STOCHASTIC MODELING OF THE *C.ACETOBUTYLICUM* AND SOLVENT PRODUCTIONS IN FERMENTATION

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Especially to my beloved family

Thanks for all the supports, sacrifices, patients and willingness to share my dreams with me…

To my honoured supervisor and all UTM lecturers

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ABSTRACT

Recent decade has seen great progress in the use of stochastic models in biological process. Researchers are now realising that stochastic models have important roles to play in biological process especially in the analysis of population dynamics. This progress encourages many researchers to develop new methods and techniques to improve the stochastic model. In recent study, logistic equations have been used to model the cell growth of *C.acetobutylicum* while the Luedeking-Piret equation incorporating the logistic equation used to model the formation of solvent. However, it was found that the Luedeking-Piret equation is not adequate for modeling the production of acetone and butanol. In this study, stochastic power law logistic model has been considered to model the cell growth of *C.acetobutylicum* and the solvent production in five different yeast cultures. In order to solve the SDEs, simulated maximum likelihood estimation method and Euler-Maruyama approximation method have been used. Finally, the stochastic models and deterministic models are compared by using their root mean square errors of the growth model and solvent productions model. The stochastic models have smaller value of root mean square errors, thus showed that the stochastic power law logistic models are better models than their deterministic counterparts to describe the growth of *C.acetobutylicum* and solvent productions in fermentation.

ABSTRAK

Kebelakangan dekad ini telah menyaksikan kemajuan yang hebat dalam penggunaan model stokastik dalam bidang biologi. Para penyelidik sekarang menyedari bahawa model stokastik memainkan peranan yang penting dalam proses biologi terutamanya dalam menganalisis populasi dinamik. Kemajuan ini mendorong lebih ramai penyelidik untuk mempraktikkan kaedah dan teknik yang baru untuk memajukan lagi model stokastik. Dalam kajian sebelum ini, persamaan logistik telah digunakan untuk memodelkan pertumbuhan sel *C.acetobutylicum* sementara persamaan Luedeking-Piret digunakan untuk memodelkan pembentukan bahan larut. Walau bagaimanapun, didapati bahawa persamaan Luedeking-Piret tidak cukup tepat untuk memodelkan penghasilan aseton dan butanol. Dalam kajian ini, model *stochastic power law logistic* telah dipertimbangkan untuk memodelkan pertumbuhan sel *C.acetobutylicum* dan penghasilan bahan larut di dalam lima kultur yis yang berbeza. Untuk menyelesaikan persamaan pembezaan stokastik, kaedah kebolehjadian maksimum dan kaedah penghampiran Euler-Maruyama telah digunakan. Akhir sekali, model stokastik dengan model deteministik telah dibandingkan melalui ralat min punca kuasa dua. Hasil kajian menunjukkan model stokastik mempunyai nilai ralat min punca kuasa dua yang lebih kecil. Kesimpulannya model stokastik lebih baik untuk memodelkan pertumbuhan *C.acetobutylicum* dan penghasilan bahan larut di dalam penapaian berbanding model deteministik.

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LIST OF SYMBOLS

t	-	Time
T	-	Maturity date
$\Im_t$	-	Filtration
W_t	-	Wiener process
$\boldsymbol{M_t}$	-	Martingale process
S_t	-	Stochastic process
Ω	-	Sample space
P	-	Probability
$\Re$	-	Set of real numbers
$X(t)$	-	Stochastic population size at time t
μ	-	Initial specific growth rate
x	-	cell concentration (g/L)
$x_{\max}$	-	Maximum attainable biomass concentration
s	-	The exponent of cell concentration
$\mu_{\max}$	-	Maximum specific growth rate
ε	-	Parameter for diffusion process
r_P	-	Formation rate
A	-	Acetone concentration (g/L)
a	-	Growth associated coefficient for acetone formation (g/g cell)
b	-	Non-growth associated coefficient for acetone formation (g/g cell)
B	-	Butanol concentration (g/L)
c	-	Growth associated coefficient for butanol formation (g/g cell)
e	-	Non-growth associated coefficient for butanol formation (g/g cell)
$(\,.\,)^T$	-	Tranpose

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CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Biological process is probably the oldest area in mathematical biology especially population of biology. This situation helps the field to be well equipped with mathematical tools for better understanding of the biological population. In recent years, the study of population has motivated many researchers in biological world not to ignore the importance of mathematics in analyzing the concept of population.

