Department of Chemical Engineering

Modeling and Control of Non-Ideally Mixed Bioreactors

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Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Signature: …….

Date: 01st August 2011

To my beloved Family

Abstract

Mixing plays a substantial role in determining the overall performance of a bioreactor. Well mixing in bioreactor, especially for ethanolic fermentation process is important for the homogenization of miscible and immiscible liquids, gas dispersion and suspension of solid particles. Improper mixing will eventually affect the biological and kinetics reactions occurring in the bioreactor and subsequently deteriorate the bioreactor performance. Currently, most modeling and control applications of bioreactors have been devoted to ideally mixed assumption, for simplicity. This is not realistic in practical applications. Furthermore, the strength and accuracy of the bioreactor models reflect their performance and subsequently its control strategy. Therefore, it is vital to consider the imperfect mixing for the control of bioreactor.

In this study, a batch, micro-aerobic bioreactor for ethanolic fermentation process will be considered for modeling. Up to date, not much study has been conducted in exploiting the mixing mechanism for controlling this type of bioreactor. Traditionally, only the bioreactor conditions such as temperature and pH are controlled for such a batch bioreactor. Other parameters, such as aeration rate and stirrer speed are not used to control the bioreactor. Thus, it is difficult to improve the bioreactor performance as the bioreactor performance is less sensitive to both temperature and pH than to the mixing mechanism. However, the mixing behaviour of the bioreactor needs to be captured if we are to employ both aeration rate and stirrer speed for the control of such a batch bioreactor. It is known that aeration rate and stirrer speed could significantly affect the biological and kinetics reactions. Therefore, both aeration rate and stirrer speed are suggested in this work as

manipulated variables in the modeling of batch bioreactor. Thus, with this approach the ideally mixed assumption will be relaxed.
The models proposed will be implemented for control studies. New control strategies will be established for continuous bioreactor, whereby dilution rate and substrate concentration are considered as disturbance variables and both aeration rate and stirrer speed are suggested as manipulated variables. With this approach, the practicability of the proposed models could be investigated.

The aims of this research have therefore been as follows:

1. To experimentally study the impact of aeration rate and stirrer speed on the bioreactor performances, i.e. yield and productivity.
2. To develop an integrated bioreactor model to allow us to employ the aeration rate and stirrer speed as manipulated variables for control design.
3. To establish new control strategies for bioreactor without the ideally mixed assumption.

A systematic approach has been proposed to develop the non-ideally mixed bioreactor model and to design the control strategy of the lab-scale fermentation process. Three modeling approaches are employed, i.e. data-based, kinetics hybrid and kinetics multi-scale models for the analysis of the impacts of both aeration rate and stirrer speed on the performance of bioreactor. Using the three models, the aeration rate and stirrer speed are also used to analyze the mixing mechanism in the bioreactor.

Furthermore, new control strategies are then proposed for the bioreactor. By using the proposed control strategies, the effect of both aeration rate and stirrer speed on the overall performance could be analyzed in the face of disturbances on other process parameters. Furthermore, the stability and achievable performance of the control strategies could be compared for different models. Hence, the proposed control strategies would lead to a better operation of the bioreactor.

The study highlighted the following main findings:

1. It is identified that both aeration rate and stirrer speed could affect significantly the overall performance of the bioreactor. Therefore, both

aeration rate and stirrer speed rather than temperature and pH could be used as manipulated variables for controlling the bioreactor. The ideally mixed assumption is relaxed where the mixing mechanism of the bioreactor is included in the proposed model.

2. The main issue in modeling is the complexity of the microbial reactions and kinetics of the bioreactor performance for the non-ideally mixed behaviour of the bioreactor. Thus, it is important to identify the main reactions and kinetics which actually affect the bioreactor performance. In this study, Monod's kinetics has been employed with the implementation of both aeration rate and stirrer speed. It is shown that the kinetics multi-scale model demonstrated good predictions of the mixing mechanism of bioreactor. Different conditions of aeration rate and stirrer speed influence the mixing mechanism and thus, contribute to the dynamics and kinetics within the bioreactor. These show that both aeration rate and stirrer speed play important role in studying the non-ideally mixed mechanism of the bioreactor.
3. Optimization results, however, suggest that the kinetics hybrid model gives the most comparable values of maximum yield and productivity. Thus, this model is suggested for the determination of the optimum conditions of the bioreactor operation due to its simplicity in model construction, as compared to the kinetics multi-scale model.
4. The control strategy of bioreactor using the data-based model does not always produce good performance, especially in the face of large disturbances. This implies that the use of models with ideally mixed assumptions would not always give good overall performance. Therefore, the controllability of the bioreactor performance is further improved with the implementation of the proposed non-ideally mixed bioreactor model. It is observed that both data-based and kinetics hybrid models are able to keep the controlled variables in their set-point values by manipulating both aeration rate and stirrer speed for low disturbance changes.

Hence, this research contributes on the understanding of mixing phenomena in micro-aerobic fermentation process from which a set of optimal operational conditions and control strategies to enhance its performance are developed.

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Publications

E.W.T. Liew, J. Nandong and Y. Samyudia, *''Unification of Mixing and Cellular Metabolism in Bioreactor Modeling: A Multi-scale Approach''*, 15th Regional Symposium on Chemical Engineering (RSCE) in Conjunction with 22nd Symposium of Malaysian Chemical Engineering (SOMChE): Innovation*s* for Sustainable Future, Malaysia, 2nd-3rd December 2008

E.W.T. Liew, J. Nandong and Y. Samyudia, *"Experimental Investigation on the Impact of Aeration Rate and Stirrer Speed on Micro-Aerobic Batch Fermentation"*, 3rd International Conference on Chemical and Bioprocess Engineering (ICCBPE) in Conjunction with 23rd Symposium of Malaysian Chemical Engineers (SOMChE): Advanced Product Design, Development and Recovery for Natural Resources, Malaysia, 12th – 14th August 2009

E.W.T. Liew, J. Nandong and Y. Samyudia, *"Studies on the Impact of Aeration Rate and Stirrer Speed on Ethanol and Glycerol Yields in Micro-aerobic Fermentation of Cassava Starch"*, Chemeca 2009: Engineering our Future: Are We Up to the Challenge?, Australia, 27th – 30th September 2009

E.W.T. Liew, P. Kumar and Y. Samyudia, *"CFD Approach for Non-Ideally Mixed Bioreactor Modeling"*, Curtin University of Technology Engineering and Science 2nd International Conference (CUTSE): Progress in Science and Engineering for Sustainable Development, Malaysia, 24th-25th November 2009

E.W.T. Liew, J. Nandong and Y. Samyudia, *"Optimization of Fermentation Process Using A Multi-Scale Kinetics Model"*, The 5th International Symposium on Design, Operation and Control of Chemical Processes (PSE Asia), Singapore, 25th-28th July 2010

L.W.T. Emily, J. Nandong and Y. Samyudia, *"Experimental Investigation on the Impact of Aeration Rate and Stirrer Speed on Micro-Aerobic Batch Fermentation"*, Journal of Applied Sciences, 2009, **9**(17), p.3126-3130

E.W.T. Liew, P. Kumar and Y. Samyudia, *"Computational Fluid Dynamics of Mixing in Aerated Bioreactors"*, 2010 International Conference on Biology, Environment and Chemistry (ICBEC), Hong Kong, 28th-30th December 2010

E.W.T. Liew and Y. Samyudia, *"Nonlinear Model-Based Control of Non-Ideally Mixed Fermentation Processes"*, 2010 International Conference on Biology, Environment and Chemistry (ICBEC), Hong Kong, 28th-30th December 2010

E.W.T. Liew and Y. Samyudia, *"Optimization of Aeration and Agitation for Bioethanol Production"*, Curtin University 3rd International Conference (CUTSE): Innovative Green Technology for Sustainable Development, Malaysia, 8th-9th November 2011

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Nomenclature

°C	degree Celcius
D	Dilution rate
P	Final product concentration of fermentation medium
S	Final substrate concentration of fermentation medium
S_o	Initial substrate concentration of fermentation medium
X	Final biomass concentration of fermentation medium
AR	Aeration Rate
BT	Batch Time of fermentation medium
DO	Dissolved Oxygen
sp	Set-point of output variables
SS	Stirrer Speed
rpm	revolution per minute
CCD	Central Composite Design
CFD	Computational Fluid Dynamics
CMA	Compartment Model Approach
GMC	Generic Model Control
LPM	litre per minute
OUR	Oxygen Uptake Rate
PID	Proportional-Integral-Derivative
PFD	Process Flow Diagram
RSM	Response Surface Methodology
FF-FB	Feedforward-Feedback
MIMO	Multiple-Input and Multiple-Output
ANOVA	Analysis of Variance

e_t	Current error trajectory
g/L	Gram per litre
k	Tuning parameter for desired closed-loop responses
u_t	Optimal inputs
u_{t-1}	Past inputs
y_{mt}	Current measurements of output
y_{sp}	Set-point of outputs
y_t	Current output
y_{t-1}	Past predicted outputs

Chapter 1 Introduction

1.1 BACKGROUND AND MOTIVATION

The modeling and control of a bioreactor for ethanol production has been of interest many researchers due to its complexity of biological and mechanical processes. The biological process involves the kinetics of the fermentation process, which is determined by the properties of microorganisms as well as the cultivation conditions and media. On the other hand, the mechanical process involves the mixing mechanism of the bioreactor, whereby the metabolic activities of microorganisms are highly affected by the distribution of nutrients to the microbial cells via a mixing process. The engagement of both processes leads to a very complex dynamics of the microbial cells. This makes the bioreactor performance difficult to achieve a high operational stability of the fermentation process, as well as constant product quality and yield. Therefore, it is vital to simultaneously consider both kinetics and mixing mechanism of the bioreactor to achieve a better bioreactor performance so as to attain a better yield and productivity of the fermentation process.

Depending on the flow of medium to or from the bioreactor, or the supply of oxygen, the operational mode of a bioreactor can be classified into several groups, i.e. batch, fed-batch and continuous cultivation. Each mode demonstrates complex interaction between mixing and transport, fast metabolic reactions and cellular growth [1]. In practice, fed-batch and continuous operations are preferable. These operations can avoid problems with strain stability and sterility that may arise during prolonged cultivations. On the other hand, a batch cultivation is limited because there is no continuous feeding of nutrients into the culture medium. After inoculation, the microbial cells are growing continuously until the essential medium component is

exhausted or the accumulation of inhibiting products ceases the growth. Therefore, fed-batch cultivation is often operated since nutrients such as oxygen and nitrogen source, are continuously fed for continuous microbial growth and product formation [1].

In order to study the operation of a batch bioreactor, it is important to investigate which parameters are involved in a batch bioreactor operation. Figure 1-1 shows a general schematic diagram of a batch bioreactor operation.

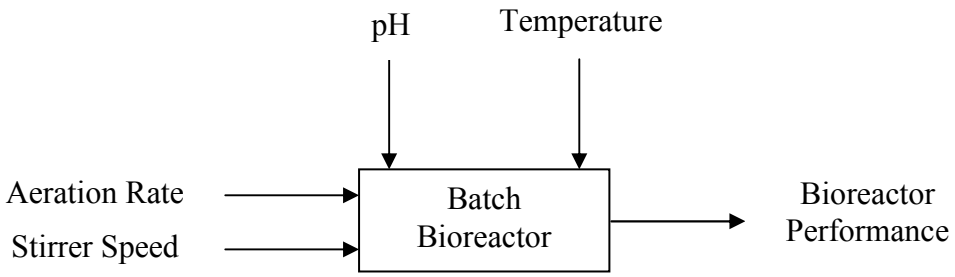


Figure 1-1 Schematic Diagram of a Batch Bioreactor Operation

Referring to Figure 1-1, there are generally four parameters that are involved in operating a batch cultivation, i.e. temperature, pH, aeration rate and stirrer speed. In previous studies, only temperature and pH are manipulated in order to achieve desired bioreactor performance. Aeration rate and stirrer speed are not used and often maintained at constant rate throughout the fermentation process. It is known that both temperature and pH are less sensitive in the mixing intensity of bioreactor as compared to that of both aeration rate and stirrer speed. Both aeration rate and stirrer speed are affecting the mixing intensity of bioreactor since both could disperse and increase the interface between gas and liquid phases in a bioreactor with momentum transfer. High turbulence is necessary for high mixing intensity, which could ensure uniform environmental conditions in the bioreactor for optimum growth of microorganisms [1]. Since in the previous work both temperature and pH are manipulated instead of aeration rate and stirrer speed, the mixing behaviour of the bioreactor is not captured. Ideally mixed assumption is therefore considered.

Up to date, no studies have been conducted to investigate the manipulation of both aeration rate and stirrer speed in modeling and control a bioreactor. This is due to the complexity of dealing with the mixing mechanism of bioreactor whereby it is very difficult to adequately describe the kinetics and dynamics of the fermentation process. With the manipulation of both aeration rate and stirrer speed for the control of a bioreactor, the mixing mechanism should be considered. Hence, non-ideally mixed mechanism is assumed for both modeling and control of bioreactor, and thus, further improvement could be made in the operation of the bioreactor.

Nowadays, researchers are still investigating how to incorporate the detailed mixing knowledge in modeling and control of a bioreactor. The understanding and modeling of the complex interactions between biological reaction and hydrodynamics are a key problem when dealing with a non-ideally mixed bioreactor. It is fundamental to accurately predict the hydrodynamics behaviour of bioreactors, especially with different bioreactor size and environmental conditions, and its interaction with the biological reaction. Since the detailed mixing effects of bioreactor are not being explored efficiently, it is imperative to explore ways and approaches in dealing with this matter.

One of the ways to incorporate the non-ideally mixed behaviour in bioreactor is by utilizing Computational Fluid Dynamics (CFD). CFD has been used over the decades for the integration of mixing details. The mixing details are obtained computationally and an efficient process simulation would allow the exploration of detailed mixing effects in a variety of new ways. For example, a detailed representation of a bioreactor could be feasibly embedded into the upstream and downstream of the bioreactor. The entire process in the bioreactor can be divided into three stages:

Stage I: Upstream processing which involves preparation of liquid medium, separation of particulate and inhibitory chemicals from the culture medium, sterilization, air purification, etc.

Stage II: Fermentation which involves the conversion of substrates to desired product with the help of biological agents such as microorganisms; and

Stage III: Downstream processing which involves separation of cells from the fermentation broth, purification and concentration of desired product and waste disposal or recycle.

Therefore, the effects of detailed flow, temperature and composition involved could be addressed efficiently. Such application has been pursued to modeling complex product distributions and controller design [2]. This approach has shown its substantial benefits in process modeling and control design. However, the integration of both aeration and agitation has not been considered using such methods. Furthermore, to accurately describe situations in which aeration and agitation greatly influence the biological reactions, combining mixing details with CFD would be advantageous. This would allow the prediction of the mixing behaviour that depends highly on spatial variations in the multiphase environment. Thus, the possible benefits gained from this approach would be significant for the control of non-ideally mixed bioreactor.

1.2 CASE STUDY

For the proposed research, a case study based on available equipment and facilities in the laboratory is proposed and described here. In this study, a micro-aerobic batch fermentation process is suggested as a case study for the development of models. Micro-aerobic process is a preferable pathway in ethanol production. This process can improve the viability of the yeast and the bioreactor performance. *Saccharomyces cerevisiae* is used as the cultivation microbe since this type of yeast is the most commonly utilized for cell growth in fermentation [1]. *Saccharomyces cerevisiae* is used as the cultivation microbe since this type of yeast is the most commonly utilized for cell growth in fermentation. This is due to the fact that this type of yeast has an active glucose transport system, whereby it metabolizes glucose through the glycolytic pathway, a metabolic pathway to convert glucose to pyruvate, Reduced Nicotinamide Adenine Dinucleotide (NADH) and energy [3]. Next, the yeast cells will convert pyruvate to acetaldehyde and carbon dioxide, then to ethanol. Roughly 98% of glucose is metabolized during fermentation, while the remaining 2% of it is made into cell materials. Due this factor, *Saccharomyces cerevisiae* is

used in fermentation to enhance the cell growth and productivity for maximum yield and productivity.

On the other hand, continuous fermentation process is suggested as a case study for control. This is to investigate the potential of the proposed models as controllers with the manipulation of aeration rate and stirrer speed with the account of disturbance variables, i.e. dilution rate and substrate concentration. Both dilution rate and substrate concentration are required to be supplied continuously into the fermentation medium for continuous growth. Without any supply of dilution rate and substrate concentration into the medium, the microorganisms' growth will deteriorate and the production rate will cease.

In order to conduct such study, a 2 litre, BIOSTAT® A Plus, double impeller Rushton turbine bioreactor is utilized in the laboratory, which is shown in Figure 1-2.

