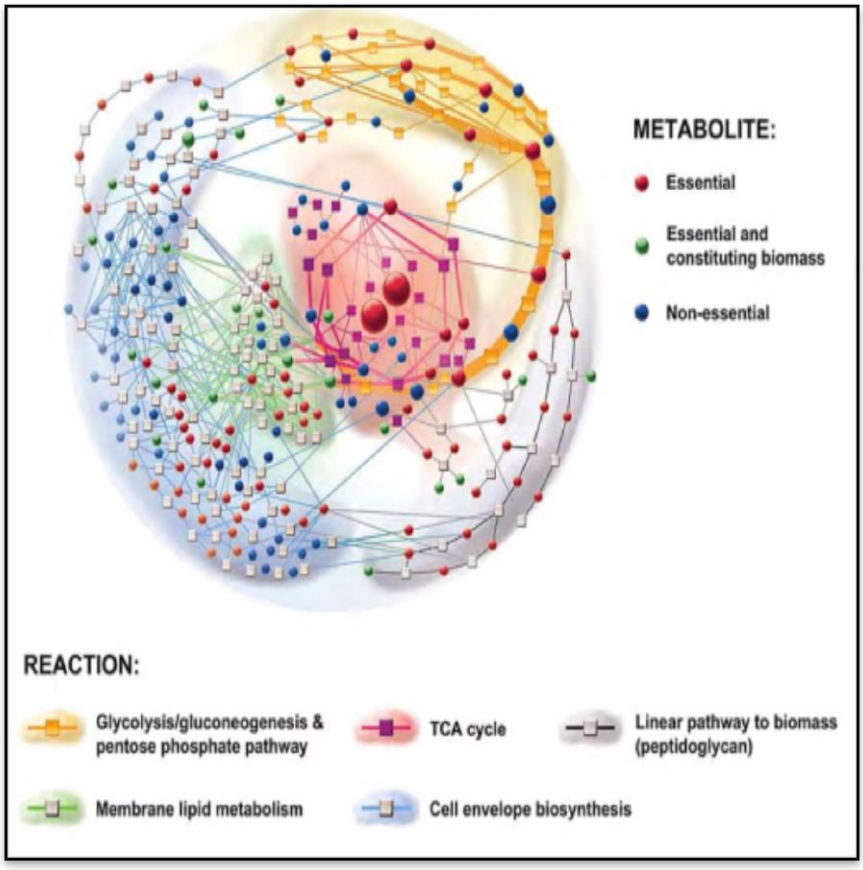

Figure 1.1 An example of E. coli metabolism map (Kim et al, 2016).

The aforementioned issues relate to the algorithmic used by previous researchers to study in silico metabolic engineering. With regards to the complexity of the metabolic network as shown in figure 1.1, this directly results in low production yield due to the presence of competing for non-desirable compounds. This can relate to the low accuracy of existing algorithms in constructing solutions to acquire the best value. There are numbers of developed algorithms used to simulate flux distribution of particular metabolites from cell factories of the genome-scale model that can directly be applicable for many industrial purposes either by standard or hybrid algorithms (Arieef *et al*.,2017).

These limitations also affect the lack of effective genome model constructed in order to generate better value for the objective function. In addition, wild-type model can only predict low flux value. Thus, an improved modelling algorithm with new modified genome model is important to be highlighted. FVA (Müller and

Bockmayr, 2013) outperform MOMA and FBA as it calculates the full range of flux distribution value while maintaining high growth rate and optimizing the objective function. In addition, FVA explores alternate solution in the optimal flux space but there is not only one optimal flux distribution can be found (Hay and Schwender, 2011). This indicates that FVA methodology is more detailed and demonstrate realistic flux value in predicting possible by-product production rates under maximal biomass production (Müller and Bockmayr, 2013).

In order to clarify the potential of metabolites strain production, several computational algorithms have been introduced. This is because in silico or computational simulation is preferred as less time is required, no labor involved, less research expenditure, and eventually, impose cost reduction (Salleh et al., 2015).

