HYBRID APPROACH FOR METABOLITES PRODUCTION USING DIFFERENTIAL EVOLUTION AND MINIMIZATION OF METABOLIC ADJUSTMENT

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All praises to Allah the Almighty for the strengths and His blessing in completing this thesis.

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ABSTRACT

Microbial strains can be optimized using metabolic engineering which implements gene knockout techniques. These techniques manipulate potential genes to increase the yield of metabolites through restructuring metabolic networks. Nowadays, several hybrid optimization algorithms have been proposed to optimize the microbial strains. However, the existing algorithms were unable to obtain optimal strains because the nonessential genes are hardly to be diagnosed and need to be removed due to high complexity of metabolic network. Therefore, the main goal of this study is to overcome the limitation of the existing algorithms by proposing a hybrid of Differential Evolution and Minimization of Metabolic Adjustments (DEMOMA). Differential Evolution (DE) is known as population-based stochastic search algorithm with few tuneable parameter control. Minimization of Metabolic Adjustment (MOMA) is one of the constraint based algorithms which act to simulate the cellular metabolism after perturbation (gene knockout) occurred to the metabolic model. The strength of MOMA is the ability to simulate the strains that have undergone mutation precisely compared to Flux Balance Analysis. The data set used for the production of fumaric acid is *S. cerevisiae* whereas data set for lycopene production is *Y. lipolytica* metabolic networks model. Experimental results show that the DEMOMA was able to improve the growth rate for the fumaric acid production rate while for the lycopene production, Biomass Product Coupled Yield (BPCY) and production rate were both able to be optimized.

ABSTRAK

Strain mikrob boleh dioptimumkan menggunakan kejuruteraan metabolik yang melaksanakan teknik-teknik penyingkiran gen. Teknik ini memanipulasi gen yang berpotensi untuk meningkatkan hasil metabolik melalui penyusunan semula rangkaian metabolik. Kini, beberapa algoritma pengoptimuman hibrid telah dicadangkan untuk mengoptimumkan strain mikrob. Walau bagaimanapun, algoritma sedia ada tidak berupaya mendapatkan strain optimum kerana gen yang tidak penting sukar untuk dikenal pasti dan perlu disingkirkan kerana rangkaian metabolik mempunyai tahap kekompleksan yang tinggi. Oleh itu, matlamat utama kajian ini adalah untuk mengatasi kekangan yang dihadapi oleh algoritma sedia ada dengan menghibridkan Evolusi Kebezaan dan Peminimuman Pelarasan Metabolik (DEMOMA). Evolusi Kebezaan (DE) dikenali sebagai carian stokastik algoritma yang berasaskan populasi dengan beberapa kawalan parameter kawalan. Peminimuman Pelarasan Metabolik (MOMA) adalah salah satu algoritma berasaskan kekangan yang bertindak untuk mensimulasikan metabolisme sel selepas pengusikan (penyingkiran gen) berlaku kepada model metabolik. Kekuatan MOMA adalah keupayaan untuk mensimulasikan strain yang telah menjalani mutasi dengan tepat berbanding algoritma Analisis Keseimbangan Fluks (FBA). Set data yang digunakan untuk pengeluaran asid fumarik adalah *S. cerevisiae* manakala set data untuk pengeluaran likopena adalah model rangkaian metabolic *Y. Lipolytica*. Keputusan eksperimen menunjukkan bahawa DEMOMA itu dapat meningkatkan kadar pertumbuhan bagi kadar pengeluaran asid fumarik manakala bagi pengeluaran likopena, kadar hasil ganding biojisim produk dan kadar pengeluaran dapat dioptimumkan.

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LIST OF ABBREVIATIONS

ABCMOMA	-	Artificial Bee Colony Minimization of Metabolic Adjustment
ACO	-	Ant Colony Optimization
BA	-	Bees Algorithm
BiGG	-	Biochemical Genetic and Genome
BPCY	-	Biomass Product Coupled Yield
COBRA	-	**Co**nstraint-**b**ased **R**econstruction and **A**nalysis
CR	-	Crossover Rates
DE	-	Differential Evolution
dFBA	-	Dynamic Flux Balance Analysis
FA	-	Fumaric Acid
FBA	-	Flux Balance Analysis
GA	-	Genetic Algorithm
GACOFBA	-	Genetic Ant Colony Optimization Flux Balance Analysis
GRN	-	Gene Regulatory Network
KEGG	-	Kyoto Encyclopedia of Genes and Genomes
LP	-	Linear Programming
MATLAB	-	Matrix Laboratory (Mathworks, Inc.)
MFA	-	Metabolic Flux Analysis
MILP	-	Mixed Integer Linear Programming
MOMA	-	Minimization of Metabolic Adjustment
NP	-	Population Size

PSO	-	Particle Swarm Optimization
QP	-	Quadratic Programming
RAM	-	Random Access Memory
ROOM	-	Regulation On/Off Minimization
S. cerevisiae	-	*Saccharomyces cerevisiae*
SBML	-	System biology mark-up language
Y. lipolytica	-	*Yarrowia lipolytica*

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CHAPTER 1

INTRODUCTION

1.1 Introduction

This chapter reviewed the fundamental study of the research which comprises production of fumaric acid and lycopene, Differential Evolution and Minimization of Metabolic Adjustment. The obstacle and difficulties confronted in producing fumaric acid and lycopene is expressed in problem background. Other than that, problem statement is diagnose to accomplish the objectives of this research. Research goal, objectives, scopes, motivations and summary are also expressed in this chapter.