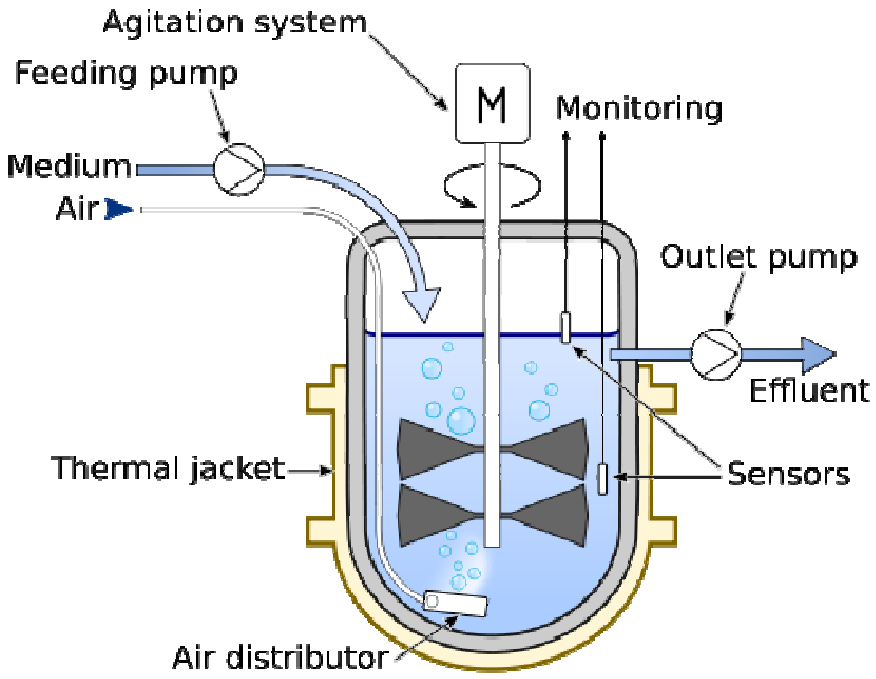


Figure 1-2 Schematic Diagram of a Double Impeller Rushton Turbine Bioreactor [4]

The bioreactor is fully instrumented to measure and maintain optimum conditions for the microbial cells. The related control loops for temperature and pH control as well

as air pump for sufficient supply of oxygen are ensured throughout the whole fermentation process. Figure 1-3 shows the operational system of the bioreactor utilized in the laboratory.

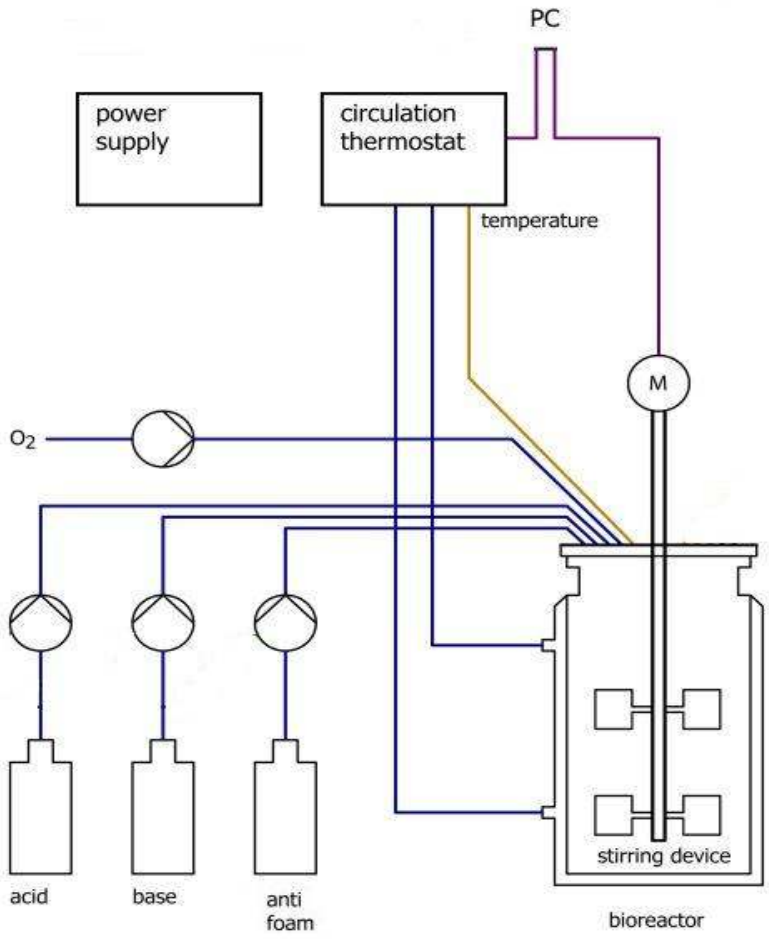


Figure 1-3 General Layout of the Laboratory Ethanolic Fermentation Process Bioreactor

The operation of the bioreactor for ethanol production begins with the addition of inoculum, i.e. a small amount of actual living cells (*Saccharomyces cerevisiae*), to the culture medium containing essential nutrients required for the growth of microbial cells.

In this study, glucose and cassava are used as the main substrates of ethanol production, in two separate case studies. This is to compare the yield and

productivity of the fermentation process for different substrates. In addition to that, it is also vital to study and compare the dynamics and kinetics of both substrates for the same mixing. The inoculum is added through the feeding tube and is pumped into the culture medium. The inoculum is only added during the beginning of the process since the fermentation process is operated at batch mode. The inlet oxygen concentration is limited to 1LPM for the cultivation and growth of the microorganisms since the fermentation process is operated under micro-aerobic operation. During the cultivation process, both aeration rate and stirrer speed are continuously controlled according to the desired optimum criterion in order to achieve maximum yield and productivity. Throughout the fermentation process, both acid and base are added in the case of maintaining the pH of the culture medium at 5. Temperature is also maintained at 30°C throughout the whole process with continuous supply of cooling water. Anti foam is, however, not added throughout the cultivation process, but we monitor the bubble foaming condition throughout the fermentation process. Monitoring and controlling the bioreactor is done throughout the cultivation process with the aid of a centralized computer system which is connected to the bioreactor.

1.3 RESEARCH QUESTIONS

To achieve the objectives of the research, some research questions are raised as follows:

1.3.1 Could we employ the aeration rate and stirrer speed for controlling the bioreactor performance?

In order to know this, we have to study how sensitive the bioreactor performance is to both aeration rate and stirrer speed. A set of experiments under different conditions of aeration rate and stirrer speed are to be conducted. Other parameters, such as temperature and pH are maintained at constant values throughout the fermentation process. The bioreactor performance, i.e. yield and productivity, are to be measured and evaluated at the end of each experiment. If the bioreactor performance of each conditions of aeration rate and stirrer speed differ significantly, thus it is suggested that both aeration rate and stirrer speed would be good candidates as manipulated variables as the bioreactor performance are sensitive to their changes.

Therefore, it is essential to study and investigate the influence of both aeration rate and stirrer speed to the bioreactor performance by evaluating its yield and productivity. We will experimentally investigate this.

The results will be presented and discussed in Chapter 3, where this chapter provides a detailed experimental methodology to study the effect of both aeration rate and stirrer speed on bioreactor performance. Also, the dynamics of the fermentation process with a non-ideal mixing condition will be exploited.

1.3.2 How to incorporate both aeration rate and stirrer speed as manipulated variables into the dynamic modeling of the batch bioreactor system?

By employing aeration rate and stirrer speed as manipulated variables for bioreactor control, the mixing mechanism of the fermentation process should be considered. From the experimental data, the kinetic parameters of substrate concentration, product concentration and biomass growth could be determined. The kinetic parameters will be used for the development of kinetics model. The kinetics model will be used to develop the non-ideally mixed bioreactor model, which will be validated against the experimental data in predicting the bioreactor performance. Three strategies, namely data-based, kinetics hybrid and kinetics multi-scale modeling, will be adopted in the development of new bioreactor models. As a result, the traditional assumption of ideally mixed bioreactor is relaxed. The developed dynamic model of the bioreactor will then be used for designing a model-based controller for the bioreactor, whereby the bioreactor performance will be controlled by manipulating both aeration rate and stirrer speed. The results of this work will be presented and discussed in Chapter 4.

1.3.3 How to apply the developed models for the optimization and control of bioreactor?

In the current study, a number of parameters, including yield, productivity, aeration rate and stirrer speed are determined to optimize the performance of the integrated system. Using the developed models, the optimum conditions of both aeration rate and stirrer speed in achieving maximum yield and productivity will be determined and compared to previous studies whereby optimization is done using models with ideally mixed assumption. Statistical methods using experimental design and the

Response Surface Methodology (RSM) will be employed to analyze the optimization results. The validation of the RSM-based optimization will be carried out experimentally. The results of this work will be presented and discussed in Chapter 5.

For the obtained optimum operating conditions, further studies will be directed towards the control of bioreactor. Previous studies to address the non-ideally features of bioreactor have been found in literatures. However, due to the complexity of the microbial activities and experimental difficulties associated with large-scale identification of enzyme kinetics, the employed models are limited to primary metabolic pathways. These models are not suitable for capturing the whole-cell metabolism, which has some impacts on the cellular growth and product synthesis rates. As a result, these models have not been used for bioreactor control design.

With the proposed non-ideally mixed bioreactor models, the control strategy for bioreactor control could be implemented. Nonlinear control is considered in this study as a control strategy for the non-ideally mixed bioreactor. This is due to the highly nonlinear operation of bioreactor which consists of multi-cellular activities of microorganisms. Chapter 6 illustrates a detailed application of the models for bioreactor control.

1.4 SPECIFIC OBJECTIVES AND GOALS OF THE THESIS

Specific objectives and goals of this thesis can be summarized as follows:

- To study the use of both aeration rate and stirrer speed as manipulated variables to control the bioreactor performance, i.e. yield and productivity.
- To develop new dynamic models of a bioreactor with the incorporation of both aeration rate and stirrer speed so that the assumption of ideally mixed behaviour in the bioreactor is relaxed.
- To determine the optimal operating conditions of both aeration rate and stirrer speed using the developed models, and to validate the results experimentally.
- To develop new control strategy for the bioreactor by employing both the aeration rate and stirrer speed as manipulated variables.

All of these specific objectives can ensure in achieving the goals of the thesis:

- To develop new dynamic models of batch bioreactor without assuming ideally mixing conditions
- To develop new approaches of controlling a bioreactor

1.5 ASSUMPTIONS AND SCOPE

The kinetics, dynamics, optimization and control analysis presented in this thesis are based on both macroscopic and microscopic biological models of the ethanolic fermentation process. The validity of the analysis depends on how well these models represent the true dynamic behaviour of the process, i.e. the non-ideally mixed mechanism of the overall ethanolic fermentation process.

Due to the nonlinearities and complexity of the bioreactor system, it is assumed that the bioreactor operation is constrained to operate within aeration rate of 1.0 to 1.5LPM and stirrer speed of 150 to 250rpm, whereby the system dynamics are represented by the proposed non-ideally mixed bioreactor model. Two case studies are carried out, i.e. with the utilization of glucose and cassava respectively, and are prepared in a 2 litre bioreactor. These case studies are carried out to investigate the dynamic behaviour of both substrates under the non-ideally mixing conditions in the bioreactor.

This thesis only addresses the effect of aeration rate and stirrer speed in the proposed model. Other parameter interactions such as temperature, pH, volume and substrate concentration, are beyond the scope of this thesis as they are set at constant values. Both glucose and cassava are utilized as the main substrate for experimental investigation, but only experimental results on glucose are used for kinetics modeling due to the incompatibility of cassava experimental results with the proposed kinetics model. Hence, the process optimization analysis is aimed at the interaction of both aeration rate and stirrer speed to achieve maximum yield and productivity. In addition, the application of the designed control systems for the glucose as the main substrate is also studied. In control studies, the continuous fermentation system is investigated.

1.6 THESIS STRUCTURE

This thesis is organized into seven chapters as outlined below. The structure of the thesis is graphically presented in Figure 1-4.

- ***Chapter 1*** defines the motivation, overall aim, scope and the structure of this thesis. This chapter also reviews the research questions, whereby the issues and gaps to be addressed in this thesis are discussed.
- ***Chapter 2*** reviews the issues in modeling and control of non-ideally mixed bioreactor. Included in this chapter is a review of relevant literatures, previous work on modeling and control design, and control strategies for bioreactor. In this chapter, we also identify the existing research gaps. As a result, a framework for the modeling and control analysis is proposed and is used as a basis of the work presented in subsequent chapters of this thesis.
- ***Chapter 3*** summarizes the research methodology, as well as experimental and analytical techniques employed in this study. Two case studies are conducted to compare the yield and productivity between glucose and cassava as the main substrate for ethanol production. Further, the dynamics and kinetics of both case studies are studied with respect to their mixing behaviour within the bioreactor.
- ***Chapter 4*** discusses the modeling approach, model development and its validation against experimental data. The models are developed for the implementation of both aeration rate and stirrer speed as manipulated variables.
- ***Chapter 5*** presents the study on the optimization of yield and productivity with respect to both aeration rate and stirrer speed. The Response Surface Method (RSM) is employed for this purpose.
- ***Chapter 6*** addresses the model-based control strategies to achieve a better bioreactor performance in the face of disturbances. The achievable performance of the control strategies using different models is examined in this chapter.
- ***Chapter 7*** draws conclusions from this study and outlines the recommendations for future research.

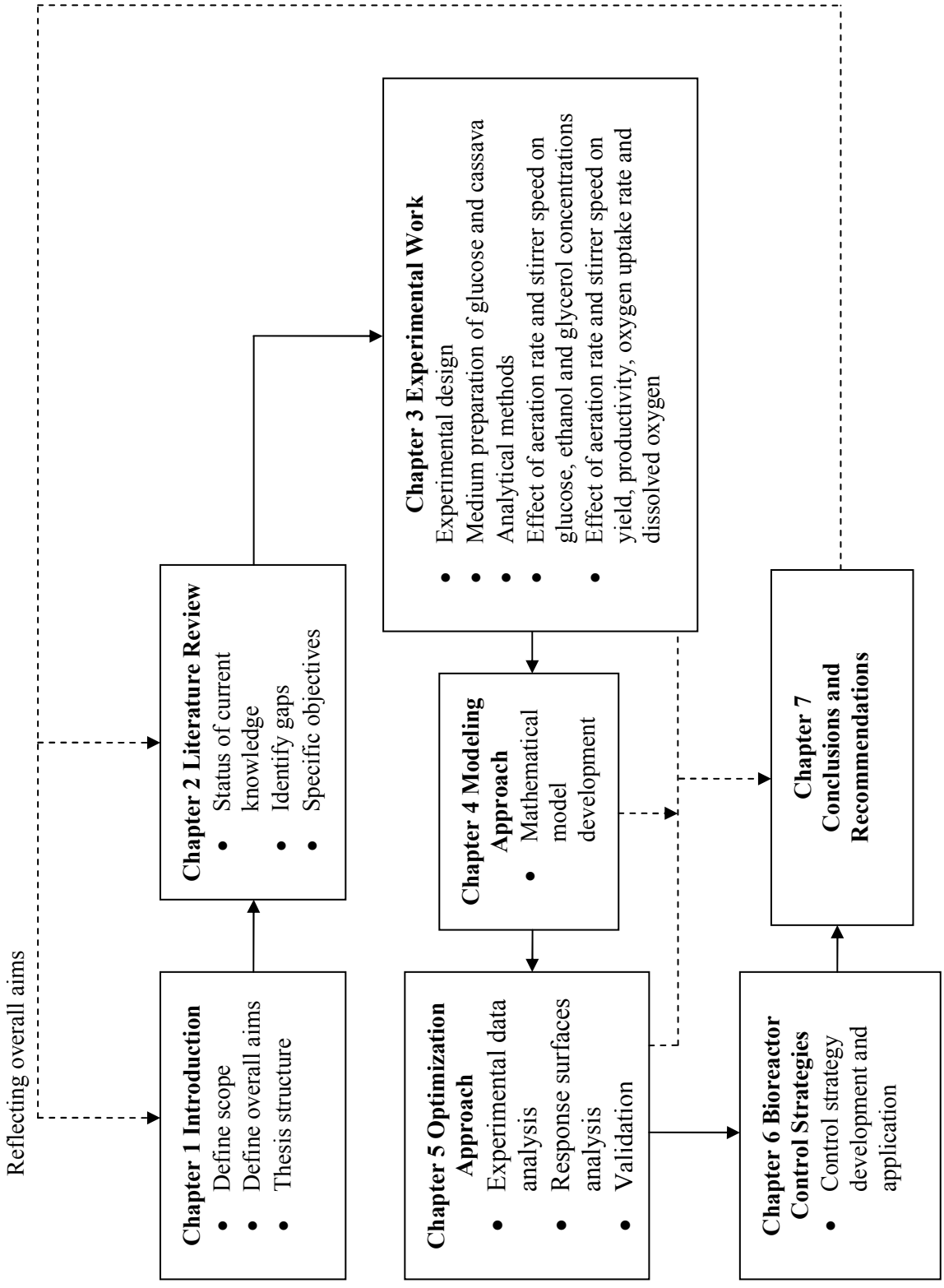


Figure 1-4 Thesis Presentation

Chapter 2 Literature Review

2.1 INTRODUCTION

A fermentation process, either small or large scale, is made up of bioreactor. Generally, the operation of bioreactor is classified into batch, fed-batch and continuous cultivation. Each bioreactor operation consists of oxidation and/or reduction of feed (or substrate) by microorganisms, such as yeast or bacteria. The complexity of the bioreactor operation and their interactive nature presents an extraordinary challenge in modeling and control of bioreactor. Nevertheless, such a challenge needs to be accounted for, in order to obtain satisfactory bioreactor operation and control over the entire fermentation process.