1.4 Problem Statement

To conduct gene knockout experiment in wet laboratories is very time consuming and costly because it deals with numerous number of genes and various microorganism strain. The intuitive experiments may also lead to generate undesirable results and sometimes the production is lower than the theoretical yield. An experiment conducted on a strain cannot be transferred to another strain thus the whole process need to be repeated again. In silico modelling approaches are required in order to aid the process of strain improvement so that optimal production of desired metabolites can be achieved. Some of the information of the genes within these microbial strain such as the function are generally known however, the problem arise when the knockout requires the combination of these genes in order to obtain the optimal design. For that reason, the modelling approaches are required to predict the best set of candidate genes from the genome scale model to ensure feasible solution can be obtained in shorter time and avoid the intuitive experiments.

Previous approaches are limited to cases that the lower-level optimization problem is linear (Gomez et al. 2014; Harwood et al. 2016; Hoeffner et al. 2013). For nonlinear objective functions, one cannot directly apply the existing approaches. Typical nonlinear objective functions include, the minimization of the overall intracellular flux and the maximization of ATP yield per flux unit (Mori et al. 2016). A solution algorithm for DFBA model with embedded LP has been previously proposed (Gomez et al. 2014; Harwood et al. 2016; Hoeffner et al. 2013), in which the lower-level optimization problem contains a sequence of LP. This solution algorithm is based on the concept of basic feasible points of LP and therefore it cannot be straightforwardly adapted to solve DFBA models with NLP embedded. Therefore this study proposed DOA and SOA method in FVA to overcome the problems.

1.5 Research Questions

The main problem of this research is the complex and large metabolic network and the challenging to optimize the production of the microbial strains. Thus, this research intends to address the aforementioned problems based on the following research questions:

i. How to reduce the metabolic network complexity in order to optimize the succinate and lactate production?

ii. How to analyze the performance of the proposed algorithm in optimizing the metabolites production?

1.6 Objectives

To achieve the aim of this research, the objectives are specified as follows:

1. To enhance the Flux Variability Analysis algorithm by implementing Dynamic Optimization Approach and Static Optimization Approach in order to optimizing the succinate and lactate production.

2. To improve the accuracy of growth rate and production rate in *E. coli* and *S.cerevisae.*

1.7 Scope of Study

Based on the objectives mentioned above, the overall research only focus on this scope of research:

i. Data that is used are taken from Biochemical Genetic and Genome (BiGG).

ii. Format: System Biology Markup Languange (SBML) (Hucka,2004).

iii. Dataset*:* *E. coli* (iJR904) and *S.cerevisae* (iFF708)

iv. Software: MATLAB, Constraints Based Reconstructions and Analysis (COBRA) Toolbox (Schellenberger et al, 2011).

1.8 Significance of Study

This study is conducted to study and improve the production of metabolites by implementing a computational framework for simulating metabolites synthesis in *E. coli* and *S.cerevisae*. The significant of this research is as follows:

i. Investigate the potential improvement of succinate and lactate in *E.coli* and *S.cerevisae*.

ii. Give a clear insight of metabolites production from *E. coli* and *S.cerevisae* by using computational modelling and analysis that provide better understanding.

iii. Development of an in silico modelling and analysis approach that provide a prediction of succinate and lactate production in *E. coli* and *S.cerevisae* that improve the metabolites yield.

1.9 Thesis Organization

i) Chapter two presents literature reviews retrieved from published journals and other available sources on the existing algorithms that are used in analyzing the genome-scale metabolic model. Details about metabolic engineering that consist of different groups related to it such as constraint-based analysis, optimization algorithm, and modeling framework are discussed comprehensively. Reading materials that relate to this research topic with beneficial and helpful information, such as journals, articles, and conference working papers are listed, too.

ii) Chapter three discusses the research methodology as a planning form used to conduct this research. The detailed descriptions of activities involved are presented and divided according to particular phases for easy understanding. The information about the data set is clarified in this study. Basic requirements of hardware and software and performance measurement that is used for this research are also presented in this chapter.

iii) Chapter four thoroughly discusses the implementation of the proposed algorithm, which is DFVA. Pre-processing step and preparation of the chosen data sets were also clarified in this chapter. The steps involved in DFVA is also discussed.

iv) Chapter five discusses the results of the proposed algorithm accompanied with thorough explanation about the list of reactions and genes suggested to be deleted are also included in this presentation. The comparison between this method and previous works are also presented in this chapter.

v) Chapter six summarizes the content of previously discussed chapters. Conclusion, contributions, and limitations of this research are also being discussed.