In this section, the production of fumaric acid and lycopene from the cell factory of a microbial strain is reviewed briefly. The importance of optimizing fumaric acid and lycopene is also described. Then, an introduction to the fundamental of metabolic engineering, Differential Evolution and Minimization of Metabolic Adjustment is presented to provide an idea of the overall research.

1.1.1 Microbial Cell Factory for Metabolite Production

As part of the economically important component of food processing, preparing medicinal drugs and industrial materials, yet the present procedure to fabricate fumaric acid are unsustainable and facing with environmental problem. Basically, fumaric acid presently generated in large amount through three distinct strategies; chemical synthesis, enzymatic catalysis or fermentation (Xu *et al.*, 2012a). It requires high cost to generate FA using chemical synthesis while converting an enzyme derived from petroleum to form into fumarate can bring ecological problem. Even though, it has been proven that fermentation process effectively produced FA, yet this process is inadequate to fulfill the industrial need. This is because, the fungi used to be fermented in producing FA are difficult to grow and their structure highly influence the amount of production generated. This issue becomes the motivation to produce FA using the microbial cell factory of organism that have been acknowledge to fulfill the amount of production yield at industrial scale. Therefore, yeast *S. cerevisiae* that own a good cultivation characteristic is selected to be manipulated in this research with the aim to optimize the production of fumaric acid.

There are over 800 chemical compounds represented by carotenoids. The market of carotenoids has shown great demand for its commercial values in food additives, animal feed additives, and medicinal drug as well as in beauty care products. Lycopene is one of the carotenoid pigments which gives the vegetables and fruits their red color. Other than that, it is an effective cancer prevention agent. Given its cancer prevention agent properties, significant fieldwork has been dedicated to a viable association between lycopene intake and healthy lifestyle. However, there is only a few of carotenoid types can be synthesized chemically. Therefore, the biotechnological generation of carotenoids comes into center for commercial sector since only a few types are available to be produced from natural sources (Matthäus *et al.*, 2014). Meanwhile, the products obtained from chemical process requires high expenditure and difficult to accomplish. Thus, this research investigates how lycopene production as secondary product in *Yarrowia lipolytica* can be increased and optimized for industrial purpose.

1.1.2 *In silico* Metabolic Engineering

Comprehensive insight of *in silico* metabolic engineering in enhancing metabolite production is represented in Figure 1.1. An increasing amount in production of particular chemical and biochemical compound can be achieved with the help of metabolic engineering. The adjustment made to the cell particularly the network of its metabolite so that the product is able to be produced when the ideal development rate is reached which is the main target of remodeling the metabolite (Yen, 2015).The technological properties of each organisms such as product yield and growth characteristics are the aspects to be improved through manipulation of their microbial strains that can lead to the overproduction of specific chemical compound. Deletion or addition some of genes into the metabolic network are some examples of metabolic engineering. Currently, researchers showed a formidable interest in the evolution of metabolic engineering to optimize the yield of target metabolites.