Batch and fed-batch cultivations are preferable in ethanol production in practice. However, most studies in modeling and control has been done in fed-batch cultivation rather than batch and continuous cultivations. Not much study has been conducted to improve the batch and continuous bioreactor operations. Additionally, in the previous studies the mixing behaviour of the bioreactor was not captured, instead an ideally mixed assumption was considered. This assumption is valid for a small scale of bioreactor, hence it is very difficult to achieve a well mixed behaviour for a large scale of bioreactor. Our study is therefore focused on the dynamical behaviour of a batch bioreactor with the assumption of non-ideally mixed mechanism in modeling. In the control of bioreactor, it would be interesting to investigate the practicability of the proposed model in continuous fermentation cultivation. Such difference is made due to the necessary implementations of continuous feed into the bioreactor in order to ensure continuous growth of microbial cells. Not only could

this describe the complexity of the mixing mechanism of bioreactor, but also improve the bioreactor performance.

Motivated by this idea, the major challenge faced in bioreactor operation is the complexity of the interrelation between the growth of microbial cells and their physical and chemical environment. It is impossible to apply linear model and control theory without severely sacrificing performance robustness properties. It is not trivial to develop reasonable accurate mathematical models with reliably estimated parameters. This is essential for optimization and design of a high performance control system. Mismatch between the developed model and the true process dynamics may degrade the bioreactor performance and can lead to serious control stability problems, especially when the process is nonlinear. Therefore, it is our objective to integrate the process nonlinearity such as the mixing mechanism of bioreactor into the modeling and control of bioreactor.

In this chapter, the issues in mixing mechanism of bioreactor are addressed. The fundamental bioreactor problems and challenges are reviewed. Issues and approaches to addressing the mixing mechanism of bioreactor are reviewed. This review includes previous work on the modeling and control of bioreactor with respect to the inclusion of mixing process. Finally, suitable kinetic models and alternative control strategies are proposed in order to address the non-ideally mixed mechanism of bioreactor.

2.2 MIXING AND CHALLENGES OF BIOREACTORS

The bioreactor performance of an ethanolic batch fermentation process is complex due to the complicated interrelations between the microbial cells and the governing environment. The exact description of flow movement by a simple model is not possible since the flow caused by the stirrer/impeller is overlapped by turbulence fluctuations. The situation is more complicated with the presence of two or more phases. Therefore, a more accurate description of the biological, chemical and physical processes and their interrelation in mixing environment is impossible [1]. Considerable abstraction is necessary which is based on the knowledge of the influence of various parameters on the bioreactor performance.

Early attempts to handle mixing in batch bioreactor were done by considering single-phase flow, i.e. perfect mixing is assumed and density variations are neglected [5]. It is only through this assumption that it is possible to achieve effective response to large bioreactor variations and thus achieving overall control objective. To do so, traditionally, operating conditions, such as temperature and pH are manipulated to achieve desirable bioreactor performance. The operation of bioreactor is often based on an off-line optimized profile for the manipulated variables. In this situation, all dynamics and chemical reactions occurring in the cultivation media conforms over all the ranges of temperature and pH.

In view of the ideally mixed assumption considered in bioreactor, numerous work has been done in studying the non-ideally mixed mechanism of bioreactor in terms of kinetics. Most kinetics is limited to macro-kinetics, i.e. the interactions of the microenvironment around the microbial cells with its dependency of the biological reaction are not taken into account. The metabolism of microorganisms is very complex, whereby the metabolism varies during the cycle of cell growth and replication. These phenomena cause inhomogeneity of the microorganisms' population. There might be morphological differentiation of microbial cells accompanied by changes in the cell metabolisms. Thus, what is observed is only an averaged behaviour over a great number of cells in different states. It is tough to establish a very detailed model to describe all the microbial metabolic activities. This will influence the control strategy of the bioreactor performance. It is hard to obtain precise kinetics in describing the inhomogeneous conditions of the microorganisms.

Further, in view of the difficulties faced in kinetics modeling, it is obvious that it is impossible to apply linear control theory. This is due to the highly nonlinear behaviour of the bioreactor operation. In this situation, other schemes such as nonlinear control or other forms of robust control are considered in tackling with the trajectory problem of bioreactors. The control of bioreactors demands extra design effort to compensate for their inherent time varying process characteristics. In fermentation cultivation, the biomass increases during the course of fermentation. Thus, the amount of heat generated and the total amount demand of oxygen increase as well [6]. Therefore, growth related process variables such as substrate concentration and temperature, are not sufficient to monitor the bioreactor

conditions. This situation is only applicable to systems that are mildly nonlinear [7]. Therefore, it is required to distinguish other process variables which affect the bioreactor conditions due to changes in dynamics.

Operating conditions such as aeration rate and stirrer speed other than temperature and pH are yet to be considered as manipulated input variables in bioreactor system. According to García-Ochoa, Santos and Alcón (1995) [8], pH does not seem to affect the production rate significantly. Comparing to that of temperature, the influence of temperature has been studied even more intensively [9]; [10]; [11]; [12]; [13]; [14]; [15]; [16]; [17]; [18]; [19]; [20]; [21]; [22]. However, according to García-Ochoa and Gomez (2009) [23], the most important among them are aeration rate and stirrer speed used in an aerated bioreactor. This is because in stirred tank bioreactor, high values of mass and heat transfer rates are attained. Oxygen mass transfer is influenced by both aeration rate and stirrer speed [8]. On the other hand, stirrer speed is used to carry out cell growth but not for production because dissolved oxygen will be exhausted. Thus, a compromise between oxygen mass transfer and mechanical stress in cells resulting from stirrer speed must exist [8]. Therefore, both aeration and stirrer speed offer more effect via the mixing mechanism of bioreactor as compared to temperature and pH since both affect the mass, heat and oxygen transfer throughout the bioreactor and both provide more turbulence in the bioreactor.

As the influence of mixing towards fermentation conditions cannot be overemphasized, it is very important to relax the ideally mixed assumption [24]. The control problems will be further enhanced by the implementation of non-ideally mixed mechanism in developing mathematical models of bioreactor.

2.3 CONTROL OF BIOREACTORS

Considering the problems and challenges faced in the bioreactor operation, automatic control for the optimization of product efficiency, product quality improvement and detection of disturbances in fermentation process operation is required. Schugerl and Bellgardt (2000) [1] as well as Rani and Rao (1999) [25] suggest that automatic control of biotechnological processes such as fermentation process, is developing slowly and there are two reasons for this:

- Accurate process models are rarely available due to the complexity of the underlying biochemical processes. Many problems of methodology in modeling remain to be solved. In order to produce reliable model, the modeling effort is often tedious as a great number of experiments are required. It is tough to reproduce experiments due to the difficulty in obtaining the same environmental conditions, as these processes involve living microorganisms. Their dynamic behaviour is strongly nonlinear and unsteady. Thus, lack of accuracy of measurements will lead to identifiability problems.
- In most cases, cost is one of the considerations which are taken into account, especially in purchasing cheap but reliable instrumentation and equipment. To date, the market offers very few sensors which are capable of providing reliable online measurements of the biological and biochemical parameters in order to implement high performance automatic control strategies. The cost and duration of analyses limit the frequency of the measurements. However, basic sensors such as pH, temperature, foam/level and dissolved oxygen (DO) probes, are necessary for monitoring the bioreactor.

Therefore, many design issues in dealing with this matter have been proposed to capture model uncertainties. The current strategy for dealing with this matter is by the utilization of adaptive control approach [1]. The application of this approach has greatly improved the bioreactor performance of control systems. But it has soon become apparent that due to the highly nonlinear behaviour of the microbial activities in the bioreactor, there is a problem in plant-model mismatch. Accurate models are not routinely available or even impossible to obtain, especially current models in mixing bioreactors are mostly ideally-mixed assumed. It had become clear that dealing with plant modeling errors in model-based control design is required. Therefore, as proposed, it is important to study how sensitive aeration rate and stirrer speed in the control of bioreactor performance.

2.3.1 Agitation and Aeration Rate for Bioreactor Control

In this study, both aeration rate and stirrer speed are considered as manipulated variables for bioreactor control. Practically, mechanically agitated aerated bioreactors are widely used rather than aerated only bioreactors. It is identified that aerated only

bioreactors are insufficient to promote medium turbulence necessary for air bubble generation. This is because the interactions of both aeration and agitation are important for fully mixed bioreactor to promote cell growth in order to achieve higher yields. Both aeration and agitation supply oxygen for the microbial cells in fermentation processes and to mix the fermentation medium, in order to make a uniform suspension of the microbes and nutrients [26].

In fermentation context, aeration is the process by which air is circulated through the mixed fermentation medium. Aeration is normally facilitated by using an air sparger, whereby air is allowed into the culture medium from the bottom of the bioreactor tank. The aeration efficiency depends on the oxygen solubility and diffusion rate into the fermentation medium on the bioreactor capacity to satisfy the oxygen demand of the microorganisms. The efficiency of aeration depends on oxygen solubilization, diffusion rate into cultivation media and bioreactor capacity to satisfy the oxygen demand of microbial population. These processes are important as microorganisms require oxygen for growth. It is also beneficial for the performance of the microbial cells to meet their requirements at any stage in the fermentation process [27]. For example, with the utilization of yeasts, like *Saccharomyces cerevisiae* in fermentation process, the microbial yeast cells require molecular oxygen. Oxygen is required especially in the synthesis of sterols and unsaturated fatty acids that are present at suboptimal concentrations [24]. The yeasts can only synthesize sterols and unsaturated fatty acids when there is exogenous supply of these compounds under strict anaerobic conditions and aerobic conditions. Failure to supply sufficient amount of oxygen may lead to undesirable changes in enzymatic makeup [28] or death of the living cells, with lower yield of the desired product [27]. Therefore, it is important to note the importance of aeration in fermentation process.

Besides aeration, agitation also plays important role in the mixing mechanism of the bioreactor. In fermentation context, agitation is the process whereby the fermentation medium is put into motion by shaking or stirring, with the aid of a stirring device known as a stirrer. Agitation is very important in mixing the fermentation medium to ensure that the medium is in homogeneous state. This is vital for equal distribution of nutrients and oxygen to the microbial population, as well as uniform distribution of energy. Energy introduced by the rotation of the stirrer, is converted into kinetic

energy of liquid and subsequently lost as turbulence dissipation. Energy balance around the stirrer is of interest since energy dissipation rate is the key variable that influences the characteristics of the impeller stream. Eventually, this also influences the characteristics of the rest of the bioreactor tank. Agitation also increases the interfacial area between the gas and liquid which improves the efficiency of aeration [26]. Shear forces are created which affect the microbial cells in several ways, causing morphological changes, growth variation and product formation [29]. Although agitation could maintain the oxygen available in the fermentation medium, poor oxygen transfer will be attained without appropriate agitation rates. The oxygen diffusion would deviate away from optimal conditions if the agitation rates are either too high or too low. This can inhibit microbial cell growth due to the heterogeneous mixing and shear stress effect [30].

Numerous biochemical reactions and interactions could not be captured entirely by experiments and computer simulations subjected to both aeration rate and stirrer speed. The complexity of the multiphase interactions and biochemical reactions even with relatively simple geometries, requires intensive research in detail. In addition, the unavailability of the related flow distribution under dynamic inflow modulation has not been reported in open literature [31]. The development of the non-ideally mixed bioreactor models is implemented in stages. However, it requires heavy modeling and computational effort to properly engaging microbial activities along with mixing. Such approach could not provide rational explanations for the performance enhancements. Thus, the target in achieving the overall production and control objective is not possible.

2.3.2 Control Strategies for Continuous Bioreactor

Control strategies for continuous bioreactors have had limited success since they are only applicable to systems that are mildly nonlinear that are not applicable in some practical situations. These approaches relatively included less robustness properties since the only manipulated variable considered is either feed or dilution rate. In this situation, control structures based on a nominal model without taking into account the inherent nonlinearities of the fermentation process. On the other hand, lack of measurements, difficulty to define product quality and the uncertainties or failure in

variations in the parameter values are also encountered [7]. Thus, it is expected to improve the operation of bioreactors by using nonlinear model-based strategy.

Model-based control is strategically using an explicit, separately identifiable dynamic model to successfully model a wide range of nonlinear processes [32]. With the inherent nonlinear nature of chemical processes, the utility of a control structure which incorporates fundamental process models becomes evident. In the past three decades, model-based controller design has attracted much attention in the control field. This is due to the fact that this control technique could greatly improve the performance of controlled systems compared with traditional PID controllers [33]. Implementation of linearized model based control may cause a lack of robustness or even instability within the operating range of these processes [34]. Thus, nonlinear model-based control has created vast interest to yield robust control for a wide range of process nonlinearity and a degree of process mismatch. Several control techniques have been proposed, engaging particular features to improve the control processes by employing a process model, for instance Model Predictive Control (MPC) [35]; [36] and Generic Model Control (GMC) [37].

Numerous advancements have been made in model-based process control. The use of this strategy strongly depends on how strong the interactions between variables, i.e. aeration rate and stirrer speed, which affect the overall bioreactor performance and the process-model mismatch. This implies that the effect of interactions and robustness on the overall performance needs to be measured.

2.3.3 Control Algorithms for Continuous Bioreactors

Using model-based control techniques, a nonlinear control algorithm is presented in this section. Before presenting the nonlinear control strategy, previous control strategies for continuous bioreactors are studied first in order to gain an insight on the control problem. Conventional fixed gain PID feedback controllers have been used in continuous bioreactor system [38]. Due to the inherent non stationary behaviour of a bioreactor, proper tuning of the PID controller to provide satisfactory regulatory performance is required. Oxygen uptake rate (OUR) and dissolved oxygen (DO) are chosen as measured variables. The change due to process dynamics, however could not be compensated completely solely through PID control action.

Therefore, it is suggested to apply two simple controller designs, feedforward-feedback (FF-FB). In this approach, a feedforward controller is added to detect any load changes and to take corrective action in an existing feedback control system. Figure 2-1 shows a block diagram to illustrate this control scheme.

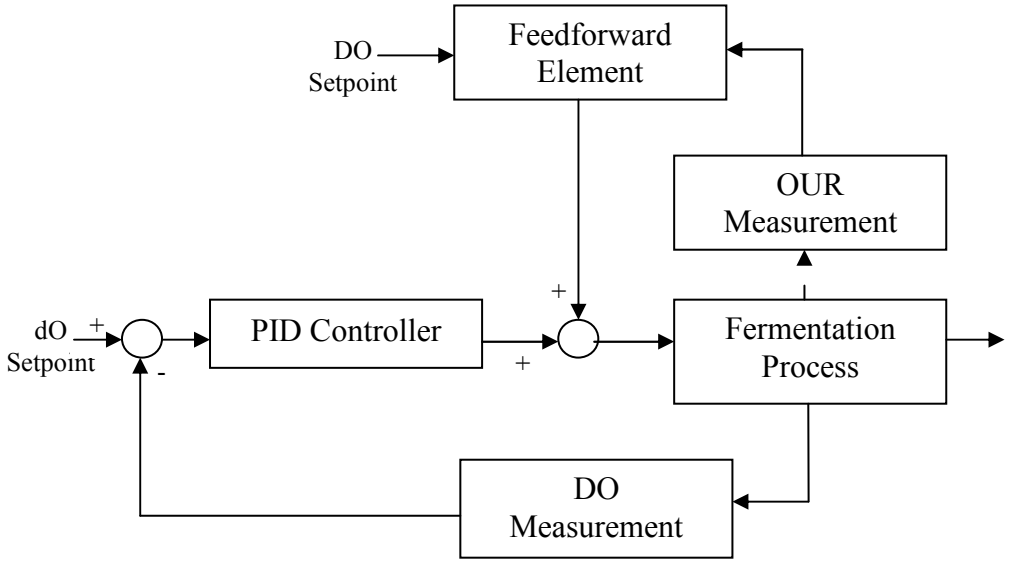


Figure 2-1 Block Diagram of a Feedforward-Feedback (FF-FB) PID Controller

Since the OUR increases monotonically, an offset in the controlled variable is expected from classical control theory. A controller purposely tuned for low OUR to ensure system stability throughout the fermentation process become sluggish as time progresses. On the other hand, a controller tuned for high OUR that produces good response and small offset may be unstable at the beginning of the process. This clearly indicates the need for the increasing of the controller gain as a function of OUR to maintain good control performance. Therefore, the addition of a feedforward controller is to detect any load changes and to take corrective action. The OUR will be monitored and the controller will adjust according to the desired values accordingly. Therefore, OUR measurement is fed to feedforward block. On the other hand, the DO setpoint is fedforward to make the plant automation system respond immediately towards the setpoint value. The feedforward element can move the process to match the flow and conditions, for example temperature and composition, of all important process streams on a process flow diagram (PFD) for a given product

and production rate. Thus, this will enforce material, component and energy balances for continuous processes [39]. The feedforward element not only provides corrective action for production rate enabling PID controllers to provide consistent product quality, but also provide immediate achievement of optimum conditions.