REFERENCES

Alpher, H., Jin, Y.S., Moxley,J.F. and Stephanopoulos, G. (2005). Identifying gene targets for the metabolic engineering of lycopene biosynthesis in Escherichia Coli. *Metabolic Engineering*. 7:155-164.

Andreozzi, S., Chakrabarti, A., Soh, K. C., Burgard, A., Yang, T. H., Van Dien, S. & Hatzimanikatis, V. (2016). Identification of metabolic engineering targets for the enhancement of 1, 4-butanediol production in recombinant E. coli using large-scale kinetic models. *Metabolic engineering*, 35, 148-159.

Apaydin, M., Zeng, B., & Qian, X. (2016). A reliable alternative of OptKnock for desirable mutant microbial strains. *In Biomedical and Health Informatics (BHI), 2016 IEEE-EMBS International Conference on (pp. 573-576).* IEEE.

Arieef, M. F., Kasim, S., Choon, Y. W., Mohamad, M. S., Deris, S., & Napis, S. (2017). A Hybrid of Integer Differential Bees and Flux Balance Analysis for Improving Succinate and Lactate Production. *International Journal on Advanced Science, Engineering and Information Technology, 7(4-2),* 1615-1620.

Asadollahi, M.A., Maury, J., Patil, K.R.,Schalk, M,. Clark, A. and Nielsen, J. (2009). Enhancing sesquiterpene production in Saccharomyces cerevisae through in silico driven metabolic engineering. *Metabolic Engineering*. 11: 328-334.

Azhar, A. H., Mohamad, M. S., Deris, S., Napis, S., & Sinnott, R. (2016). A Hybrid of Ant Colony Optimization and Flux Variability Analysis to Improve the Production of L-Phenylalanine and Biohydrogen. *International Journal of Advances in Soft Computing & Its Applications, 8(2).*

Baart, G. J., and Martens, D. E. (2012). Genome-scale metabolic models: reconstruction and analysis. *Neisseria meningitidis: Advanced Methods and Protocols, 107-126.*

Borodina, I., & Nielsen, J. (2014). Advances in metabolic engineering of yeast Saccharomyces cerevisiae for production of chemicals. *Biotechnology journal*, 9(5), 609-620.

Brochado, A.R, Matos, C., Moller, B.L., Hansen, J., Mortesen, U.H. and Patil, K.r. (2010). Improved vanillin production in Baker's Yeast Through in silico design. *Microbial Cell Factories*. 9:84.

Chapman, S. P., Paget, C. M., Johnson, G. N., & Schwartz, J. M. (2015). Flux balance analysis reveals acetate metabolism modulates cyclic electron flow and alternative glycolytic pathways in Chlamydomonas reinhardtii. *Frontiers in plant science, 6, 474.*

Chen, Q., Wang, Z. and Wei,D.Q (2010). Progress in the application of flux analysis of metabolic networks. *Chinese Science Bulletin.* 55(22):2315-2322.

Choon YW, Mohamad MS, Deris S, Illias RM, Chong CK, et al. (2014) Differential Bees Flux Balance Analysis with OptKnock for In Silico Microbial Strains Optimization. *PLoS ONE 9(7): e102744. doi:10.1371/journal.pone.0102744.*

Curran, K. A., Crook, N. C., and Alper, H. S. (2012). Using flux balance analysis to guide microbial metabolic engineering. *In Microbial Metabolic Engineering (pp. 197-216): Springer*.

Feist AM, Herrga° rd MJ, Thiele I, Reed JL, Palsson BØ (2009) Reconstruction of biochemical networks in microorganisms. *Nat Rev Microbiol* 72, 129–143.

Fowler, Z.L., Gikandi, W.W. and Koffas, M.A.G. (2009). Increased malonyl coenzyme a biosynthesis by tuning the Escherichia Coli metabolic network and its application to flavanone production. *Applied and Environmental Microbiology. 5831-5839.*

Garcia Sanchez, C.E.,Vargas Garcia, C.A., and Torres Saez, R.G. (2012). Predictive potential of Flux Balance Analysis of Saccharomyces cerevisae using as optimization function combinations of cell compartmental objectives. *PLoS ONE*. 7(8):e43006.

Goldstein, Y. A., & Bockmayr, A. (2015). Double and multiple knockout simulations for genome-scale metabolic network reconstructions. *Algorithms for Molecular Biology*, 10(1), 1.