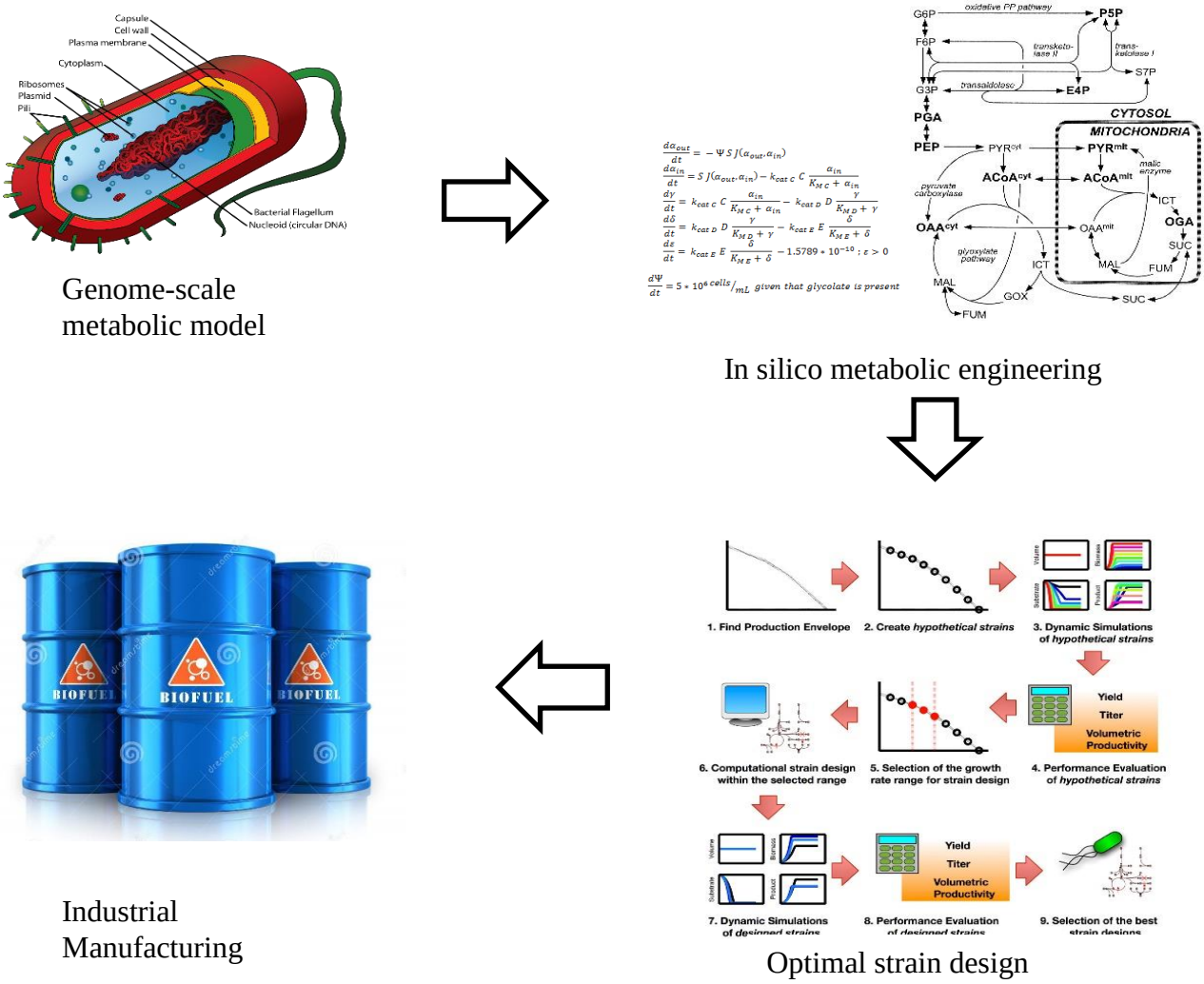

Figure 1.1 Overall view of *in silico* metabolic engineering

The genome of microorganism is represented in the form of metabolic network. The metabolic network comprises of thousands of reaction and genes. Figure 1.2 demonstrate how the metabolic network is entangled as it comprised of plentiful metabolites. The modification that aims to increase the production of specific metabolites can be conducted once a thorough finding have been done. This is because an adjustment to the network gives an impact since the gene is interdependent. Conventionally, the scientist has to perform the same experiments numerous times in the laboratory to test each combination of gene to obtain the optimal set of gene to be knockout. This scenario requires a high budget to be spent for the materials used in the laboratory.

The power of computational simulation used on metabolic engineering is strongly helpful to aid in enhancing the production of chemical compound since the wet-lab experiments is time-consuming and high cost to conduct the experiments. The improved phenotypes obtained from the identification of gene knockout strategies are the result of the algorithms proposed. The rising of available genome-scale metabolic model is also contributing to the effectiveness of computational simulation. Generally, there are recognized algorithms that widely in use for implementing metabolic engineering such as optimization algorithms, modeling algorithms and modeling framework. These kinds of algorithms are utilized to aid in triggering the cellular metabolism that leads an enhancement in production of target metabolites expressed in genome scale model.

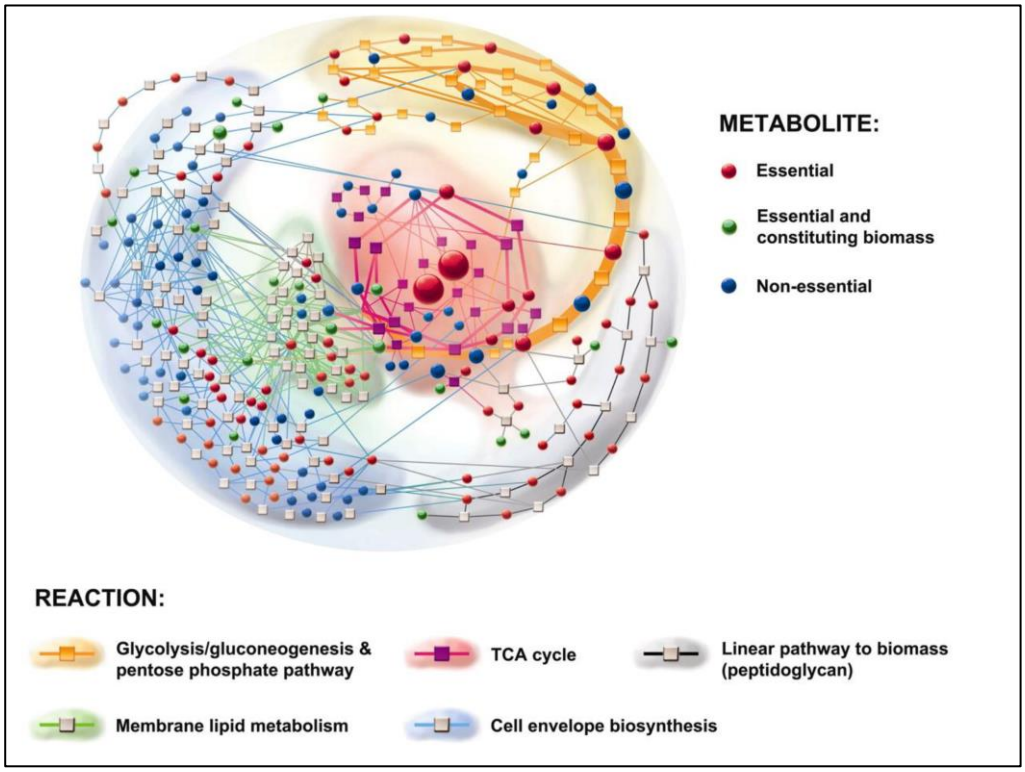

Figure 1.2 Characteristics of essential and nonessential metabolites in E. coli metabolism (Kim *et al.*, 2007).