Based on this control approach, results suggest that the response of the process is greatly enhanced by the addition of the feedforward loop. However, unsatisfactory regulatory performance is observed. The stability of the process is unaffected. In addition, the FF-FB controller is unable to diminish the process offset. The offset in the dissolved oxygen concentration decreases with increasing controller gain, and that the FF-FB controller cannot compensate for this offset. The FF-FB controller is also rather insensitive to small errors in modeling. This is observed when the performance remains the same when the OUR is purposely underestimated or overestimated. Subject to this effect, this eventually erodes controller performance since it is left uncompensated [6]. On the other hand, the control problems are further deteriorated by the fact that the mathematical models describing the process is assumed to be well-mixed. Thus, the control approach is unable to measure or predict the desired set-points accurately.

Given these problems, it is suggested to propose a control strategy, which could address the desired set-points implemented into the control system for a continuous bioreactor process. Therefore, the parameters which significantly affect the mixing mechanism of the bioreactor are identified.

In this study, a multivariable control (multiple-input and multiple-output MIMO) strategy is used, whereby both aeration rate and stirrer speed are varied in response to yield and productivity. In these control loops, temperature and pH are not allowed to be manipulated and to be remained constant. All the control loops have the objective of achieving optimum yield and productivity at the end of the fermentation process. Model-based control is utilized instead of PID control, in order to describe the non-ideal features of bioreactor by using mathematical models. Figure 2-2 shows the illustration of the proposed control strategy for non-ideally mixed continuous bioreactor control design in this study.

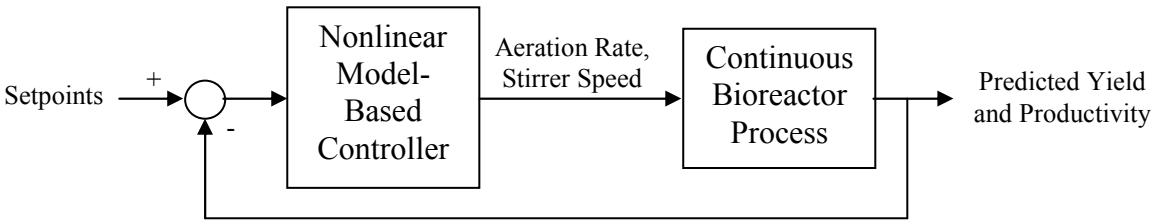


Figure 2-2 Block Diagram of Proposed Control Strategy for Non-Ideally Mixed Continuous Bioreactor

Based on Figure 2-2, the proposed algorithm for modeling non-ideally mixed bioreactor can be formulated as follows.

Procedure 1:

Step 1: Generate non-ideally mixed batch bioreactor model by considering aeration rate and stirrer speed as manipulated variables. The algorithm of the development of non-ideally mixed bioreactor model will be discussed in the next section.

Step 2: Ensure that the proposed non-ideally mixed bioreactor model is accurate and precise by performing statistical analysis. Next, investigate the effect of interaction for both aeration rate and stirrer speed on overall bioreactor performance. Statistical analysis and experimental validations are required to study the interaction. In the case whereby there is interaction between aeration rate and stirrer speed on bioreactor performance, this indicates that both interact with one another, affecting the mixing mechanism of bioreactor. Therefore, this interaction affects the bioreactor performance.

Step 3: Next, set both aeration rate and stirrer speed as input variables; yield and productivity as output variables; initial feed substrate and dilution rate as disturbance variables. These setups are set to investigate the controllability of both aeration rate and stirrer speed in the highly nonlinear bioreactor performance, i.e. yield and productivity, with the presence of disturbances.

Step 4: To design the nonlinear model-based controller, an optimization approach is employed which requires an explicit nonlinear model in the form of [40]:

$$y_t = f(y_{t-1}, u_{t-1}, \theta) \tag{1}$$

where y_t is the current predicted output and y_{t-1} is the past predicted outputs; u_{t-1} is the past inputs; Θ is the process parameters.

Equation 1 is used in solving a constrained or unconstrained nonlinear optimization problem that minimized the following objective function:

$$\Delta u_t^* = \arg\{\min_{\Delta u_t} (\Delta y_t - e_t)^2 + (\Delta u_t)^2\} \tag{2}$$

Subject to:

$$\Delta y_t = y_t - y_{mt} \tag{3}$$

$$u_{\min} \le u_t \le u_{\max} \tag{4}$$

$$\Delta u_{\min} \le \Delta u_t \le \Delta u_{\max} \tag{5}$$

where y_{mt} are the current measurements of the outputs; $u_t = u_{t-1} + \Delta u_t^*$ are the optimal inputs and e_t is the current error trajectory defined as:

$$e_t = k\int_0^T (y_{sp} - y_{mt})dt \tag{6}$$

y_{sp} is the set-point of the outputs and k is the tuning parameter for desired closed-loop responses.

Step 5: The proposed nonlinear model-based controller is implemented in a closed-loop system. This nonlinear model-based controller has the following control structure implementation as follows:

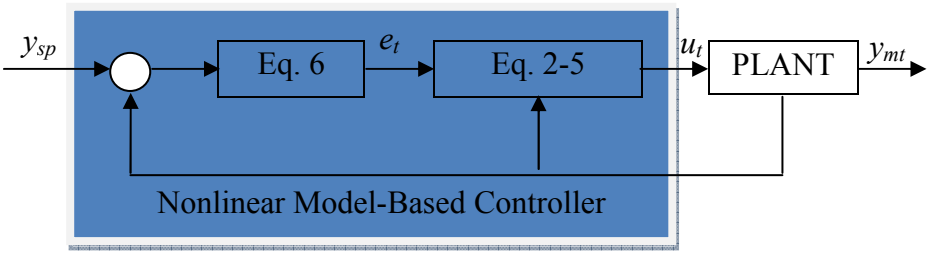


Figure 2-3 Nonlinear Model-Based Controller of Fermentation Process

Note that when there are no constraints, i.e. Equation 4 and Equation 5 do not exist, the optimal solution for the nonlinear optimization will have an explicit form as follows:

$$\Delta u_t^* = -[\Delta y_t - k\int_0^T (y_{sp} - y_{m_t})dt] \tag{7}$$

which is a PI type controller, but the gain is adjusted using the nonlinear model of Equation 1 so it is nonlinear gain.

It is noted that the nonlinear gain as shown in Equation 7 is obtained by comparing the measured and predicted outputs, as indicated in Equation 3. In the case of no plant-model mismatch, a pure integrator controller with the design parameter *k*, can be used to achieve the desired close-loop performance, i.e. zero offset. But, it is clear that no plant-model mismatch is an ideal situation model.

On the other hand, the higher the value of Δy_t, the higher the plant-model mismatch. This implies that a lower gain is required to make the system more robust, subject to modeling errors. This would limit the choice of *k* that represents the desired closed-loop performance.

As a summary, if Equation 1 is implemented to design the nonlinear controller, the optimization problem should be solved as follows:

1. Given $y_{m_t}; y_{t-1}; u_{t-1}$
2. Compute $\Delta y_t = y_{t-1} - y_{m_t}$
3. Solve the optimization problem for Δu_t^*

4. Use Δu_t^* to compute $u_t = u_{t-1} + \Delta u_t^*$ and then apply it in the steady state model, i.e. Equation 1, to get y_t
5. Repeat 2 and 3.

In the implementation of Procedure 1, the following modeling problem is defined:

Problem 1:

1. How to develop the non-linear model for fermentation process by considering both aeration rate and stirrer speed as manipulated variables.

2.4 MODELING OF BATCH BIOREACTORS

With the account of the control strategy and algorithm identified from Section 2.3, it is vital to study previous work done in modeling of non-ideally mixed bioreactor. This is to make further enhancements and progression in developing the non-ideally mixed bioreactor, without engaging perfect mixing assumption.

2.4.1 Previous Modeling Work on Batch Bioreactors

Despite of the complexity of developing non-ideally mixed bioreactor models, efforts have been done over these decades. Subsequently, there are advancements in modeling the mixing mechanism of bioreactor. In this section, previous modeling work in the non-ideally mixed bioreactor mechanism is addressed.

For example, Harvey III and Rogers (1996) [41] utilize a multi-block grid generation to generate computation of mixing bioreactors. This approach indicates that there are limitations in attaining results accuracy for unsteady and multiphase flows. This is because the laminar flow is considered throughout the computation process. Therefore, single phase flow is assumed and thus, ideally mixed assumption is considered.

On the other hand, Venneker et al. (2002) [42] evaluate that the mass, momentum and turbulence transport properties, such as density variations and mixture heat capacity are assumed to be constant. Each of these properties has to be solved for each individual phase by utilizing the population balance modeling. The use of this

approach would face a difficulty in defining the properties for each individual phase. This is due to the lack of universal agreement as to provide a generally valid formulation of the interfacial transfer terms. From an application point of view, this method is applicable, but problems arose due to the complexity of the actual microbial activities which is hard to be captured. Therefore, up to date, ideally mixed assumption is still considered.

Up to date, in batch fermentation process, Monod expression has been used to describe the microbial kinetics of bioreactor. Yet, ideally mixed assumption is still considered in describing the dynamics of the biomass, substrate and dissolved oxygen concentrations. On the other hand, the inlet air flowrate is assumed to be equal to the outlet air flowrate. For simplicity in process modeling, the Monod expression is solely dependent on the substrate concentration, which is employed to describe the cell growth. Thus, all assumptions are made without comprising the dynamics and bioreactor performance.

Based on the review of previous work on process modeling of non-ideally mixed bioreactor, the following remarks can be made.

1. Mixing has been an extensive research in the field of bioreactor in both single and multiphase flows.
2. Ideally mixed assumption is still considered in current practice for computation simplicity purposes in terms of modeling. This is because it is hard to capture and define all microbial activities in modeling and control.
3. The implementation of both aeration rate and stirrer speed has yet to be investigated in process modeling in batch bioreactors. Therefore, the mixing mechanism of bioreactor has yet to be implemented in process modeling. Due to this fact, the effects on the achievable overall objective, i.e. achieving maximum yield and productivity, have not been met. It would be of great interest to investigate the effect of both in process modeling and indicate the need for both variables to maintain good control performance.

2.4.2 Computational Fluid Dynamics (CFD) for Batch Bioreactors

Despite on the ideally mixed assumption on bioreactor modeling, computational fluid dynamics (CFD) is being utilized over the years to aid in solving mixing problems in bioreactor. This is due to its ability in establishing the relationship between the hardware and resulting fluid dynamics. CFD has proven to be useful in engineering fluid flow systems [43] which have been used for modeling mixing problems in recent years [44]. It is a useful tool that has become well-liked in the study of industrial fluid flow processes recently, which involves the usage of high-speed digital computer [41]. CFD codes normally facilitate the visualization of flow phenomena. It is beneficial when it is impractical within the fluid domains for the measurement of parameters such as pressure and velocity [45]. On the other hand, CFD requires relatively few restrictive assumptions and gives a complete description of the flow field for all variables [46]. Complex configurations can be treated and the methods are relatively easy to apply, whereby a variety of processes can be incorporated simultaneously.

The current approach in investigating the mixing mechanism of bioreactor through the utilization of CFD is by simulating flow within and outside the impeller region. This is done either with the combination of moving and deforming or sliding mesh [47;48;49;50;51] or iterative methods using rotating coordinate system [50;52]. These approaches show a promising view of utilizing CFD as a design tool without requiring any experimental inputs. However, it soon became apparent that these approaches could not be used as design tool due to the following reasons.

1. These approaches relyon solution of full-varying flow in mixing bioreactors. Thus, the computational requirements of these are much greater than those required by the steady-state simulations. Therefore, the utilization of such an approach as a design tool to screen various configurations becomes tough.
2. There are restrictions on number of computational cells that can be used for simulations due to excessive computational requirements. Such limitations will allow some variable predictions difficult.
3. Results obtained using these approaches are yet to be validated. According to Harris et al., (1996) [50], it is reported that there is severe underprediction of turbulence characteristics in simulations of flow generated by stirrer using sliding mesh approach.

In order to overcome these limitations, computational snapshot approach has been suggested to simulate flow generated in a fully baffled bioreactor [51]. This approach has been proven to be useful in capturing the key features of flow in single phase applications. However, this approach has yet to be extended in the application of multiphase flows. This is due to the fact that in the present computational model, coalescence is not modeled. Hence, the model is not able to simulate the formation of gas cavities behind impeller blades. This suggests that it is worthwhile to explore the potential of snapshot approach by considering the possibility of simulating flow within stirrer blades without excessive computations.

Another alternative approach to study the mixing mechanism in bioreactors is the general hybrid multizonal methodology. This method is proposed by Bezzo, Macchietto and Pantelides (2003) [53], whereby the non-ideally mixed behaviour in a bioreactor is represented by a multizone, which divides the equipment volume into a network of interconnected zones. Each zone is addressed to be ideally mixed and a population balance equation is incorporated to describe the phenomena in detail.

A general structure for hybrid multizonal approach is illustrated in Figure 2-4 below.

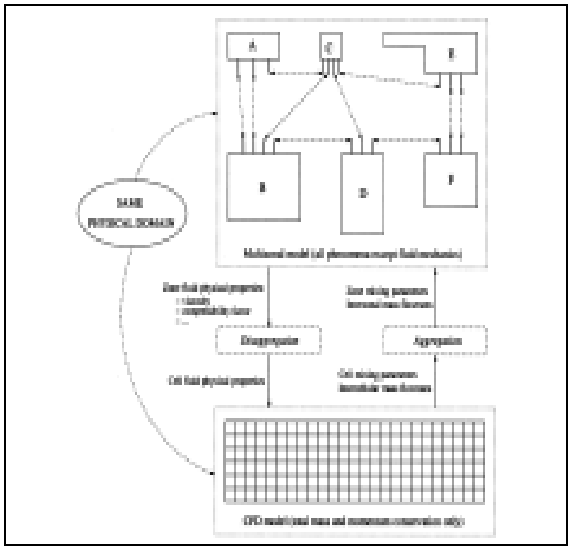


Figure 2-4 General Structure of Multizonal Approach [53]

Based on the general structure of the multizonal approach, fluid-flow prediction is solely being focused by dividing the space into a relatively large number of cells and solving total mass and momentum conservation equations only. Thus, intensive

properties such as composition and temperature are ignored. Superficial air velocity is assumed to be constant and uniform throughout the domain. Based on this ignorance, fluid-flow phenomena operate on a shorter time scale than other phenomena. Although the computational burden has been decreased, but the results attained would not be able to deal simultaneously with multiphase flow and non-Newtonian liquid behaviour.

Another technique which is employed to study the mixing behaviour of bioreactor is Compartment Model Approach (CMA). Several compartment models are available from literature, but most of them were based on artificial flow parameters without relation to the hydrodynamics of the process [54]. This approach is not feasible in the study of non-ideally mixed bioreactor as it does not provide independent knowledge of hydrodynamics. Therefore, it is difficult to extend this model with respect to non-ideality of mixing bioreactor. Vrabel (1999) [54] identifies that CMA is based on simple compartment structure and is expressed by fundamental correlations of fluid dynamics. Studies are well verified on small scale bioreactors. However, for large scale bioreactors, it is not sufficient to draw conclusions about the effect of model predictions.

Based on the review of previous work of CFD in mixing bioreactor, the following statements can be made.

- Numerous innovations and advancements have been made in CFD approach to study the non-ideally mixed behaviour of bioreactor. Considerable effort has been applied to develop efficient way for the simulation of complex multiphase flows of a bioreactor. However, up to date, most results obtain are only well validated on steady single phase flow, whereby most of these investigations have treated the rotating stirrer as a black box.

As a conclusion, considerations are required to be done in terms of high effort of modeling if CFD were to be considered for further studies in the non-ideally mixed behaviour of bioreactor. Considering the advances made in CFD application in single phase flow, it would be therefore a challenge to apply advanced technique in the analysis of multiphase flow of bioreactor. In addition, the design of control strategies to deal with nonlinear kinetics of fermentation process is also a challenge to be faced.

2.4.3 Modeling Approach for Batch Bioreactors

Based on the previous work done in modeling mixing mechanism of bioreactor, an algorithm is proposed in this section for the integration of modeling the non-ideality of mixing in bioreactor. The integration of modeling the mixing mechanism of bioreactor is done by considering the characteristics of stirred bioreactor, to achieve optimum yield and productivity. Models are proposed and generated by the analysis of the effects of interactions, i.e. aeration and agitation on the achievable bioreactor performance. Based on critical literature review and by considering the characteristics of the non-ideally mixed behaviour of bioreactor, CFD will be implemented. On the other hand, CFD will be used as a basis of the control strategy development. Hence, the proposed model would lead to a systematic approach to control analysis.

The proposed algorithm for modeling non-ideally mixed bioreactor can be formulated as follows.

Procedure 1:

Step 1: Study the bioreactor setup and determine the operating conditions. In this study, both aeration rate and stirrer speed are considered as input variables. Yield and productivity are considered as output variables. These setups are proposed to study the effects of both aeration rate and stirrer speed on the mixing mechanism and performance of bioreactor. To determine how well the performance of bioreactor is, both yield and productivity are measured. High yield and productivity indicate good bioreactor performance.

Step 2: Based on literature studies, Monod kinetic expressions such as growth rate, substrate utilization rate and product formation rate are suggested to describe the reaction rates of bioreactor. Monod kinetic expressions are utilized since it is the simplest kinetic formulation. Mass and heat transfer bioreactor models are identified as well to describe the dynamics of bioreactor.