Gomez, J. A., Höffner, K., & Barton, P. I. (2014). DFBAlab: a fast and reliable MATLAB code for dynamic flux balance analysis. *BMC bioinformatics*, 15(1), 409.

Gonzalez, J. E., Long, C. P., & Antoniewicz, M. R. (2017). Comprehensive analysis of glucose and xylose metabolism in Escherichia coli under aerobic and anaerobic conditions by 13 C metabolic flux analysis. *Metabolic*

engineering, 39, 9-18.

Gu, D., Zhang, C., Zhou, S., Wei, L., & Hua, Q. (2016). IdealKnock: a framework for efficiently identifying knockout strategies leading to targeted overproduction. *Computational biology and chemistry*, 61, 229-237.

Gudmundsson, S., & Thiele, I. (2010). Computationally Efficient Flux Variability Analysis. *BMC bioinformatics*, 11(1), 489.

Gupta, S. K., & Shukla, P. (2016). Advanced technologies for improved expression of recombinant proteins in bacteria: perspectives and applications. *Critical reviews in biotechnology*, 36(6), 1089-1098.

Harwood S, Höffner K, Barton P (2016) Efficient solution of ordinary differential equations with a parametric lexicographic linear program embedded. *Numer Math* 133(4):623–653.

Hay, J., and Schwender, J. (2011). Metabolic network reconstruction and flux variability analysis of storage synthesis in developing oilseed rape (Brassica napus L.) embryos. *The Plant Journal*, 67(3), 526-541.

Höffner, K., Harwood, S. M., & Barton, P. I. (2013). A reliable simulator for dynamic flux balance analysis. *Biotechnology and bioengineering*, 110(3), 792-802.

Jantama, K., Polyiam, P., Khunnonkwao, P., Chan, S., Sangproo, M., Khor, K., ... & Kanchanatawee, S. (2015). Efficient reduction of the formation of by-products and improvement of production yield of 2, 3-butanediol by a combined deletion of alcohol dehydrogenase, acetate kinase-phosphotransacetylase, and lactate dehydrogenase genes in metabolically engineered Klebsiella oxytoca in mineral salts medium. *Metabolic engineering*, 30, 16-26.

Karp, P. D., Paley, S. M., Krummenacker, M., Latendresse, M., Dale, J. M., Lee, T. J., Kaipa, P., Gilham, F., Spaulding, A., Popescu, L., Altman, T., Paulsen, I., Keseler I. M., and Caspi, R. (2010). Pathway tools version 13.0: integrated software for pathway/genome informatics and systems biology. *Brief Bioinform*. 11(1), 40–79.

Kauffman, K.J., Prakash,P., and Edwards,J.S. (2003). Advances in Flux Balance Analysis. *Current Opinion in Biotechnology*. 14:491-496.

Kim, B., Kim, W. J., Kim, D. I., and Lee, S. Y. (2015). Applications of genome-scale metabolic network model in metabolic engineering. *Journal of industrial microbiology and biotechnology*, 42 (3), 339-348.

Kim, J., and Reed, J. (2010). OptORF: Optimal metabolic and regulatory perturbations for metabolic engineering of microbial strains. *BMC Systems Biology*, 4(1), 53.

Kim, J., Fabris, M., Baart, G., Kim, M. K., Goossens, A., Vyverman, W., & Lun, D. S. (2016). Flux balance analysis of primary metabolism in the diatom Phaeodactylum tricornutum. *The Plant Journal*, 85(1), 161-176.

King, Z. A., Lloyd, C. J., Feist, A. M., & Palsson, B. O. (2015). Next-generation genome-scale models for metabolic engineering. *Current opinion in biotechnology*, 35, 23-29.

Kronen, M., & Berg, I. A. (2015). Mesaconase/fumarase FumD in Escherichia coli O157: H7 and promiscuity of Escherichia coli class I fumarases FumA and FumB. *PloS one*, 10(12), e0145098.

Kuepfer, L., Sauer, U., & Blank, L. M. (2005). Metabolic functions of duplicate genes in Saccharomyces cerevisiae. *Genome research*, 15(10), 1421-1430.

Lisha, K., and Sarkar, D. (2014). Dynamic flux balance analysis of batch fermentation: effect of genetic manipulations on ethanol production. *Bioprocess and biosystems engineering*, 37(4), 617-627.

Long, M. R., Ong, W. K., and Reed, J. L. (2015). Computational methods in metabolic engineering for strain design. *Current opinion in biotechnology*, 34, 135-141.