Population dynamics is the study of marginal and long term changes in numbers, individual weights and age composition of individuals in one or several populations, biological and environmental processes influencing those changes. It has traditionally been played a major part in mathematical biology, which is recently rapidly developed.

Microbiology is the study of living organism of microscopic size. Microbiology is very interesting and important in term of science. During the last 40 years, microbiology has emerged as a very significant field of biology. Today, microorganisms are used by researchers in the study of practically all major biological phenomena including fermentations. Since prehistoric times, humans have been controlling the fermentation process. People use fermentation in converting juice into wine and grains into beer. The earliest evidence of winemaking dates from eight thousand years ago, in Georgia, in the Caucasus area. In modern world, people use fermentations to obtain something more valuable from the fermentations process, known as the products.

Earlier on, people just use trial and error approach in fermentations before their discovery in how to use mathematical modeling. What is modeling? Modeling is an attempt to describe, in a precise way, an understanding of the elements of a system of interest, their states, and their interactions with other elements. Biologists have traditionally preferred to work with systems of ordinary or partial differential equations (ODEs or PDEs) which are deterministic. These have the advantages of being more precise and fully quantitative, but also have a number of disadvantages. The problem in microbiology of course, does bacteria vary in number continuously and deterministically. Wilkinson (2006) found that they vary discretely and stochastically. Stochastic will allow us to understand the distribution associated with the time to extinction, something that simply is not possible when using a deterministic framework.

Mathematical modeling has played it role in fermentation process. Fermentation involves bacteria in order to complete the process. As we mention earlier, microorganism vary stochastically, hence deterministic is no longer suitable to model the fermentation process. Therefore this thesis describes a study on modeling the fermentations process using a stochastic approach and it will focus on microbial growth and the productions of fermentation process.

1.2 Problem Statement

Modeling population dynamics in random environment is a way of studying the fluctuation of population size that was affected by the stochasticity of the environmental factors. When we study stochastic, it means being or having a random variable. A stochastic model is a tool for estimating probability distributions of potential outcomes by allowing for random variation in one or more inputs over time. The random variation is usually based on fluctuations observed in historical data for a selected period. Therefore this thesis describes a study of stochastic modeling in population dynamics and its focus is on microbial growth dynamics and solvent productions dynamics.

Madihah (2002) used logistic and Leudeking-Piret equations to describe growth of *C.acetobutylicum* and solvent production in fermentation using gelatinized sago starch. The Logistic equation $\frac{dx}{dt} = [\mu_{\max}(1 - \frac{x}{x_{\max}})x]$ is used to model the cell growth of *C.acetobutylicum*, where x is cell concentration (g/L), $x_{\max}$ is the maximum cell concentration (g/L) and $\mu_{\max}$ is the maximum specific growth rate (h^{-1}). The Luedeking-Piret equation $\frac{dA}{dt} = a\frac{dx}{dt} + bx$ and $\frac{dB}{dt} = c\frac{dx}{dt} + dx$ was used to model the formation of acetone and butanol respectively. Acetone concentration is represented by *A*, *a* represents growth associated coefficient for acetone formation(g/g cell) and *b* is the non-growth associated coefficient for a acetone formation(g/g cell), and *B* represents butanol concentration, *c* is the growth associated coefficient for butanol formation (g/g cell) and *d* is the non-growth associated coefficient for butanol formation (g/g cell).