Step 3: Both aeration rate and stirrer speed are implemented into the kinetic models by using linear regression analysis. Three modeling approaches are implemented to develop the non-ideally mixed bioreactor model, i.e. data-based, kinetics hybrid and

kinetics multi-scale models. The proposed models are regarded as non-ideally mixed bioreactor model since both aeration rate and stirrer speed are implemented as input variables to describe the mixing mechanism of the bioreactor.

Step 4: Next, it is important to investigate whether a bioreactor, no matter small or large scale, exerts non-ideally mixed behaviour in any operating conditions of aeration rate and stirrer speed. This step is important as many consider small scale bioreactors to be well-mixed. Therefore, it is vital to determine whether both small and large scale bioreactors exhibit insufficient mixing, in order to successfully validate the non-ideally mixed bioreactor models by using experimental data. Experimental validation is important to ensure that the proposed non-ideally mixed bioreactor models are accurate and precise, before implementation into control strategy. Once validated, the proposed non-ideally mixed bioreactor models are ready to be utilized for control purposes.

In the implementation of the above steps, the following problems are considered:

Problem 2:

1. Identification of kinetic and general bioreactor models for developing non-ideally mixed bioreactor model.
2. Effect of both aeration rate and stirrer speed in the mixing mechanism of bioreactor. This effect is to be observed in CFD.
3. Prediction quality of bioreactor performance using the developed models.

2.5 REMARKS

Based on the reviews, we noted the following remarks:

- Knowledge on modeling the mixing mechanism of batch bioreactor is still limited due to the complexity of the microbial activities. The dynamics of fermentation process is still not fully understood. A better approach to the modeling of bioreactors remains open for research. In order to produce a reliable model, the modeling effort is often tedious as a great number of experiments are required. It is tough to reproduce experiments due to the

difficulty in obtaining the same environmental conditions as these processes involve living organisms. Their dynamic behaviour is strongly nonlinear and unsteady. Thus, lack of accuracy of measurements will lead to identifiability problems.

- Since only temperature and pH are considered as manipulated variables, the bioreactor model is often assumed to be ideally mixed. All microbial cell activities are assumed to be identical. This will lead to severe loss in yield and changes in microbial physiology since the mixing mechanism is not considered for control.
- Both aeration and agitation play important roles in the mixing mechanism of bioreactor. Aeration is beneficial to the growth and performance of the living cells. This is done by the improvement of the mass transfer characteristics with respect to substrate and product, as well as providing the amount of oxygen to the yeast strain and its growth requirements. On the other hand, agitation satisfies the oxygen demand of a fermentation process.
- Implementation of linearized model based control may cause a lack of robustness or even instability. The implementation of nonlinear model-based control would be of great advantage, in order to yield robust control for a wide range of process nonlinearity and a degree of process mismatch.
- So far, temperature and pH are considered as manipulated variables in the control system. Other operating conditions such as aeration rate and stirrer speed are yet to be considered. It is identified that both aeration rate and stirrer speed has more effect on the mixing mechanism of bioreactor as compared to pH. Thus, it is suggested to utilize both aeration rate and stirrer speed as manipulated variables in the control system. This leads subsequently to the need of the development of non-ideally mixed bioreactor model.
- Most of the previous work done on CFD for mixing bioreactors are based on steady state analyses. Thus, the required degree of accuracy of predicted results will be affected.

Despite different models investigated in literature, no single model seems adequate to describe the non-ideally mixed behaviour of bioreactor. Therefore, it would be of our interest to investigate the effects of both aeration rate and stirrer speed in the

mixing mechanism of bioreactor. Through previous studies performed on investigating the mixing mechanism of bioreactor, it is vital to understand how its effect on a fermentation process. Therefore, there is a need to perform further research studies to investigate the non-ideally mixed behaviour of bioreactor.

The specific objectives of the present study are therefore:

- To study the mixing mechanism of bioreactor with the account of both aeration and agitation experimentally.
- To develop non-ideally mixed bioreactor models with the implementation of both aeration rate and stirrer speed as manipulated variables.
- To investigate the application of CFD in the development of non-ideally mixed bioreactor model.
- To develop control strategies for the implementation of non-ideally mixed bioreactor model into fermentation processes.

Finally, the outcomes of the present research will be integrated bioreactor model and control, which is evaluated against experimental data and practical needs. This is important so that the understanding and applicability of non-ideally mixed bioreactor model can be explored. The subsequent chapters (Chapters 4-6) will focus on the development of mathematical models to solve Problem 2. In addition, the issue of control analysis will be addressed in developing the control strategy on the development of an approach to solve Problem 1. Hence, both problems would lead to a systematic approach to modeling and control of non-ideally mixed bioreactor.

Chapter 3 Effects of Aeration Rate and Stirrer Speed on Bioreactor Performance

3.1 INTRODUCTION

The objective of this chapter is to study the effect of both aeration rate and stirrer speed on batch bioreactor performance. This chapter outlines the methodology, experimental design and analytical techniques from which the effects of both aeration rate and stirrer speed on the micro-aerobic batch fermentation process are investigated experimentally.

Two case studies are considered, i.e. the use of glucose and cassava as main substrates. Two-factor factorial designs are conducted to design experiments for both case studies, whereby in each experiment, the substrate, product, byproduct and biomass concentrations are measured. These experimental results will also be used for kinetics modeling, optimization and control strategy in the following chapters.

This chapter is divided into four sections. Section 3.2 explains the definition of the parameters involved in experimental studies. Section 3.3 describes the experiment design approach to study the effect of aeration rate and stirrer speed on bioreactor performance. Section 3.4 outlines the case studies involved in the experimental investigation. In Section 3.5, the experimental results for both case studies are presented. In this section, the effect and interaction of aeration rate and stirrer speed on the bioreactor performance for the case studies are discussed. Finally, concluding remarks are presented at the end of this chapter.

3.2 DEFINITIONS

In our study, there are a number of parameters employed in the experimental study of bioreactor performance. It is imperative to define those parameters for clarity. Listed below is the definition of those parameters:

- Aeration rate (AR) is defined as the rate of air circulating through mixed fermentation medium. Unit used is LPM (liter per minute).
- Stirrer speed (SS) is defined as the speed of the bioreactor impeller to mix the fermentation medium in circular motions. Rushton turbine is utilized in our study. Unit used is RPM (revolution per minute).
- Biomass concentration is defined as microbial cell density in the fermentation medium. Unit used is g/L.
- Glucose concentration is defined as the amount of feed supplied into the fermentation medium for microbial growth. Unit used is g/L.
- Ethanol concentration is defined as the amount of ethanol produced in the fermentation medium. Unit used is g/L.
- Glycerol concentration is defined as the amount of glycerol produced in the fermentation medium. Unit used is g/L.
- Dissolved oxygen (DO) is defined as the relative measure of the amount of oxygen that is dissolved or carried in a given fermentation medium
- Oxygen Uptake Rate (OUR) is defined as the microorganisms consumption rate of oxygen
- Yield is defined as the highest amount of product obtained from initial amount of substrate introduced in a fermentation process. It is calculated as:

$$\text{Yield} = \frac{P}{S_0 - S} \times 100\% \tag{8}$$

  where P is the product concentration, S_0 is the initial substrate concentration and S is the final substrate concentration (g/L) of the fermentation medium
- Productivity is defined as the measured product concentration within the fermentation process time. It is calculated as:

$$\text{Productivity} = \frac{P}{BT} \tag{9}$$

where P is the product concentration (g/L) and BT is the batch time of the whole fermentation process

- Bioreactor performance is referred to as the amount of yield and productivity. High amount of yield and productivity indicate that the bioreactor performance is good, and vice versa.

3.3 EXPERIMENTAL DESIGN

The effect of aeration rate (*AR*) and stirrer speed (*SS*) on the bioreactor performance will be studied experimentally. In this section, the theoretical background of the design experiment will be presented for completeness, and then applied to our case studies to generate sets of experimental data. Statistical test, i.e. "Prob > F" is applied to the experiment data from which the effect of aeration rate and stirrer speed on bioreactor performance are studied and analyzed. "Prob" indicates probability and "F" indicates F test. F test is a statistical test to identify the effect of a factor on a response [55]. The "Prob > F" value is required to be less than 0.05 for significance. The smaller the "Prob > F", the more significant is the corresponding coefficient [56]. The significance of the "Prob > F" value will imply that both aeration rate (*AR*) and stirrer speed (*SS*) have significant effects towards the output variables.

3.3.1 Preliminaries

In the design experiment, the selection of response variables, factors, levels and ranges play an important role in generating reliable data sets. The selection depends on the objective of the study by viewing the process or system as follows:

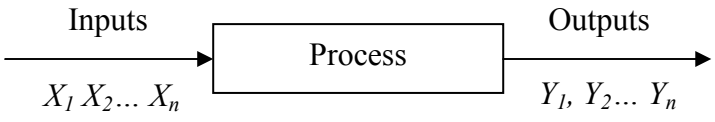


Figure 3-1 General Model of a Process or System

The objectives of the experiment may include the following [57]:

1. Determining which variables X are most influential on the response Y.
2. Determining where to set the influential X's so that Y is almost always near the desired nominal value.

3. Determining where to set the influential *X*'s so that the variability in *Y* is small.

For our micro-aerobic fermentation process, previous work suggest that both aeration rate and stirrer speed are the most influential variables on the response of yield and productivity on bioreactor performance [58]. Therefore, both aeration rate (X_1) and stirrer speed (X_2) are chosen as the input variables while Yield (Y_1) and Productivity (Y_2), on the other hand, are chosen as output responses. As a result, the process yield or productivity can be modeled as a function of the levels of aeration rate and stirrer speed, i.e.:

$$Y_n = f(X_1, X_2) + \varepsilon \tag{10}$$

where ε represents the error in the response Y_n, i.e. Yield (Y) or Productivity (P). If the expected response is denoted by $E(y) = f(X_1, X_2) = \eta$, then the surface represented by $\eta = f(X_1, X_2)$ is called as a response surface.

$$\text{Therefore, } y = \begin{bmatrix} Y \\ P \end{bmatrix} = \begin{bmatrix} f_1(X_1, X_2) \\ f_2(X_1, X_2) \end{bmatrix} + \begin{bmatrix} \varepsilon_1 \\ \varepsilon_2 \end{bmatrix} \tag{11}$$

Once the factors are determined, the next step is to determine the specific levels and ranges over these factors. Both levels and ranges are determined based on the equipment limitations in the laboratory. Particularly, it is based on the limitations of both aeration rate and stirrer-speed so that the bioreactor could achieve the desired bioreactor performance.

3.3.2 Factorial Design

The choice of experimental design involves the consideration of sample size (number of replicates), the selection of suitable run order for experimental trials, and the determination of whether randomization is involved. In general, factorial designs are the most efficient in the study of the effects of two or more factors. It reveals interactions of factors. Furthermore, factorial designs also allow the effects of a factor to be estimated at several levels of the other factors, yielding conclusions that

are valid over a range of experimental conditions. All possible combinations of the levels of the factors in each complete replication of the experiment are investigated. For example, if there are *a* levels of factor *A* and *b* levels of factor *B*, each replicate contains all *ab* treatment combinations.

In the general case, let y_{ijk} be the observed response when factor *A* is at the *i*th level (*i* = 1, 2,…, *a*) and factor *B* is at the *j*th level (*j* = 1, 2,…, *b*) for the *k*th replicate (*k* = 1, 2,…, *n*). A two-factor factorial experiment will appear as in Table 3-1. The order in which the *abn* observations are taken is selected at random so that this design is completely randomized design.

Table 3-1 General Arrangement for a Two-Factor Factorial Design

		Factor *B*			
		1	2	…	*b*
Factor *A*	1	$y_{111}, y_{112}, \ldots, y_{11n}$	$y_{121}, y_{122}, \ldots, y_{12n}$		$y_{1b1}, y_{1b2}, \ldots, y_{1bn}$
	2	$y_{211}, y_{212}, \ldots, y_{21n}$	$y_{221}, y_{222}, \ldots, y_{22n}$		$y_{2b1}, y_{2b2}, \ldots, y_{2bn}$
	…				
	a	$y_{a11}, y_{a12}, \ldots, y_{a1n}$	$y_{a21}, y_{a22}, \ldots, y_{a2n}$		$y_{ab1}, y_{ab2}, \ldots, y_{abn}$

In our study, a regression model is used since this model is useful when one or more of the factors in the experiment are quantitative [57]. A regression is performed to describe the data collected whereby an observed response is approximately based on a functional relationship between the estimated variable, *y* and one or more input variable $X_1, X_{2, \ldots,} X_i$.

Since there are two manipulated variables taken into account in this research, i.e. aeration rate and stirrer speed, it would be easier to utilize 2nd order terms instead of 3rd order terms in developing the regression model. On the other hand, it is of interest to fit the developed model by using Central Composite Design (CCD) for

optimization purpose. CCD is chosen for optimization in this research since it is the most commonly used method for optimizing fermentation processes. Furthermore, CCD is known to be the most popular class of designs used for fitting 2nd order models efficiently [57].

Thus, a regression model representation of the two-factor factorial experiment could be written as:

$$y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \varepsilon \tag{12}$$

where y is the response, the β's are parameters whose values are to be determined, X_1 is a variable that represents factor A, X_2 is a variable that represents factor B, X_1X_2 represents the interaction between X_1 and X_2. ε is a random error term.

The parameter estimates in this regression model turn out to be related to the effect estimates. Specifically, it is of interest in testing hypotheses about the equality of row treatment effects, i.e.

$$H_0 : \tau_1 = \tau_2 = ... = \tau_a = 0$$
$$H_1 : \text{at least one } \tau_i \neq 0 \tag{13}$$

and the equality of column treatment effects, i.e.

$$H_0 : \beta_1 = \beta_2 = ... = \beta_b = 0$$
$$H_1 : \text{at least one } \beta_j \neq 0 \tag{14}$$

It is also of interest in determining whether row and column factors (or treatments) interact. Thus,

$$H_0 : (\tau\beta)_{ij} = 0 \quad \text{for all } i, j$$
$$H_1 : \text{at least one } (\tau\beta)_{ij} \neq 0 \tag{15}$$

On the other hand, there must be at least two replicates ($n \geq 2$) to obtain an error sum of squares. The total corrected sum of squares can be written as:

$$SS_T = SS_A + SS_B + SS_{AB} + SS_E \tag{16}$$

The number of degrees of freedom associated with each sum of squares is:

Effect	**Degrees of Freedom**
A	$a - 1$
B	$b - 1$
AB interaction	$(a - 1)(b - 1)$
Error	$ab(n - 1)$
Total	$anb - 1$

Each sum of squares divided by its degrees of freedom is a mean square. The expected values of the mean squares are:

$$E(MS_A) = E\left(\frac{SS_A}{a-1}\right) = \sigma^2 + \frac{bn\sum_{i=1}^{a}\tau_i^2}{a-1} \tag{17}$$

$$E(MS_B) = E\left(\frac{SS_B}{b-1}\right) = \sigma^2 + \frac{an\sum_{j=1}^{b}\beta_j^2}{b-1} \tag{18}$$

$$E(MS_{AB}) = E\left(\frac{SS_{AB}}{(a-1)(b-1)}\right) = \sigma^2 + \frac{n\sum_{i=1}^{a}\sum_{j=1}^{b}(\tau\beta)_{ij}^2}{(a-1)(b-1)} \tag{19}$$

and

$$E(MS_E) = E\left(\frac{SS_E}{ab(n-1)}\right) = \sigma^2 \tag{20}$$

Notice that if the null hypotheses of no row treatment effects, no column treatment effects, and no interaction are true, then the expected values of the mean squares are all estimate of σ^2. However, if there are differences between row treatment effects, then MS_A will be larger than MS_E. Similarly, if there are column treatment effects or interaction present, then the corresponding mean squares will be larger than MS_E. Thus, to test the significance of both main effects and their interaction, divide the corresponding mean square by the error mean square. Large values of this ratio imply that the data do not support the null hypothesis.

If the model (see Equation 12) is adequate and that the error terms are normally and independently distributed with constant variance, then each of the ratios of mean

squares are distributed as F with $(a-1)$, $(b-1)$ and $(a-1)(b-1)$ numerator degrees of freedom, respectively and $ab(n-1)$ denominator degrees of freedom.

3.4 APPLICATION AND CASE STUDIES

In our study, a two-factor factorial design is selected since aeration rate and stirrer speed is the two factors of interest to study on the effect of bioreactor performance. Aeration rate (X_1, LPM) and stirrer speed (X_2, rpm) are considered as input variables. Yield (Y_1, %) and productivity (Y_2, g/L.hr) are considered as output variables. The levels of the input variables are selected based on the range of reasonable formulations since the interpretation of the results are valid only within experimental limits in the laboratory available. Three levels are coded as -1, 0 and +1, which corresponded to the lower, middle and higher values respectively. The experiments are chosen to realize every possible combination between the variables, with the levels coded.

Based on the above two-factor factorial design theory, the two-factor factorial design of experiment for this study is proposed. Aeration rate is varied between 1.0 LPM and 1.5LPM and stirrer speed ranged between 150 rpm and 250rpm. Both aeration rate and stirrer speed ranges are set according to the specifications and limitations of the bioreactor in the laboratory. Table 3-2 shows the input variables and levels employed.