1.1.3 Differential Evolution

Differential evolution is a simple and effective optimization algorithm and commonly being used for solving continuous optimization problem. DE belongs to Evolutionary Algorithms that is inspired by the nature of species evolves. Basically, the operation employed by DE in discovering the optimal solutions is through a population of candidate solutions which represent the individuals of the population must be initialized first instead of working just on a single solution. The perturbation of solutions with a scaled difference of two randomly selected population vectors eventually generates the offspring. Then, each of these individuals is compared to each other to be included in the next generation. The selected individuals is the vector that outperforms the objective function value of its corresponding parent. The performance of DE in solving a continuous optimization problem is affected by the proper initialization of population size and their associated control parameter values. Apart from metabolic engineering, DE has been successfully applied in diverse fields such as mechanical engineering (Rogalsky *et al.*, 2000), communication (Storn, 1996) and pattern recognition (Ilonen *et al.*, 2003). The implementation of DE in this research is to predict a near-optimal set of gene to be knocked out that leads to overproduction of metabolites.

1.1.4 Minimization of Metabolic Adjustment

Minimization of metabolic adjustment (MOMA) is a constraint-based modeling algorithm used to model and mimic biological processes for phenotype prediction. The common use of MOMA is to forecast the aftereffect of perturbation introduced to the metabolic network, for example gene knockout. The behavior of perturbed metabolic network can be predicted precisely since MOMA find the minimal distance between solutions of the mutant strain relative to the wild type solution. The defined objective function is solved using quadratic programming. However, MOMA

is not able to predict the optimal set of gene to be removed in order to achieve the optimal production. Thus, MOMA is then hybridized with DE to overcome the limitations aforementioned.

1.2 Background of Problem

There are a series of modeling frameworks such as OptKnock, OptGene and OptReg have been developed. These frameworks highlighted continuous efforts on the advancement of *in silico* metabolic engineering. Optknock, the first systematic optimization-based method is developed for suggesting gene knockout strategies for biochemical overproduction by coupling the production of a desired compound with cellular (Burgard *et al.*, 2003). OptReg which is the upgraded version of OptKnock also include the modulation on pathways by up- or down-regulating reactions besides knocking them out to maximize the production of desired compound.

From a metabolic engineering perspective, such models can be used for computer-aided design of optimal genetic and culture condition manipulation strategies to improve the production of industrially relevant compounds (Machado and Herrgård, 2015). However, given the size of metabolic networks, the exhaustive analysis of multiple simultaneous genetic manipulations becomes computationally infeasible. The aforementioned properties are also the cause to the drawbacks of these approaches which it tends to fall into premature convergence and takes high computational time to find the global optima.

OptKnock, the bilevel optimization where the outer optimization layer maximizes the product yield, while the inner layer optimizes for the cellular growth. The limitation of this framework is the degeneracy in the solution of inner problem, which sometimes result in the overly optimistic predictions and lead to strain designs

that are not effectively growth-coupled. This drawback has laid the foundation for a diversity of bilevel methods for rational strain designs that take the consideration to ensure that the production of desired compound is produced in maximum amount without abandoning the growth characteristic of the production host.

These common used framework employ Mixed Integer Linear Programming (MILP) to formulate the problem where it can be used to find a globally optimal solution. This formulation can lead to worst case for the computational cost, increase exponentially with the number of reaction deletions. The approach that implement heuristic optimization strategies to cope with complex optimization problems cannot guarantee to find global optimal solutions. It often finds sufficiently good solutions with a reasonable computational cost. This drawback indicates that the approach is lack in the accuracy to predict the optimal solutions.

The hybrid algorithm of DE and MOMA is proposed in this study to solve the aforementioned drawback of previous approach. This hybrid algorithm is the combination of evolutionary algorithm and constraint based method. The combinatorial problem can be solved with the implementation of DE while to predict the effect of knocking out genes is portrayed by MOMA. DE is known to be one of the algorithm commonly used to solve a complex problem by having operator (crossover, mutation and selection) that can predict the optimal solution within the minimum computation cost. The probability of having overly optimistic predictions can be encounter with the help of implementing MOMA, as the objective function is to reduce the flux distribution between wild type and mutant. This feature shows that genetic perturbations that occur to the metabolic network is being considered by MOMA.

1.3 Statement of Problem

The overproduction of desired compound can be achieved with the advent of in silico metabolic engineering method. However, it is a challenging task in identifying the optimal genes to be knockout that eventually become the obstacle to optimize the production to its maximum yield. The process of identification nonessential genes suffers from problems such as premature convergence, high computational cost and the accuracy of optimal solutions cannot be guaranteed.

The complexity of metabolic network has made the process to identify optimal genes to be knockout difficult. This is because the network is entangled and the modification of the genes cannot be done without a thorough study on the genome. High number of reactions available in the genome-scale metabolic model led to a combinatorial problem and cause high computational cost in order to converge to global optimal solutions.

Therefore, the main problem in this research is the unoptimised metabolites production because the nonessential genes that prevent the production to be optimized are hardly to be diagnosed and need to be knockout. The difficulty to discover the optimal genes to be knockout is due to high complexity of metabolic network. Thus, this research intends to address the aforementioned problems based on the following research questions:

i. How to reduce the complexity of metabolic network in order to optimize the fumaric acid and lycopene production?
ii. How to evaluate and validate the performance of the proposed hybrid algorithm in optimizing the metabolites production?

1.4 Research Goal

The goal of this research is to propose a hybrid of Differential Evolution and Minimization of Metabolic Adjustment to reduce the complexity of metabolic network by identifying a near optimal set of gene knockout that leads to overproduction of fumaric acid and lycopene.