However, the Luedeking-Piret equation is not adequate for modeling the production of aceton and butanol. In 2005, Ponciano presented a novel application of a stochastic ecological model to the study and analysis of microbial growth dynamics as influenced by environmental conditions in an extensive experimental data set. The implication of his study is that the inadequacy of the above-mentioned equation maybe due to random environmental influence.

Works by Bahar (2005), proposed a stochastic power law logistic model in the study of population dynamics. It is hoped that by introducing the stochasticity through the $-\frac{\mu_{\max}}{x_{\max}}$, the modeling of acetone and butanol formation will be more realistic. That is $\frac{\mu_{\max}}{x_{\max}} \rightarrow \frac{\mu_{\max}}{x_{\max}} + \varepsilon x\, dw/dt$, where *dw/dt* is the Brownian noise. The propose equation is

$$\frac{dx}{dt} = [\mu_{\max}(1 - \frac{x^s}{x_{\max}}) + \varepsilon x \frac{dw}{dt}]x.$$

Thus, this research will validate the effectiveness of stochastic power law logistic model in biological system and especially in describing growth of *C.acetobutylicum* in fermentation using gelatinized sago starch and the solvent productions (acetone and butanol) during the fermentation process.

1.3 Objectives of the Study

The study was conducted to achieve the following objectives:

i. To simulate the stochastic power law logistic model for growth of *C.acetobutylicum* of fermentation of sago starch and solvent production (acetone and butanol).
ii. To estimate the parameters of the model using simulated maximum likelihood.
iii. To validate the appropriateness of stochastic power law logistic model.
iv. To propose stochastic power law logistic model as general model for describing cell growth and solvent production.

1.4 Scopes of the Study

This study focused on application of stochastic modeling in population dynamics and analysis of the fermentation of sago starch by a microb (*Clostridium Acetobutylicum P262*) in a batch culture using an extensive experimental data. The selected data which was obtained from Madihah (2002) will be used for analysis. The data showed biomass of *C.acetobutylicum* in five different cultures which are yeast culture, yeast with ammonium suphate culture, yeast with ammonium phosphate culture, yeast with ammonium chloride culture and yeast with ammonium nitrate culture and the production of acetone and butanol for each culture. The parameter then estimated using the Euler-Maruyama approximation method. The scopes also focused on stochastic power law logistic model and Ito stochastic differential equation.

1.5 Significance of the Study

By the end of this study, hopefully we can have a better model in studying the population dynamics especially in cell growth and solvent production. By doing this study, we can also show that stochastic modelling in cell growth is better than the deterministic model thus contributing this study into biological community.

1.6 Research Framework

This thesis is organized into six chapters. Chapter 1 is the introduction of the thesis. The intoduction is based on the background of the study. Objectives and scopes of this study is also defined in this chapter. The significance of this study is also mentioned in this chapter.

Chapter 2 outlines the literature review on this study. The literature review is focus on fermentation process and the development of the fermentation process during recent decades generally and on the fermentation of sago starch specifically.

Chapter 3 includes the basic definitions and of the stochastic process, martingales and stochastic differential equation. This chapter also defines the Brownian motion and stochastic integral including Ito formula and some examples. For the parameter estimation part, the parametric estimation method involving the Euler-

Maruyama approximation method is defined in this chapter.

Chapter 4 presents the data analysis for the cells growth of the fermentation process. Experimental data collected by Madihah (2002) will be used for analysis. The stochastic growth will be analized and compared to the deterministic growth.

Chapter 5 presents the data analysis for solvent production. The solvents are acetone and butanol. This chapter will prove that stochastic power law logistic model is more adequate to model the solvent productions compared to the Luedeking-Piret equations.

Lastly, in Chapter 6 the summary and conclusions of this study will be disscussed. At the end of the chapter, few recommendations for the future studies of stochastic modeling will be presented.