Machado, D., and Herrgård, M. (2015). Co-evolution of strain design methods based on flux balance and elementary mode analysis. *Metabolic Engineering Communications.*

Mahadevan, R., Edwards, J. and Doyle, F. (2002). Dynamic Flux Balance Analysis of Diauxic Growth in Escherichia coli. *Biophysical Journal*, 83(3), 1331-1340.

Mahalik, S., Sharma, A. K., & Mukherjee, K. J. (2014). Genome engineering for improved recombinant protein expression in Escherichia coli. *Microbial Cell Factories*, 13(1), 1.

Maia, P., Rocha, M., & Rocha, I. (2016). In silico constraint-based strain optimization methods: the quest for optimal cell factories. *Microbiology and Molecular Biology Reviews*, 80(1), 45-67.

Mannan, A. A., Toya, Y., Shimizu, K., McFadden, J., Kierzek, A. M., and Rocco, A. (2015). Integrating Kinetic Model of E. coli with Genome Scale Metabolic Fluxes Overcomes Its Open System Problem and Reveals Bistability in Central

Metabolism. *PLoS ONE*, 10(10), e0139507.

Martins Conde, P. D. R., Sauter, T., & Pfau, T. (2016). Constraint Based Modeling Going Multicellular. *Frontiers In Molecular Biosciences*, 3.

Mienda, B. S., Shamsir, M. S., and Salleh, F. M. (2015). In silico Evaluation of the Effect of pfl Gene Knockout on the Production of D-lactate by Escherichia coli Genome Scale Model using the OptFlux Software Platform. *Indian Journal of Science and Technology*, 8(2), 172-177.

Mienda, B. S., Shamsir, M. S., Shehu, I., Deba, A. A., and Galadima, I. A. (2014). In silico metabolic engineering interventions of Escherichia coli for enhanced ethanol production, based on gene knockout simulation.*Gene*,23 (24), 25.

Mori, M., Hwa, T., Martin, O. C., De Martino, A., & Marinari, E. (2016). Constrained allocation flux balance analysis. *PLoS computational biology*, 12(6), e1004913.

Müller, A. C., and Bockmayr, A. (2013). Fast thermodynamically constrained flux variability analysis. *Bioinformatics*, btt059.

Nielsen, J., & Keasling, J. D. (2016). Engineering cellular metabolism. *Cell*, 164(6), 1185-1197.

Nilsson, A., & Nielsen, J. (2017). Genome scale metabolic modeling of cancer. *Metabolic engineering*, 43, 103-112.

O'Brien, E. J., Monk, J. M., & Palsson, B. O. (2015). Using genome-scale models to predict biological capabilities. *Cell*, 161(5), 971-987.

Ohno, S., Shimizu, H., and Furusawa, C. (2014). FastPros: screening of reaction knockout strategies for metabolic engineering. *Bioinformatics*, 30(7), 981-987.

Ortho, J.d., Thiele, I., and Palsson. (2010).What is Flux Balance Analysis. *National Biotechnology.* 28(3):245-248.

Patil, K.R., Rocha, I., Forster, J., and Nielsen, J. (2005). Evolutionary programming as a platform for in silico metabolic engineering. *BMC Bioinformatics*. 6:308.

Pereira, R., Nielsen, J., & Rocha, I. (2016). Improving the flux distributions simulated with genome-scale metabolic models of Saccharomyces cerevisiae. *Metabolic Engineering Communications*, 3, 153-163

Ren, S., Zeng, B., and Qian, X. (2013). Adaptive bi-level programming for optimal gene knockouts for targeted overproduction under phenotypic constraints. *BMC bioinformatics*, 14(2), 1.

Rocha, I., Maia, P., Evangelista, P., Vilaça, P., Soares, S., Pinto, J. P. & Rocha, M.

(2010). OptFlux: an open-source software platform for in silico metabolic engineering. *BMC systems biology*,4 (1), 45.

Roeva, O., Fidanova, S., and Paprzycki, M. (2015). Population size influence on the genetic and ant algorithms performance in case of cultivation process modeling. *In Recent Advances in Computational Optimization* (pp. 107-120).\

Rosano, G. L., & Ceccarelli, E. A. (2014). Recombinant protein expression in Escherichia coli: advances and challenges. *Frontiers in microbiology*, 5, 172.