1.5 Objectives

The research target can be accomplished by conducting the following specified objectives.

i. To develop a hybrid of Differential Evolution and Minimization of Metabolic Adjustment for reducing the metabolic network complexity that leads toward optimizing production of fumaric acid and lycopene.
ii. To analyze the results of fitness values (biomass product couple yield, growth rate and production rate) of each metabolites and list of reactions deletions which correspond to the information of reactions and genes from the biological databases.

1.6 Scope of Study

The scopes of this research are listed as following:

i. Two datasets used are:
   a. Model iND750.xml downloaded from published literature by (Xu *et al.*, 2012b) derived from bigg.ucsd.edu/models/iND750
   b. Model *yli v1*.7.xml from published literature by (Nambou *et al.*, 2015)

ii. Format of dataset is in System Biology Markup Language (SBML). The biological processes of models are represented in SBML based on XML which is a readable language machine. This type of machine language gives features to any experimental data for exchanging information, storing and also fitting the parameters. The significant modifications made on genome models can be predicted accurately through this given features.

iii. The software used is Constraints Based Reconstruction and Analysis (COBRA) Toolbox for constraint-based modeling which is implemented in MATLAB.

iv. The proposed method is a hybrid of Minimization of Metabolic Adjustment and Differential Evolution to identify the near-optimal set of genes to be knocked out for production improvement.

v. Metabolites production of fumaric acid in *S. cerevisiae* and lycopene in *Y. lipolytica* are the products focused in this research.

1.7 Significant of Study

In this study, the effect of modifications made on the genome model is simulated and explored to enhance the production of fumaric acid and lycopene through the implementation of computational algorithm. The significance of conducting this research is listed below:

i. The prospective enhancement of metabolites production is explored in the microbial cell factories.
ii. Deliver better understanding of function at the cell level through the computational modeling and analysis that can lead to a better comprehension of fumaric acid and lycopene production from microbial cell factory.
iii. The metabolites yield can be improved through the development of the hybrid algorithm that combines the optimization and constraint based modeling.
iv. The near-optimal set of genes to be knockout suggested by the proposed hybrid algorithm can be reference for the researchers and biologist to conduct the laboratory experiment towards a more promising production in time effective manner.

1.8 Thesis Outline

Chapter 1 discussed a brief introduction to metabolites production and metabolic engineering included Differential Evolution and Minimization of Metabolic Adjustment. Background of problem which presented the existing issue in the related field and followed by the problem statements of this research is described in detail. The aim, objectives and scopes are also enclosed precisely.

Chapter 2 presents some reviews of previous published literary works and other available sources on the existing algorithms used to analyze the genome-scale metabolic model. In addition, some information about metabolic engineering which comprises different groups of algorithms such as constraint-based analysis, optimization algorithms and hybrid algorithms are deliberated briefly. The reference materials related to this research topic that is helpful such as journals, articles and conference working papers are listed too.

Chapter 3 explains precisely the research methodology designed to conduct this research. The comprehensive illustration of activities covered are reviewed and divided according to each phases respectively for a better understanding. The information of data set chosen is elucidated. Then, the pre-requisite hardware and software as well as performance measurements that are being practiced for this research are explicitly presented. The proposed algorithms is presented in this chapter.

Chapter 4 deliberates and reviews the flow implementation of the proposed hybrid algorithm, DEMOMA. Steps to pre-process the two data sets as a groundwork to have a compatible dataset. The discussion on the formulated steps in DEMOMA is also included. After that, the analysis of results obtained from the proposed hybrid algorithm is presented along with the explanation about the reactions and genes suggested to be removed that leads to the improvement in production of metabolites are also represented in this chapter.

Chapter 5 concludes the contents of all formerly discussed chapters. Contributions, limitations and future works that can be conducted on this research are also being explained.

1.9 Summary

This chapter elaborates on the practice of metabolic engineering that is getting more consideration and the accomplishment of *in silico* modeling utilizing refined and mimic microbial cell factory to improve coveted metabolites. The exposition on the proposed algorithm that integrates constraint-based and optimization algorithms is also included. The aim of this research can be fulfilled once the objectives have been identified. The incoming chapter reviews the information about the existing algorithms

taken from published literary works. This is crucial to determine the most applicable algorithm to be implemented in this research to achieve the proposed objectives.

REFERENCES

Aghazadeh, F., and Meybodi, M. R. (2011). *Learning bees algorithm for optimization.* Paper presented at the International Conference on Information and Intelligent Computing.

Alper, H., Jin, Y.-S., Moxley, J., and Stephanopoulos, G. (2005). Identifying gene targets for the metabolic engineering of lycopene biosynthesis in Escherichia coli. *Metabolic engineering, 7*(3), 155-164.

Amorim-Carrilho, K., Cepeda, A., Fente, C., and Regal, P. (2014). Review of methods for analysis of carotenoids. *TrAC Trends in Analytical Chemistry, 56*, 49-73.

Asadollahi, M. A., Maury, J., Patil, K. R., Schalk, M., Clark, A., and Nielsen, J. (2009). Enhancing sesquiterpene production in Saccharomyces cerevisiae through in silico driven metabolic engineering. *Metabolic engineering, 11*(6), 328-334.

Beopoulos, A., Cescut, J., Haddouche, R., Uribelarrea, J.-L., Molina-Jouve, C., and Nicaud, J.-M. (2009). Yarrowia lipolytica as a model for bio-oil production. *Progress in lipid research, 48*(6), 375-387.