Table 3-2 Input Variables and Their Levels Employed in Two-Factor Factorial Design

Factor	**Variable**	**Units**	**Low Level (-)**	**Middle Level (0)**	**High Level (+)**
X_1	*Aeration Rate*	LPM	1.0	1.25	1.5
X_2	*Stirrer Speed*	rpm	150	200	250

A total of seven experiments are conducted based on the randomized order from factorial design application. Table 3-3 summarizes the aeration rate and stirrer speed setups for the seven experimental runs, whereby all the experiments are conducted.

Table 3-3 Factorial Design Layout

Standard Order	**Run Order**	X_1**: Aeration Rate (LPM)**	X_2**: Stirrer Speed (rpm)**
7	1	1.25	200
1	2	1.0	150
5	3	1.25	200
6	4	1.25	200
3	5	1.0	250
4	6	1.5	250
2	7	1.5	150

From Table 3-3, the experiments are randomized in order to make the experimental error as small as possible. Standard Order is the order of treatment combinations based on the level indicated in Table 3-2. As observed from Table 3-3, replicates are required to be conducted for the baseline values. However, there are no replicates required for other conditions. It is designed in such a way in order to reduce the modeling error structures among the input variables [59]. Since there is no replication for other conditions, the error variance can be used as a measure of precision or accuracy of experiments [60]. This can be measured by investigating the "Prob > F" value, which has been discussed in Section 3.3 in this chapter. As long as the "Prob > F" value is significant, the experiments conducted are precisely accurate, even though the experiments are designed in such a way that there is no replication for some conditions. For a two-factor factorial design, the treatment combinations begin with the order of:

Table 3-4 Standard Order Interpretation for a Two-Factor Factorial Design

Standard Order	X_1**: Aeration Rate (LPM)**	X_2**: Stirrer Speed (rpm)**
1	-	-
2	+	-
3	-	+
4	+	+

Any replicates for the baseline values are to be included after the fourth standard order. Since there are two replicates, the baseline values are set at Standard Order 5, 6 and 7 respectively. On the other hand, Run Order is the randomized order of the Standard Order. Experiments are conducted based on the Run Order. Experimental data, i.e. glucose, ethanol, glycerol and biomass concentrations are to be measured and recorded in order to investigate the impact of both aeration rate and stirrer speed on the micro-aerobic batch fermentation process in laboratory scale.

Two case studies are chosen to measure the effects of aeration rate and stirrer speed on bioreactor performance, namely either glucose or cassava is used as the main substrate of the fermentation medium. Glucose is chosen due to its ability in producing a high amount of ethanol. Cassava, on the other hand, is chosen since it is of interest to study the effect of aeration rate and stirrer speed on more complex structure than glucose, and it is much cheaper to conduct experiments by using cassava. The experimental procedures for both case studies are available in Appendix A.

3.5 MATERIALS AND EQUIPMENTS

3.5.1 Bioreactor (BIOSTAT® A Plus 2L, MO-ASSEMBLY)

In this study, a 2 litre bioreactor, BIOSTAT® A Plus is employed, which is shown in Figure 3-2.

Figure 3-2 Bioreactor BIOSTAT® A Plus 2L, MO Assembly [61]

The height of the bioreactor is 0.24m, whereas the diameter of the bioreactor is 0.128m. To have a clearer view of the bioreactor, Figure 3-3 shows the top view of the bioreactor which indicates the location of the probes, inoculation port, sample injection pipe, sparger, aeration fitting and cooling fingers.

Figure 3-3 Bioreactor Top Plate

Basically, this bioreactor is a solid, autoclavable laboratory bioreactor system which is suitable for a wide range of research and industrial applications. Additionally, this bioreactor has oxygen enrichment capability, efficient agitation system for high cell densities as well as automatic dissolved oxygen (DO) control via agitation, substrate addition and optional oxygen. This bioreactor is applicable for:

- Microbial culture for the growth of bacteria, yeast and fungi.
- Cell culture for the growth of animal, insect and plant cells.
- Transition from shaker or tissue culture flask.
- Small scale protein expression.

Thus, this bioreactor is suitable to be utilized for the study of both aeration and agitation in mixing mechanism of bioreactor for an ethanolic fermentation process.

3.5.2 Agitation System (Rushton Turbine)

To study the effect of agitation, Rushton turbine is utilized since it is one of the most common agitator utilized in fermentation process. This type of agitator is suitable for efficient mixing and maximum oxygen transfer within the bioreactor since it could

break up a fast air stream without itself becoming flooded in air bubbles [58];[62]. Figure 3-4 shows the Rushton turbine, which is utilized in the laboratory. It consists of six discs turbine, with a series of rectangular vanes set in a vertical plane around the circumference. The diameter of the turbine is 0.03m and the thickness of the turbine is 0.001m.

A dual impeller combination is utilized in the laboratory to ensure good mixing and aeration could be achieved. The lower impeller acts as the gas dispenser and the upper impeller acts primarily as a device for aiding circulation of medium contents [58].

Figure 3-4 Rushton Turbine

3.5.3 Aeration System (Sparger)

To study the effect of aeration, a sparger is utilized as the aeration system in this study. Figure 3-5 shows a ring sparger utilized in the laboratory, whereby air from the sparger hit the underside of the Rushton turbine. Air bubbles are broken up into smaller bubbles. This type of sparger is suitable under the operation of high aeration rates.

Figure 3-5 Ring Sparger

3.6 RESULTS AND DISCUSSION

3.6.1 Glucose Substrate

3.6.1.1 Effect of Aeration Rate and Stirrer Speed on Glucose Concentration

Figure 3-6 shows the glucose concentrations measured under different conditions of aeration rate and stirrer speed.

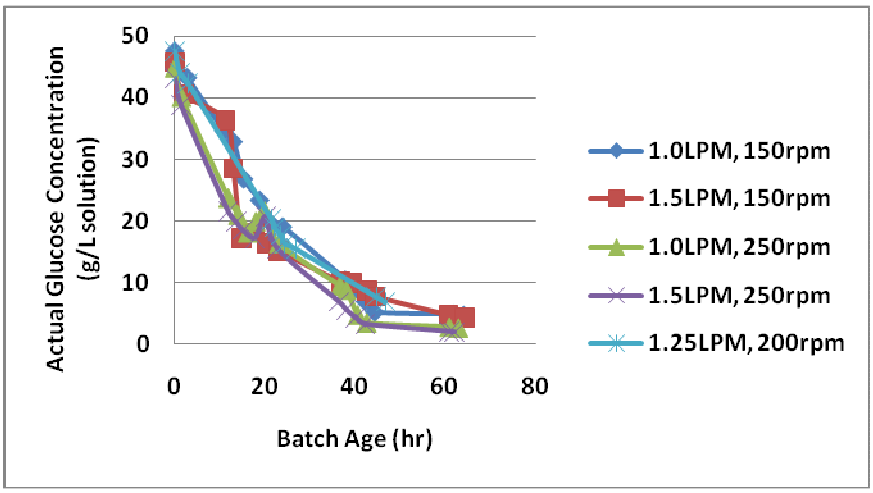


Figure 3-6 Glucose Concentration vs. Batch Age for Different Sets of Experiments

It is observed that the glucose concentrations are relatively comparable for all sets of aeration rate and stirrer speed conditions. The trends of the glucose concentrations

for all experimental conditions are expected to decrease due to the consumption of glucose throughout the fermentation process. It is also observed that the consumption of glucose is high for all conditions of aeration rate and stirrer speed. Results show that the microorganisms utilize a large amount of glucose with oxygen consumption to produce ethanol. Aerating and agitating mixed the nutrients together along with oxygen throughout the fermentation medium for microbial growth.

Based on ANOVA analysis (Refer to Appendix B.1), it is identified that there is effect of aeration rate and stirrer speed on glucose concentration. The "Prob > F" interaction value for glucose concentration is 0.0070. This value indicates that both aeration rate (*AR*) and stirrer speed (*SS*) have significant effects on glucose concentration since the "Prob > F" is less than 0.05.

3.6.1.2 Effect of Aeration Rate and Stirrer Speed on Ethanol Concentration

Figure 3-7 shows the ethanol concentration profiles under different conditions of aeration rate and stirrer speed.

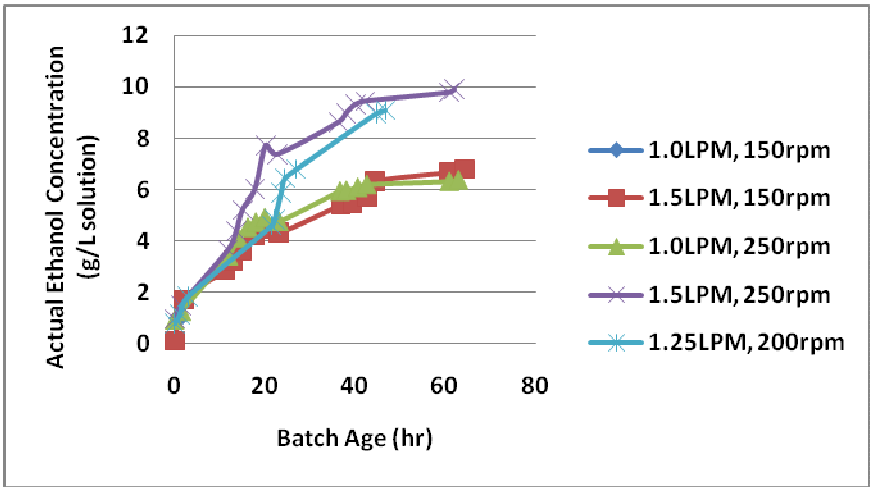


Figure 3-7 Ethanol Concentration vs. Batch Age for Different Sets of Experiments

As expected, the ethanol concentration for different conditions of aeration rate and stirrer speed increase throughout the fermentation process. It is noticed that the ethanol concentration for aeration rate of 1.5LPM and stirrer speed of 250rpm is the highest among all the conditions of aeration rate and stirrer speed. Therefore, it is suggested that in order to produce high amount of ethanol, higher aeration rate and stirrer speed is to be implemented. This phenomenon could be due to more efficient

mixing, whereby higher aeration rate and stirrer speed generate better mixing. Thus, the microbial culture is supplied with oxygen during growth at a rate sufficient to satisfy the microorganisms demand [58]. Both aeration rate and stirrer speed satisfy the oxygen demand and thus, ethanol productivity is higher.

Based on ANOVA analysis (Refer to Appendix B.1), it is identified that there is effect of aeration rate and stirrer speed on ethanol concentration. The "Prob > F" interaction value for ethanol concentration is 0.033. This value indicates that both aeration rate (*AR*) and stirrer speed (*SS*) have significant effects on ethanol concentration since the "Prob > F" is less than 0.05.

3.6.1.3 Effect of Aeration Rate and Stirrer Speed on Glycerol Concentration

Figure 3-8 displays how the glycerol concentration profile varies with different conditions of aeration rate and stirrer speed. The highest production rate of glycerol is found to coincide with the highest production rate of ethanol, i.e. at aeration rate of 1.5LPM and stirrer speed of 250rpm.

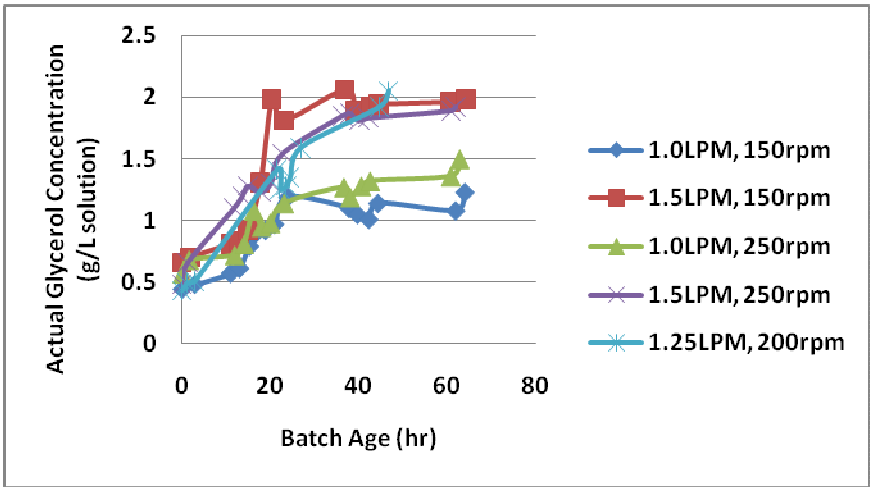


Figure 3-8 Glycerol Concentration vs. Batch Age for Different Sets of Experiments

The production of glycerol is highly affected by aeration rate. At aeration rate of 1.5LPM, the rate of glycerol productions at stirrer speed of 150rpm and 250rpm are almost comparable. This suggests that glycerol production seems to be dependent on aeration rate more rather than on the stirrer speed. This result shows that it is important to operate the bioreactor at lower aeration rate and stirrer speed to have minimum glycerol production since glycerol is only a byproduct.

Based on ANOVA analysis (Refer to Appendix B.1), it is identified that there is effect of aeration rate and stirrer speed on glycerol concentration. The "Prob > F" interaction value for glycerol concentration is 0.046. This value indicates that both aeration rate (*AR*) and stirrer speed (*SS*) have significant effects on glycerol concentration since the "Prob > F" is less than 0.05.

3.6.1.4 Effect of Aeration Rate and Stirrer Speed on Biomass Concentration

Figure 3-9 shows the biomass concentration profiles of various conditions of aeration rate and stirrer speed.

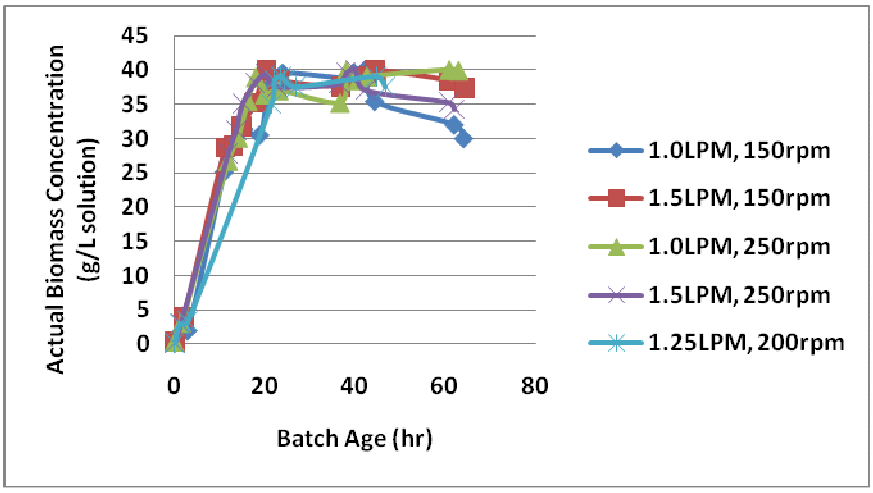


Figure 3-9 Biomass Concentration vs. Batch Age for Different Sets of Experiments

It is observed that under different conditions of aeration rate and stirrer speed, the biomass concentrations are significantly similar. The microbial growth increase steadily until the 20th hour before approaching to the stationary phase, whereby there is no net growth. This could be due to either exhaustion of substrate, or due to a balance of growth and lysis processes. The lag phase is extremely short, whereby the microbial cells grow rapidly. The more probable explanation in this case could be due to the links of growth rate and ethanol production rate to cell viability. It has been known that the higher the rate of ethanol formation during the fermentation process, the lower is the cell viability which could be due to the inhibition of ATP synthesis or leakage of metabolites from the cells while the yeast cells are metabolically inactive [63]; [64]; [65]; [66]; [67]. This activity will cause the loss of plasma membrane integrity and thus, results in the damage of the plasma membrane. The membrane integrity plays an important role in ethanol tolerance, whereby with

the loss of membrane integrity, it will lead to the decrease in phospholipid content. This will affect the level of ethanol tolerance which will eventually cause cell death [64]; [66]; [67]; [68]; [69]. Thus, both aeration rate and stirrer speed play important role in the supply of sufficient amount of oxygen for cell growth.

Based on ANOVA analysis (Refer to Appendix B.1), it is identified that there is effect of aeration rate and stirrer speed on biomass concentration. The "Prob > F" interaction value for biomass concentration is 0.033. This value indicates that both aeration rate (*AR*) and stirrer speed (*SS*) have significant effects on glucose concentration since the "Prob > F" is less than 0.05.

3.6.1.5 Effect of Aeration Rate and Stirrer Speed on Yield and Productivity

In order to study the effect of aeration rate and stirrer speed on bioreactor performance of glucose substrate, the measurements of yield and productivity are required to be measured. Experiments are conducted based on the randomized experimental layout as shown in Table 3-5.