Saa, P. A., & Nielsen, L. K. (2017). Formulation, construction and analysis of kinetic models of metabolism: A review of modelling frameworks. *Biotechnology advances.*

Salleh, A. H. M., Mohamad, M. S., Deris, S., Omatu, S., Fdez-Riverola, F., and Corchado, J. M. (2015). Gene knockout identification for metabolite production improvement using a hybrid of genetic ant colony optimization and flux balance analysis. *Biotechnology and Bioprocess Engineering*. 20: 685-693.

Sánchez, C., Quintero, J. C., & Ochoa, S. (2015). Flux balance analysis in the production of clavulanic acid by Streptomyces clavuligerus. *Biotechnology progress*, 31(5), 1226-1236.

Segre, D., Vitkup, D. and Church, G.M. (2002). Analysis of optimality in natural and perturbed metabolic networks. *Proc Natl Acad Sci USA*. 99:15112-15117.

Shaw, A. J., Lam, F. H., Hamilton, M., Consiglio, A., MacEwen, K., Brevnova, E. E. & Stephanopoulos, G. (2016). Metabolic engineering of microbial competitive advantage for industrial fermentation processes. *Science*, 353(6299), 583-586.

Shiue, E., Brockman, I. M., & Prather, K. L. (2015). Improving product yields on D- glucose in Escherichia coli via knockout of pgi and zwf and feeding of supplemental carbon sources. *Biotechnology and bioengineering*, 112(3), 579-587.

Shlomi, T., Berkman, O. and Ruppin, E. (2005). Regulatory on/off minimization of metabolic flux changed after genetic pertubations. *Proc Natl Acad Sci USA*. 102:7695-7700.

Song, B., Büyüktahtakın, I. E., Bandyopadhyay, N., Ranka, S., & Kahveci, T. (2011). Identifying enzyme knockout strategies on multiple enzyme associations. *In Bioinformatics-Trends and Methodologies. InTech*.

Varges, F.A., Pizzarro, F., Peres-Correa, J.R. and Agosin, E. (2011). Expanding a dynamic flux balance model of yeast fermentation to genome-scale. *BMC*

Systems Biology. 5:75.

Wahid, N. S. A., Mohamad, M. S., Salleh, A. H. M., Deris, S., Chan, W. H., Omatu, S., ... & Yusof, Z. M. (2016). A Hybrid of Harmony Search and Minimization of Metabolic Adjustment for Optimization of Succinic Acid Production. *In 10th International Conference on Practical Applications of Computational Biology & Bioinformatics* (pp. 183-191). Springer, Cham.

Wang, X., Miller, E. N., Yomano, L. P., Zhang, X., Shanmugam, K. T., & Ingram, L. O. (2011). Increased furfural tolerance due to overexpression of NADH-dependent oxidoreductase FucO in Escherichia coli strains engineered for the production of ethanol and lactate. *Applied and environmental microbiology*, 77(15), 5132-5140.

Wu, W.H., Wang, F.S. and Chang, .M.S. (2011). Multi-objective optimization of enzyme manipulations in metabolic networks considering resilience effects. *BMC Systems Biology*. 5:145.

Xu, Z., Zheng, P., Sun, J., and Ma, Y. (2013). ReacKnock: Identifying Reaction Deletion Strategies for Microbial Strain Optimization Based on Genome-Scale Metabolic Network. *Plos ONE*, 8(12), e72150.

Yang, X. S., Deb, S., Loomes, M., & Karamanoglu, M. (2013). A framework for self-tuning optimization algorithm. *Neural Computing and Applications*, 23: 2051-2057.

Ye, C., Xu, N., Chen, H., Chen, Y. Q., Chen, W., & Liu, L. (2015). Reconstruction and analysis of a genome-scale metabolic model of the oleaginous fungus Mortierella alpina. *BMC systems biology*, 9(1), 1.

Yen, J. Y., Tanniche, I., Fischer, A. K., Gillaspy, G. E., Bevan, D. R., & Senger, R. S. (2015). Designing metabolic engineering strategies with genome-scale metabolic flux modeling. *Clinical Epidemiology*, 7, 149-160.

Zhao, X., Noack, S., Wiechert, W., & von Lieres, E. (2017). Dynamic flux balance analysis with nonlinear objective function. *Journal of mathematical biology*, 75(6-7), 1487-1515.