Bilecová-Rabajdová, M., Birková, A., Urban, P., Gregová, K., Durovcová, E., and Mareková, M. (2013). Naturally occurring substances and their role in chemo-protective effects. *Central European journal of public health, 21*(4), 213.

Brochado, A. R., Matos, C., Møller, B. L., Hansen, J., Mortensen, U. H., and Patil, K. R. (2010). Improved vanillin production in baker's yeast through in silico design. *Microbial cell factories, 9*(1), 1.

Burgard, A. P., Pharkya, P., and Maranas, C. D. (2003). Optknock: a bilevel programming framework for identifying gene knockout strategies for microbial strain optimization. *Biotechnology and bioengineering, 84*(6), 647-657.

Cao, N., Du, J., Gong, C., and Tsao, G. (1996). Simultaneous Production and Recovery of Fumaric Acid from Immobilized Rhizopus oryzae with a Rotary Biofilm

Contactor and an Adsorption Column. *Applied and environmental microbiology, 62*(8), 2926-2931.

Chavali, A. K., D'Auria, K. M., Hewlett, E. L., Pearson, R. D., and Papin, J. A. (2012). A metabolic network approach for the identification and prioritization of antimicrobial drug targets. *Trends in microbiology, 20*(3), 113-123.

Chong, S. K., Mohamad, M. S., Salleh, A. H. M., Choon, Y. W., Chong, C. K., and Deris, S. (2014). A hybrid of ant colony optimization and minimization of metabolic adjustment to improve the production of succinic acid in Escherichia coli. *Computers in biology and medicine, 49*, 74-82.

Ciccone, M. M., Cortese, F., Gesualdo, M., Carbonara, S., Zito, A., Ricci, G., et al. (2013). Dietary intake of carotenoids and their antioxidant and anti-inflammatory effects in cardiovascular care. *Mediators of inflammation, 2013*.

Colorni, A., Dorigo, M., and Maniezzo, V. (1991). *Distributed optimization by ant colonies.* Paper presented at the Proceedings of the first European conference on artificial life, 134-142.

Dbouk, T., and Harion, J.-L. (2015). Performance of Optimization Algorithms Applied to Large Nonlinear Constrained Problems. *American Journal of Algorithms and Computing, 2*(1), 32-56.

Dorigo, M., and Stützle, T. (2010). Ant colony optimization: overview and recent advances. In *Handbook of metaheuristics* (pp. 227-263): Springer.

Duarte, N. C., Herrgård, M. J., and Palsson, B. Ø. (2004). Reconstruction and validation of Saccharomyces cerevisiae iND750, a fully compartmentalized genome-scale metabolic model. *Genome research, 14*(7), 1298-1309.

Eberhart, R. C., and Kennedy, J. (1995). *A new optimizer using particle swarm theory.* Paper presented at the Proceedings of the sixth international symposium on micro machine and human science, 39-43.

Edwards, J. S., Covert, M., and Palsson, B. (2002). Metabolic modelling of microbes: the flux-balance approach. *Environmental microbiology, 4*(3), 133-140.

Eiben, A. E., and Smith, J. E. (2003). *Introduction to evolutionary computing* (Vol. 53): Springer.

Gowen, C. M., and Fong, S. S. (2011). Applications of systems biology towards microbial fuel production. *Trends in microbiology, 19*(10), 516-524.

Haupt, R. L., and Haupt, S. E. (2004). *Practical genetic algorithms*: John Wiley & Sons.

Heider, S. A., Peters-Wendisch, P., Netzer, R., Stafnes, M., Brautaset, T., and Wendisch, V. F. (2014). Production and glucosylation of C50 and C40 carotenoids by metabolically engineered Corynebacterium glutamicum. *Applied microbiology and biotechnology*, *98*(3), 1223-1235.

Hjersted, J., and Henson, M. (2009). Steady-state and dynamic flux balance analysis of ethanol production by Saccharomyces cerevisiae. *IET systems biology, 3*(3), 167-179.

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

Huang, Z., and Chen, Y. (2013). An improved differential evolution algorithm based on adaptive parameter. *Journal of Control Science and Engineering, 2013*, 3.

Hwang, S.-F., and He, R.-S. (2006). A hybrid real-parameter genetic algorithm for function optimization. *Advanced Engineering Informatics, 20*(1), 7-21.

Ibarra, R. U., Edwards, J. S., and Palsson, B. O. (2002). Escherichia coli K-12 undergoes adaptive evolution to achieve in silico predicted optimal growth. *Nature*, *420*(6912), 186-189.

Ilonen, J., Kamarainen, J.-K., and Lampinen, J. (2003). Differential evolution training algorithm for feed-forward neural networks. *Neural Processing Letters, 17*(1), 93-105.

Kachitvichyanukul, V. (2012). Comparison of three evolutionary algorithms: GA, PSO, and DE. *Industrial Engineering and Management Systems, 11*(3), 215-223.

Kanehisa, M., and Goto, S. (2000). KEGG: kyoto encyclopedia of genes and genomes. *Nucleic acids research, 28*(1), 27-30.

Karaboga, D. (2005). *An idea based on honey bee swarm for numerical optimization*: Technical report-tr06, Erciyes university, engineering faculty, computer engineering departmento. Document Number)

Karaboga, D., Gorkemli, B., Ozturk, C., and Karaboga, N. (2014). A comprehensive survey: artificial bee colony (ABC) algorithm and applications. *Artificial Intelligence Review, 42*(1), 21-57.