Table 3-5 Summary of Yield and Productivity with Respect to Aeration Rate and Stirrer Speed (Glucose Substrate)

Standard Order	**Run Order**	X_1: **Aeration Rate (LPM)**	X_2: **Stirrer Speed (rpm)**	Y_1: **Yield (%)**	Y_2: **Productivity (g/L.hr)**
7	1	1.25	200	21.500	0.180
1	2	1.0	150	14.788	0.099
5	3	1.25	200	21.050	0.176
6	4	1.25	200	21.250	0.178
3	5	1.0	250	15.105	0.102
4	6	1.5	250	24.040	0.160
2	7	1.5	150	16.392	0.106

As shown in Table 3-5, different conditions of aeration rate and stirrer speed show different measurements of yield and productivity. From the experimental results,

yield measurement is the highest at aeration rate of 1.5LPM and stirrer speed of 250rpm, i.e. the maximum level for both aeration rate and stirrer speed. Productivity measurement is the highest at the centre point or baseline value, i.e. at aeration rate of 1.25LPM and stirrer speed of 200rpm. In particular, maximum yield and productivity are observed for different experimental conditions. Maximum productivity is not achieved for highest aeration rate and stirrer speed. Based on results presented in Figure 3-7, the final amount of ethanol produced is the highest among all the experimental conditions. However, the final amount of glycerol produced is among the highest, as observed in Figure 3-8. Therefore, it is suggested that maximum productivity is not achievable for highest aeration rate and stirrer speed due to the high production of glycerol which affect the productivity of ethanol.

In general, as ethanol productivity increases with the increment in aeration rate and stirrer speed, the production of glycerol increases as well. This statement is validated on the basis of knowledge of the biological role of glycerol by *Saccharomyces cerevisiae*. Glycerol is produced during fermentation of glucose to ethanol in order to maintain the redox balance and osmoregulation in yeast cells [67]; [70]. The yeast cells will increase the rate of glycerol productivity with respect to decreased extracellular water activity. Under this phenomenon of hyperosmotic stress in the yeast cells, glycerol is conserved within the cells to maintain osmotic equilibrium with the external environment. Thus, with the increment of ethanol production in the fermentation medium, glycerol production will also increase at the same time (but selectivity depends strongly on aeration rate). This is to overcome hyperosmotic stress within the yeast cells. This statement could be well justified, whereby a decrease in ethanol yield was observed when the glycerol formation is reduced in a micro-aerobic ethanolic fermentation by proper controlled oxygenation [67]; [71]. Therefore, with higher aeration rate and stirrer speed, higher ethanol and glycerol production is observed.

Comparing both experimental conditions, it is suggested to operate the bioreactor at higher aeration rate and stirrer speed in order to achieve maximum yield. On the other hand, for maximum productivity to be achieved, it is suggested to operate both aeration rate and stirrer speed at the baseline conditions. These results show that

aeration rate and stirrer speed play important roles in achieving high yield and productivity.

Based on ANOVA analysis (Refer to Appendix B.1), it is identified that there is effect of aeration rate and stirrer speed on yield and productivity. The "Prob > F" interaction value for yield is 0.0049. On the other hand, the "Prob > F" interaction value for productivity is 0.0096. These values indicate that both aeration rate (*AR*) and stirrer speed (*SS*) have significant effects on yield and productivity since the "Prob > F" values are less than 0.05. Aeration rate (*AR*) and stirrer speed (*SS*) have higher effect on yield than productivity as the "Prob > F" value for yield is lower than productivity.

3.6.1.6 Effect of Aeration Rate and Stirrer Speed on Dissolved Oxygen (DO) and Oxygen Uptake Rate (OUR)

Figure 3-10 shows the dissolved oxygen (DO) profiles for different conditions of aeration rate and stirrer speed conditions.

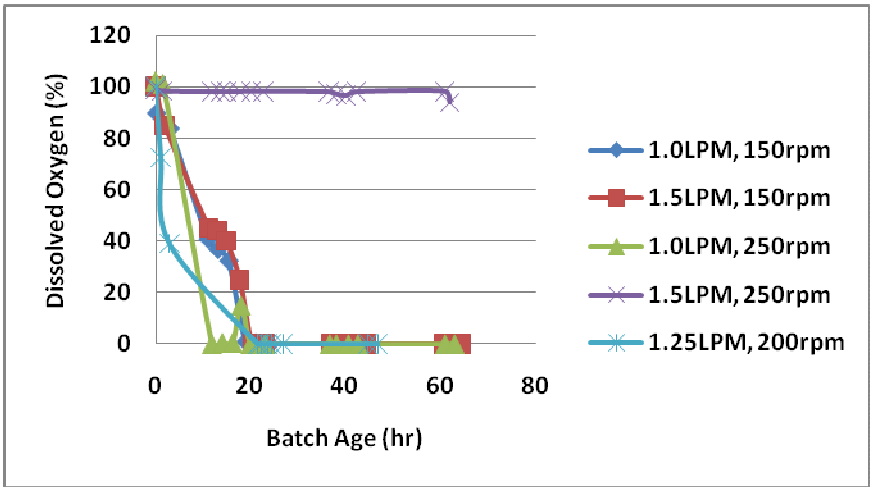


Figure 3-10 Dissolved Oxygen (DO) Profile vs. Batch Age for Different Sets of Experiments

As expected, the DO measurements tend to decrease with respect to time. The DO profiles are typically similar for all experimental conditions, except for conditions under aeration rate of 1.5LPM and stirrer speed of 250rpm. This phenomenon shows that the oxygen demand is so high under this condition that the DO measurement

does not increase further [72]. It is suggested that both aeration rate and stirrer speed lead to drastic shift from micro-aerobic to aerobic condition.

On the other hand, Figure 3-11 shows the profiles of oxygen uptake rate (OUR), whereby the OUR for each conditions of aeration rate and stirrer speed increase throughout the fermentation process.

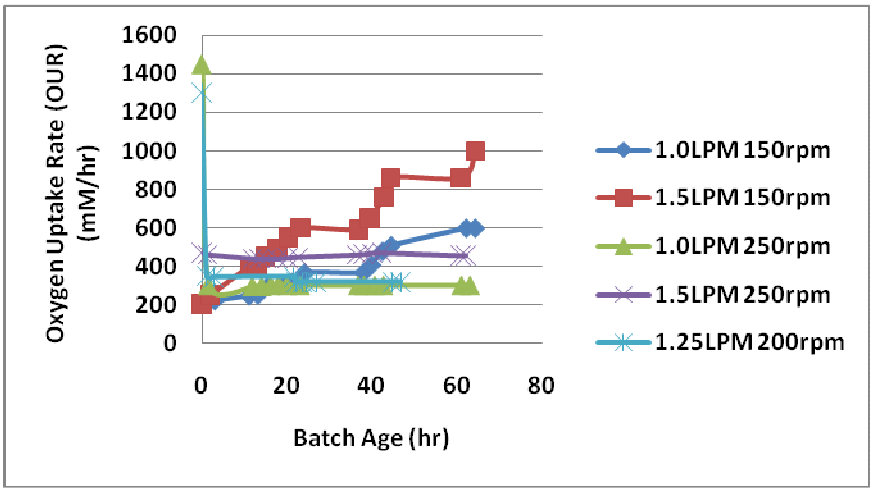


Figure 3-11 Oxygen Uptake Rate (OUR) Profile vs. Batch Age for Different Sets of Experiments

OUR increase in the exponential growth phase because in this step, a high substrate consumption rate takes place [72]. This phenomenon suggests that OUR value is higher at higher aeration rate while stirrer speed is maintained. At the same time, OUR value is also higher at lower stirrer speed when aeration rate is maintained. Thus, results suggest that in order to achieve high OUR value, higher aeration rate and lower stirrer speed are to be implemented. It is vital to ensure that OUR should not decrease because this action will decrease the metabolic activity of cells [72]. Interestingly, there is a drastic decrease of OUR under experimental conditions of 1.0LPM and 250rpm as well as 1.25LPM and 200rpm at the beginning of the fermentation process. This phenomenon could be due to the drastic change of oxygen requirement for cell growth. After inoculation at the initial stage of the fermentation process, there is a period during which it appears that no growth takes place. This period is referred to as lag phase and is considered as a time of adaptation [58]. Therefore, it is suggested that during this time, the cells are adapting to the new environment whereby oxygen requirement is considered, either low or high oxygen

requirement. Since there is a drastic decrease of OUR, it is suggested that there is no cell growth due to the adaptation period.

Based on ANOVA analysis (Refer to Appendix B.1), it is identified that there is effect of aeration rate and stirrer speed on DO and OUR. The "Prob > F" interaction value for DO is 0.0268. On the other hand, the "Prob > F" interaction value for OUR is 0.0421. These values indicate that both aeration rate (*AR*) and stirrer speed (*SS*) have significant effects on DO and OUR since the "Prob > F" values are less than 0.05. Based on these values, aeration rate (*AR*) and stirrer speed (*SS*) have higher effect on DO than OUR as the "Prob > F" value for DO is lower than OUR. This shows that aeration rate (*AR*) and stirrer speed (*SS*) highly affect the amount of oxygen that is carried in the fermentation medium.

3.6.2 Cassava Substrate

3.6.2.1 Effect of Aeration Rate and Stirrer Speed on Glucose Concentrations

Figure 3-12 shows the profiles of glucose concentrations under different conditions of aeration rate and stirrer speed for cassava substrate.

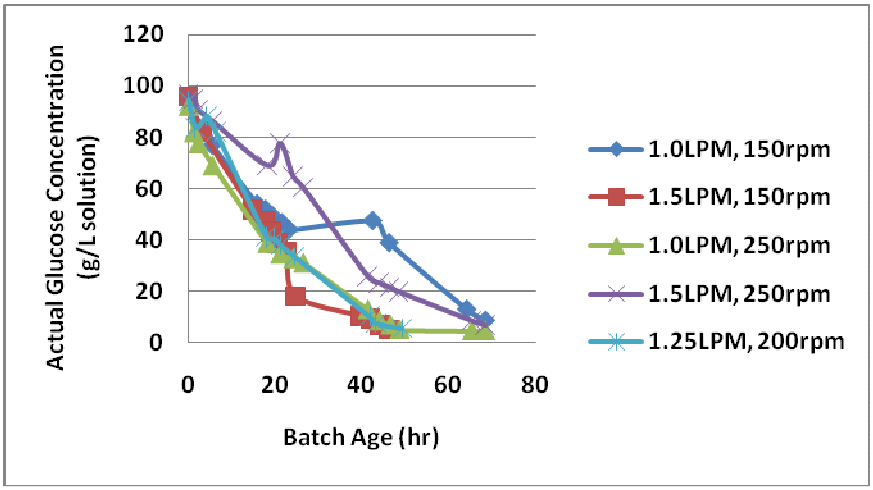


Figure 3-12 Glucose Concentration vs. Batch Age for Different Sets of Experiments

Results show that despite of the complicated structure of cassava comparing to glucose, aeration rate and stirrer speed have significant effect on glucose concentrations. Under different conditions of aeration rate and stirrer speed, different glucose concentrations are obtained. More dynamics are observed comparing to that of the glucose concentration profile for glucose substrate.

Based on ANOVA analysis (Refer to Appendix B.2), it is identified that there is effect of aeration rate and stirrer speed on glucose concentration. The "Prob > F" interaction value for glucose concentration is 0.0239. This value indicates that both aeration rate (*AR*) and stirrer speed (*SS*) have significant effects on glucose concentration since the "Prob > F" is less than 0.05.

3.6.2.2 Effect of Aeration Rate and Stirrer Speed on Ethanol Concentrations

Figure 3-13 shows the ethanol concentration profiles under different conditions of aeration rate and stirrer speed for cassava substrate.

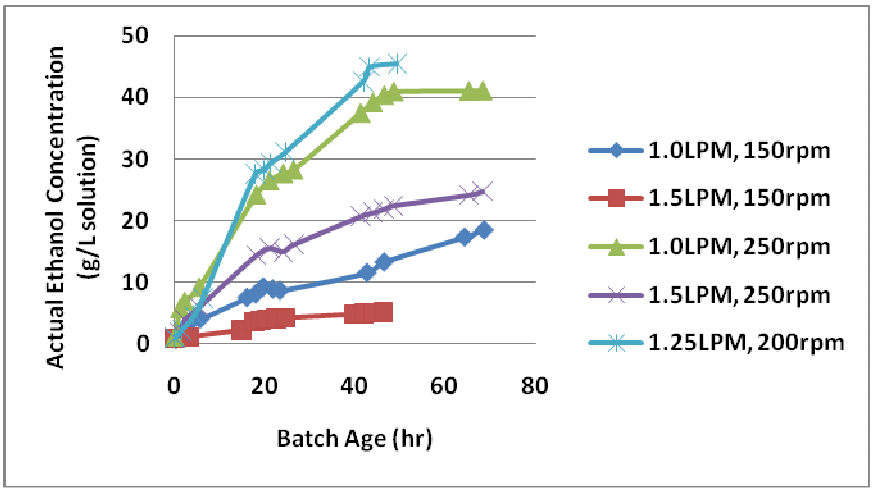


Figure 3-13 Ethanol Concentration vs. Batch Age for Different Sets of Experiments

It is observed that the highest ethanol concentration achieved is at the baseline value of the experimental range. In order to achieve high ethanol concentration, it is suggested to operate the bioreactor under high aeration rate and low stirrer speed. Different conditions of aeration rate and stirrer speed produce different amount of ethanol concentration. Therefore, overall results suggest that both aeration rate and stirrer speed rate have significant effects on ethanol concentration. Despite of the complicated structure of cassava substrate, ethanol concentration is higher, comparing to that of glucose substrate.

Based on ANOVA analysis (Refer to Appendix B.2), it is identified that there is effect of aeration rate and stirrer speed on ethanol concentration. The "Prob > F" interaction value for ethanol concentration is 0.0063. This value indicates that both

aeration rate (*AR*) and stirrer speed (*SS*) have significant effects on ethanol concentration since the "Prob > F" is less than 0.05.

3.6.2.3 Effect of Aeration Rate and Stirrer Speed on Glycerol Concentrations

Figure 3-14 displays the glycerol concentration profiles, which varied significantly with different experimental conditions of aeration rate and stirrer speed.

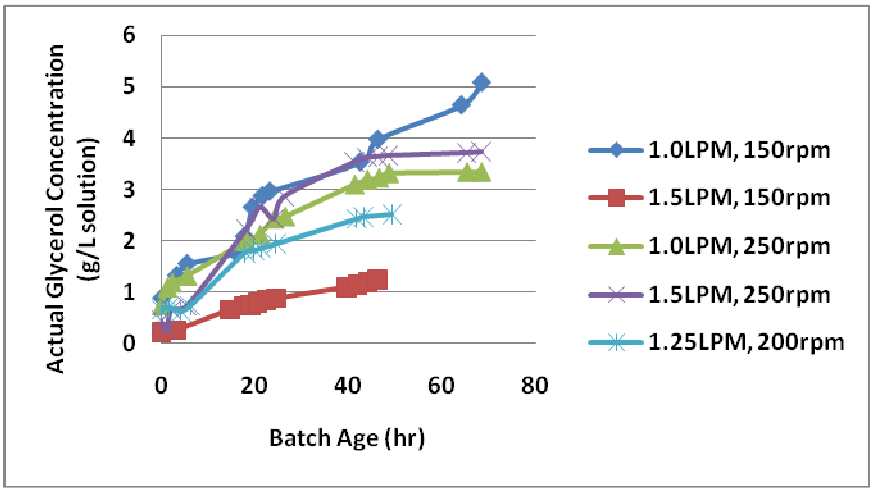


Figure 3-14 Glycerol Concentration vs. Batch Age for Different Sets of Experiments

Based on the profiles, it is observed that different amount of glycerol is produced with respect to different conditions of aeration rate and stirrer speed. In order to achieve minimum amount of glycerol, it is suggested to operate the bioreactor under high aeration rate and low stirrer speed. Other conditions of aeration rate and stirrer speed will lead to higher amount of glycerol produced, which is not desirable.

Based on ANOVA analysis (Refer to Appendix B.2), it is identified that there is effect of aeration rate and stirrer speed on glycerol concentration. The "Prob > F" interaction value for glycerol concentration is 0.0329. This value indicates that both aeration rate (*AR*) and stirrer speed (*SS*) have significant effects on glycerol concentration since the "Prob > F" is less than 0.05.

3.6.2.4 Effect of Aeration Rate and Stirrer Speed on Biomass Concentrations

Figure 3-15 shows the biomass concentration profiles for cassava substrate, whereby the biomass concentrations for each condition of aeration rate and stirrer speed are similar.

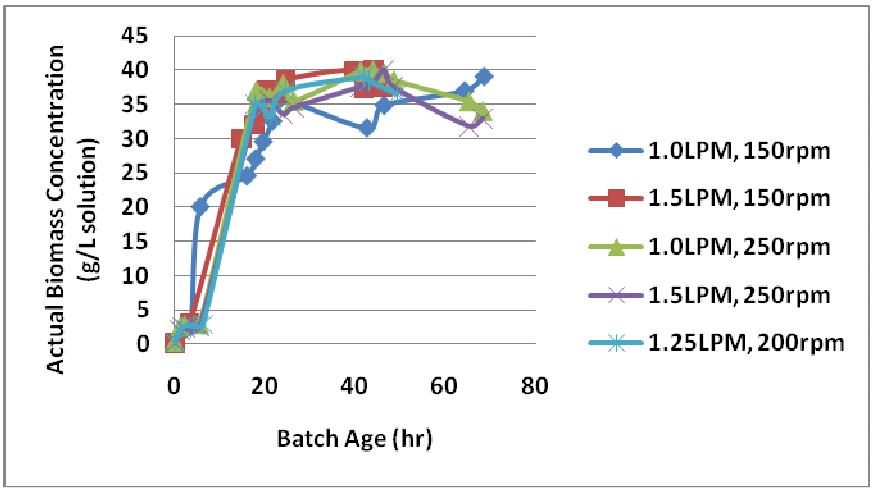


Figure 3-15 Biomass Concentration vs. Batch Age for Different Sets of Experiments

The microbial growth increase steadily until the 40th hour before approaching to the stationary phase, whereby there is no net growth. However, for glucose substrate, the microbial growth increase steadily until the 20th hour. Meaning, for cassava substrate, the microorganisms require another 20 hours to grow. This is due to the complicated structure of cassava substrate, whereby both aeration rate and stirrer speed play important role in the supply of sufficient amount of oxygen throughout the fermentation process.