Kauffman, K. J., Prakash, P., and Edwards, J. S. (2003). Advances in flux balance analysis. *Current opinion in biotechnology*, *14*(5), 491-496.

Kenealy, W., Zaady, E., du Preez, J. C., Stieglitz, B., and Goldberg, I. (1986). Biochemical aspects of fumaric acid accumulation by Rhizopus arrhizus. *Applied and environmental microbiology, 52*(1), 128-133.

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

Kim, P.-J., Lee, D.-Y., Kim, T. Y., Lee, K. H., Jeong, H., Lee, S. Y., et al. (2007). Metabolite essentiality elucidates robustness of Escherichia coli metabolism. *Proceedings of the National Academy of Sciences, 104*(34), 13638-13642.

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

Koo, C. L., Salleh, A. H. M., Mohamad, M. S., Deris, S., Omatu, S., and Yoshioka, M. (2014). *A gene knockout strategy for succinate production using a hybrid algorithm of bees algorithm and minimization of metabolic adjustment.* Paper presented at the Granular Computing (GrC), 2014 IEEE International Conference on, 131-136.

Lampinen, J., and Zelinka, I. (2000). *On stagnation of the differential evolution algorithm.* Paper presented at the Proceedings of MENDEL, 76-83.

Laporte, G., and Osman, I. H. (1996). Foreword. *Annals of Operations Research, 63*(1), 1-2.

Machado, D., and Herrgård, M. J. (2015). Co-evolution of strain design methods based on flux balance and elementary mode analysis. *Metabolic Engineering Communications, 2*, 85-92.

Mahadevan, R., Edwards, J. S., and Doyle, F. J. (2002). Dynamic flux balance analysis of diauxic growth in Escherichia coli. *Biophysical journal, 83*(3), 1331-1340.

Mahadevan, R., and Schilling, C. (2003). The effects of alternate optimal solutions in constraint-based genome-scale metabolic models. *Metabolic engineering, 5*(4), 264-276.

Matthäus, F., Ketelhot, M., Gatter, M., and Barth, G. (2014). Production of lycopene in the non-carotenoid-producing yeast Yarrowia lipolytica. *Applied and environmental microbiology, 80*(5), 1660-1669.

Mohamed, A. W., Sabry, H. Z., and Khorshid, M. (2012). An alternative differential evolution algorithm for global optimization. *Journal of Advanced Research, 3*(2), 149-165.

Moonchai, S., Madlhoo, W., Jariyachavalit, K., Shimizu, H., Shioya, S., and Chauvatcharin, S. (2005). Application of a mathematical model and Differential Evolution algorithm approach to optimization of bacteriocin production by Lactococcus lactis C7. *Bioprocess and biosystems engineering, 28*(1), 15-26.

Moradi, S., Fatahi, L., and Razi, P. (2010). Finite element model updating using bees algorithm. *Structural and Multidisciplinary Optimization, 42*(2), 283-291.

Nambou, K., Jian, X., Zhang, X., Wei, L., Lou, J., Madzak, C., et al. (2015). Flux Balance Analysis Inspired Bioprocess Upgrading for Lycopene Production by a Metabolically Engineered Strain of Yarrowia lipolytica. *Metabolites, 5*(4), 794-813.

Neri, F., and Tirronen, V. (2010). Recent advances in differential evolution: a survey and experimental analysis. *Artificial Intelligence Review, 33*(1-2), 61-106.

Nesbitt, N. M., Baleanu-Gogonea, C., Cicchillo, R. M., Goodson, K., Iwig, D. F., Broadwater, J. A., et al. (2005). Expression, purification, and physical characterization of Escherichia coli lipoyl (octanoyl) transferase. *Protein expression and purification, 39*(2), 269-282.

Neto, R. T., and Godinho Filho, M. (2013). Literature review regarding Ant Colony Optimization applied to scheduling problems: Guidelines for implementation and directions for future research. *Engineering Applications of Artificial Intelligence, 26*(1), 150-161.

Nevoigt, E. (2008). Progress in metabolic engineering of Saccharomyces cerevisiae. *Microbiology and Molecular Biology Reviews, 72*(3), 379-412.

Nookaew, I., Olivares-Hernández, R., Bhumiratana, S., and Nielsen, J. (2011). Genome-scale metabolic models of Saccharomyces cerevisiae. In *Yeast Systems Biology* (pp. 445-463): Springer.

Ohno, S., Furusawa, C., and Shimizu, H. (2013). In silico screening of triple reaction knockout Escherichia coli strains for overproduction of useful metabolites. *Journal of bioscience and bioengineering, 115*(2), 221-228.

Orth, J. D., Thiele, I., and Palsson, B. Ø. (2010). What is flux balance analysis? *Nature biotechnology, 28*(3), 245-248.

Park, J. M., Kim, T. Y., and Lee, S. Y. (2009). Constraints-based genome-scale metabolic simulation for systems metabolic engineering. *Biotechnology advances, 27*(6), 979-988.

Patil, K., Rocha, I., Förster, J., and Nielsen, J. (2005a). Evolutionary programming as a platform for in silico metabolic engineering. *BMC bioinformatics, 6*(1), 1.

Patil, K. R., Rocha, I., Förster, J., and Nielsen, J. (2005b). Evolutionary programming as a platform for in silico metabolic engineering. *BMC bioinformatics, 6*(1), 308.

Pham, D., Ghanbarzadeh, A., Koc, E., Otri, S., Rahim, S., and Zaidi, M. (2011). *The Bees Algorithm–A Novel Tool for Complex Optimisation.* Paper presented at the Intelligent Production Machines and Systems-2nd I* PROMS Virtual International Conference 3-14 July 2006, 454.

Price, N. D., Papin, J. A., Schilling, C. H., and Palsson, B. O. (2003). Genome-scale microbial in silico models: the constraints-based approach. *Trends in biotechnology, 21*(4), 162-169.

Ranganathan, S., Tee, T. W., Chowdhury, A., Zomorrodi, A. R., Yoon, J. M., Fu, Y., et al. (2012). An integrated computational and experimental study for overproducing fatty acids in Escherichia coli. *Metabolic engineering, 14*(6), 687-704.

Rashid, A. H. A., Choon, Y. W., Mohamad, M. S., Chai, L. E., Chong, C. K., Deris, S., et al. (2013). Producing succinic acid in yeast using a hybrid of differential evolution and flux balance analysis. *Int. J. Bio-Sci. Bio-Technol.(IJBSBT), 5*(6), 91-100.

Rocha, M., Maia, P., Mendes, R., Pinto, J. P., Ferreira, E. C., Nielsen, J., et al. (2008). Natural computation meta-heuristics for the in silico optimization of microbial strains. *BMC bioinformatics, 9*(1), 499.

Rogalsky, T., Kocabiyik, S., and Derksen, R. (2000). Differential evolution in aerodynamic optimization. *Canadian Aeronautics and Space Journal, 46*(4), 183-190.

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*(4), 685-693.

Segre, D., Vitkup, D., and Church, G. M. (2002). Analysis of optimality in natural and perturbed metabolic networks. *Proceedings of the National Academy of Sciences, 99*(23), 15112-15117.

Selvi, V., and Umarani, D. R. (2010). Comparative analysis of ant colony and particle swarm optimization techniques. *International Journal of Computer Applications (0975–8887), 5*(4).

Shlomi, T., Berkman, O., and Ruppin, E. (2005). Regulatory on/off minimization of metabolic flux changes after genetic perturbations. *Proceedings of the National Academy of Sciences of the United States of America, 102*(21), 7695-7700.

Sivanandam, S., and Deepa, S. (2008). Introduction to particle swarm optimization and ant colony optimization. In *Introduction to Genetic Algorithms* (pp. 403-424): Springer.

Soons, Z. I., Ferreira, E. C., Patil, K. R., and Rocha, I. (2013). Identification of metabolic engineering targets through analysis of optimal and sub-optimal routes. *PloS one, 8*(4), e61648.

Sörensen, K. (2015). Metaheuristics—the metaphor exposed. *International Transactions in Operational Research, 22*(1), 3-18.

Storn, R. (1996). *On the usage of differential evolution for function optimization.* Paper presented at the Fuzzy Information Processing Society, 1996. NAFIPS., 1996 Biennial Conference of the North American, 519-523.

Storn, R., and Price, K. (1997). Differential evolution–a simple and efficient heuristic for global optimization over continuous spaces. *Journal of global optimization, 11*(4), 341-359.

Sun, Y., Sun, L., Shang, F., and Yan, G. (2016). Enhanced production of β-carotene in recombinant Saccharomyces cerevisiae by inverse metabolic engineering with supplementation of unsaturated fatty acids. *Process Biochemistry, 51*(5), 568-577.

Tang, P. W., Choon, Y. W., Mohamad, M. S., Deris, S., and Napis, S. (2015). Optimising the production of succinate and lactate in Escherichia coli using a hybrid of artificial bee colony algorithm and minimisation of metabolic adjustment. *Journal of bioscience and bioengineering, 119*(3), 363-368.

Wang, Y., Liu, X., Robbins, K., and Rekaya, R. (2010). AntEpiSeeker: detecting epistatic interactions for case-control studies using a two-stage ant colony optimization algorithm. *BMC research notes, 3*(1), 1.

Wu, C.-Y., and Tseng, K.-Y. (2008). Topology optimization of structure using differential evolution. *J. Syst. Cybern. Inform, 6*(1), 46-51.

Xu, G., Liu, L., and Chen, J. (2012a). Reconstruction of cytosolic fumaric acid biosynthetic pathways in Saccharomyces cerevisiae. *Microbial cell factories, 11*(1), 1.

Xu, G., Zou, W., Chen, X., Xu, N., Liu, L., and Chen, J. (2012b). Fumaric acid production in Saccharomyces cerevisiae by in silico aided metabolic engineering. *PloS one, 7*(12), e52086.

Xue, X.-d., Xu, B., Wang, H.-l., and Jiang, C.-p. (2010). *The basic principle and application of ant colony optimization algorithm.* Paper presented at the Artificial Intelligence and Education (ICAIE), 2010 International Conference on, 358-360.

Yen, J. Y. (2015). Designing metabolic engineering strategies with genome-scale metabolic flux modeling. *Clinical Epidemiology, 7*, 149-160.

Yusup, N., Zain, A. M., and Hashim, S. Z. M. (2012). Evolutionary techniques in optimizing machining parameters: Review and recent applications (2007–2011). *Expert Systems with Applications, 39*(10), 9909-9927.

Zhao, Q., and Kurata, H. (2006). *Comparison of the Prediction Abilities of FBA, MOMA and ROOM for a pykF Mutant of E. coli.* Paper presented at the The 17th International Conference on Genome Informatics, 107-101.

Zhu, Q., and Jackson, E. N. (2015). Metabolic engineering of Yarrowia lipolytica for industrial applications. *Current opinion in biotechnology, 36*, 65-72.