Based on ANOVA analysis (Refer to Appendix B.2), it is identified that there is effect of aeration rate and stirrer speed on biomass concentration. The "Prob > F" interaction value for biomass concentration is 0.0074. This value indicates that both aeration rate (*AR*) and stirrer speed (*SS*) have significant effects on biomass concentration since the "Prob > F" is less than 0.05.

3.6.2.5 Effect of Aeration Rate and Stirrer Speed on Yield and Productivity

Table 3-6 summarizes the results of yield and productivity measured for cassava substrate, with respect to different conditions of aeration rate and stirrer speed.

Table 3-6 Summary of Yield and Productivity with Respect to Aeration Rate and Stirrer Speed (Cassava Substrate)

Standard Order	**Run Order**	X_1: **Aeration Rate (LPM)**	X_2: **Stirrer Speed (rpm)**	Y_1: **Yield (%)**	Y_2: **Productivity (g/L.hr)**
7	1	1.25	200	48.322	0.922
1	2	1.0	150	19.577	0.280
5	3	1.25	200	48.500	0.980
6	4	1.25	200	48.952	0.990
3	5	1.0	250	44.432	0.701
4	6	1.5	250	25.597	0.450
2	7	1.5	150	5.404	0.130

Different conditions of aeration rate and stirrer speed produce different measurements of yield and productivity for cassava substrate. The highest yield and productivity are measured at the centre point or baseline value, i.e. at aeration rate of 1.25LPM and stirrer speed of 200rpm. Comparing to other aeration rate and stirrer speed operating conditions, yield and productivity attained are quite low especially for conditions under aeration rate of 1.5LPM and stirrer speed of 150rpm. Thus, results suggest that, it is preferable to operate the bioreactor at the baseline value if cassava substrate is to be used as the main substrate, in order to achieve higher yield and productivity.

It is interesting to consider cassava as the main substrate, as it is possible to obtain higher yield and productivity as compared to that of glucose. Considering glucose as the main substrate, high yield and productivity are attained at high aeration rate and stirrer speed. However, high yield and productivity are obtained at the baseline value if cassava is considered as the main substrate. Lower aeration rate and stirrer speed are essential to operate the bioreactor in order to obtain high yield and productivity if cassava is considered. The operating conditions required by cassava to produce high yield and productivity are different than glucose could be due to the more complex structure of cassava. Since cassava has been hydrolyzed acidically before the

fermentation process to release their constituent glucose and maltooligosaccharides, both of these constituents are easily transported across the cell membrane and metabolized by the yeast cells [73]. According to Ejiofor, Chisti and Moo-Young (1996) [73], by using cassava to produce ethanol, it is expected to develop a low energy-requiring process to convert cassava starch to a fermentable medium and an exponential feeding strategy for yeast cells. Therefore, based on the results presented in Table 3-6, lower aeration rate and stirrer speed are suggested to produce high yield and productivity.

Furthermore, the yield and productivity obtained are twice the amount of yield and productivity obtained by utilizing cassava as the main substrate. Therefore, both aeration rate and stirrer speed highly affect the yield and productivity of the fermentation process, subject to different substrate. These show that both aeration rate and stirrer speed affect the yield and productivity of the fermentation process due to the complicated structure of cassava. More mixing might be required for sufficient transfer of oxygen to be absorbed by the microorganisms.

Based on ANOVA analysis (Refer to Appendix B.2), it is identified that there is effect of aeration rate and stirrer speed on yield and productivity. The "Prob > F" interaction value for yield is 0.0189. On the other hand, the "Prob > F" interaction value for productivity is 0.0303. These values indicate that both aeration rate (*AR*) and stirrer speed (*SS*) have significant effects on yield and productivity since the "Prob > F" values are less than 0.05. Aeration rate (*AR*) and stirrer speed (*SS*) have higher effect on yield than productivity as the "Prob > F" value for yield is lower than productivity.

3.6.2.6 Effect of Aeration Rate and Stirrer Speed on Dissolved Oxygen (DO) and Oxygen Uptake Rate (OUR)

Figure 3-16 shows the effect of aeration rate and stirrer speed on dissolved oxygen (DO). As observed from this figure, the DO concentrations decrease with time.

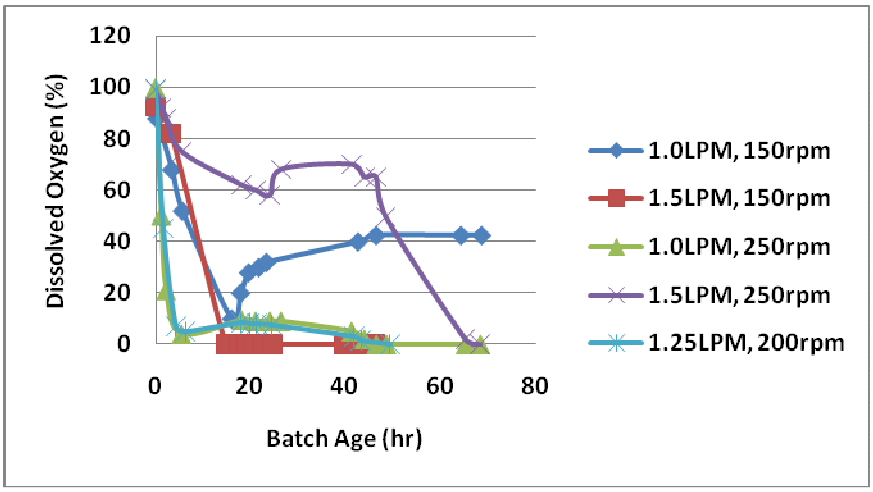


Figure 3-16 Dissolved Oxygen (DO) Profile vs. Batch Age for Different Sets of Experiments

The DO measurements decrease towards the end of the fermentation process. However, under certain conditions of aeration rate and stirrer speed, different profiles are observed. At aeration rate of 1.0LPM and stirrer speed of 150rpm, the DO measurement increased and lead to steady state. On the other hand, under aeration rate of 1.5LPM and stirrer speed of 250rpm, there is an increment of DO measurement during the 20^{th} to 40^{th} hour followed by a drastic measurement decrease towards the end of the fermentation process. It is obvious that different conditions of aeration rate and stirrer speed lead to different DO measurements. This will subsequently lead to different mass transfer rate and mechanism within the bioreactor. Besides, the dynamics of the DO are also affected by the accumulation of biomass and synthesized products, addition of acid and base solutions as well as sampling of the culture which will affect the activities of the microorganisms [74]. All of these factors affect the DO measurements, which is difficult to control accurately, owing to its intrinsic nonlinear and time variant dynamics.

Figure 3-17 shows the profile of oxygen uptake rate (OUR) for cassava substrate.

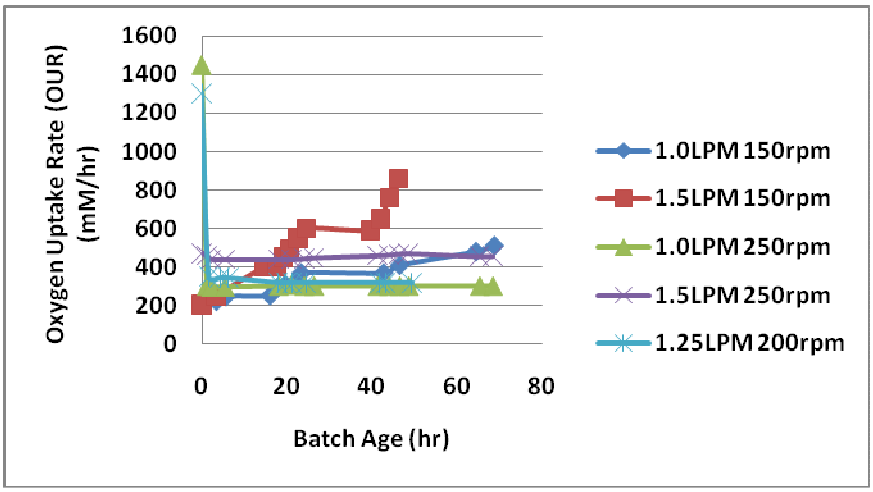


Figure 3-17 Oxygen Uptake Rate (OUR) Profile vs. Batch Age for Different Sets of Experiments

As observed from the figure, OUR increase in the exponential growth phase because in this step, a high substrate consumption rate takes place [72]. Results suggest that in order to achieve high OUR value, higher aeration rate and lower stirrer speed should be implemented. This trend is similar as OUR profile produced by using glucose as the main substrate, whereby there is a drastic decrease of OUR under experimental conditions of 1.0LPM and 250rpm as well as 1.25LPM and 200rpm at the beginning of the fermentation process. Therefore, it is suggested that there is no cell growth due to the adaptation period. Both aeration rate and stirrer speed show significant effects on OUR, since different conditions of aeration rate and stirrer speed lead to different OUR measurements.

Based on ANOVA analysis (Refer to Appendix B.2), it is identified that there is effect of aeration rate and stirrer speed on DO and OUR. The "Prob > F" interaction value for DO is 0.0268. On the other hand, the "Prob > F" interaction value for OUR is 0.0452. These values indicate that both aeration rate (*AR*) and stirrer speed (*SS*) have significant effects on DO and OUR since the "Prob > F" values are less than 0.05. Based on these values, aeration rate (*AR*) and stirrer speed (*SS*) have higher effect on DO than OUR as the "Prob > F" value for DO is lower than OUR. This shows that aeration rate (*AR*) and stirrer speed (*SS*) highly affect the amount of oxygen that is carried in the fermentation medium.

3.7 CONCLUSIONS

From our experimental studies, both glucose and cassava substrates show different dynamical behaviour and potential in producing ethanol under different aeration rate and stirrer speed conditions. Statistical analysis has showed that there is significant effect of aeration rate and stirrer speed on glucose, ethanol, glycerol and biomass concentrations as well as yield, productivity, DO and OUR measurements. The "Prob > F" values for all variables are less than 0.05, which indicate that there is significant effect of aeration rate and stirrer speed on each variable. Thus, there is significant effect of both aeration rate and stirrer speed on bioreactor performance. Specific findings from this study can be summarized as follows:

- Aeration rate and stirrer speed have effect on the glucose, ethanol, glycerol and biomass concentrations for both case studies as supported by the statistical analysis. The amount of ethanol produced from cassava substrate is around four times more than the amount of ethanol produced from glucose substrate. Glycerol production from cassava substrate is two times more than the amount of glycerol produced from glucose substrate. Based on statistical analysis, for glucose substrate, aeration rate and stirrer speed have high effect on glucose concentration compared to other concentrations since the "Prob > F" value is the lowest. For cassava substrate, aeration rate and stirrer speed have high effect on ethanol concentration instead as the "Prob > F" value is the lowest. These results show that both aeration rate and stirrer speed have different effects on the glucose, ethanol, glycerol and biomass concentrations, despite different substrates utilized.
- Aeration rate and stirrer speed have effect on yield and productivity for both case studies as well as supported by the statistical analysis. Cassava substrate is able to produce high yield and productivity, almost double the amount of yield and five times the amount of productivity as compared to that of glucose substrate. However, different conditions of aeration rate and stirrer speed are required to achieve such performance due to the difference in substrates utilized. Based on statistical analysis, aeration rate and stirrer speed has higher effect on yield than productivity as the "Prob > F" value for yield is lower than productivity for both substrates. These results show that both aeration rate and stirrer speed have higher effects on yield than productivity

regardless of the different types of substrate utilized. Overall, both aeration rate and stirrer speed have significant effects on the bioreactor performance.

- DO and OUR results show that there is significant effect of aeration rate and stirrer speed on DO and OUR measurements. Under different conditions of aeration rate and stirrer speed, different DO and OUR data are measured. Similarly, there is interaction between aeration rate and stirrer speed for both case studies as supported by the statistical analysis. The "Prob > F" value for DO is lower than that of OUR for both substrates, thus indicate that aeration rate and stirrer speed have higher effects on DO than OUR.

Both substrates result in different glucose, ethanol, glycerol and biomass concentrations, yield and productivity as well as DO and OUR profiles within the same experimental setups of aeration rate and stirrer speed. Thus, it would be interesting to explore the dynamics and kinetics of each glucose and cassava substrates. It is also observed that both aeration rate and stirrer speed have significant effects on each concentrations, yield, productivity, DO and OUR. In the next chapter, kinetics modeling of each glucose and cassava substrates are investigated and proposed to study the dynamical performance of each substrate. All of the experimental results obtained will be used for the development of kinetics models. It is to investigate the dynamical performance in order to study the non-ideally mixed effect of both aeration rate and stirrer speed within the bioreactor.

Chapter 4 Kinetics Modeling of Batch Bioreactor

4.1 INTRODUCTION

This chapter addresses the development of kinetics modeling with the account of both aeration rate and stirrer speed, to develop steady state and dynamic models of batch bioreactors. One major limitation of current kinetics models of bioreactor is that they do not take into account how the aeration rate and stirrer speed affect the kinetics. Currently, fermentation kinetic models are most commonly expressed in terms of: (1) medium temperature, and (2) medium pH. Thus, based on these facts, it is of interest to develop kinetics model, whereby both aeration rate and stirrer speed are taken into account.

The kinetics model development is based on experimental results presented in Chapter 3. The scope and assumptions of the kinetics model development are presented in Section 4.2. The modeling approach is presented in Section 4.3. Three modeling approaches are considered, i.e. data-based, kinetics hybrid and kinetics multi-scale models. Two case studies are suggested and outlined in Section 4.4, whereby the developed models are to be implemented into the case studies. This is to investigate the applicability of the developed models into batch bioreactors. Analytical results for the modeling approaches are discussed in detail in Section 4.5. In section 4.6, discussions on model applications are made. Finally, concluding remarks were presented at the end of this chapter.

4.2 SCOPE AND ASSUMPTIONS

Traditionally, aeration rate and stirrer speed have not been considered in the kinetics model development for batch bioreactors. Therefore, in our study, it is of interest to

investigate the applicability of both aeration rate and stirrer speed in kinetics model development for batch bioreactors. Figure 4-1 shows the schematic diagram of a batch bioreactor operation, whereby both aeration rate and stirrer speed are considered as input variables. Yield and productivity are considered as output variables. The developed models are only applicable within the experimental range since the models are developed by using experimental data within the experimental range.

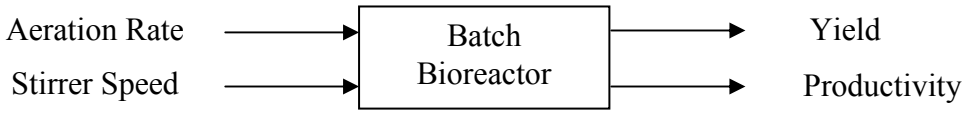


Figure 4-1 Schematic Diagram of Batch Bioreactor Kinetics Model Development

4.3 MODELING APPROACH

The majority kinetics of ethanol fermentation utilize a formal (macro) approach to describe microbial growth, whereby they are empirical and based on either Monod's equation or on its numerous modifications which take into account the inhibition of microbial growth by a high concentration of product and/or substrate [75].

4.3.1 Data-Based Model

A data-based model is a theory or specification to express a set of operations that can be performed on the data available. In engineering perspective, a data-based model is proposed based on regression analysis, whereby it is a general approach to fitting empirical models, i.e. an interpolation equation for the response variable in the process. Figure 4-2 shows a schematic diagram of an ethanol fermentation data-based model which is utilized in this study, whereby both aeration rate and stirrer speed are considered as the inputs of the fermentation process.

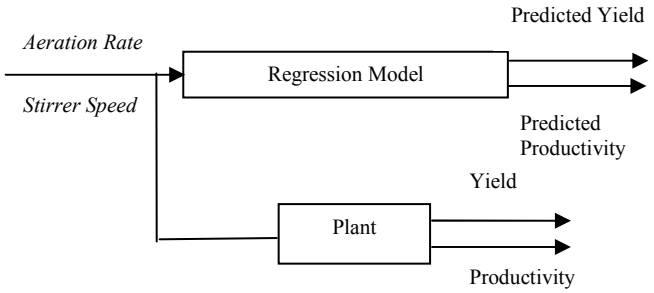


Figure 4-2 Schematic Diagram of Ethanolic Fermentation Data-Based Model

Data-based model is developed based on a correlation model from experimental data obtained. This is the simplest model for yield and productivity predictions. Experimental data obtained are implemented for the development of regression model. Response Surface Method (RSM) is utilized in the analysis of problems in which the response of interest is influenced by variables, i.e. aeration rate and stirrer speed, and the objective is to optimize these responses.

In our study, supposed that the levels of aeration rate (X_1) and stirrer speed (X_2) to maximize the yield or productivity of the fermentation process, the process yield or productivity is a function of the levels of aeration rate and stirrer speed, say:

$$y = f(X_1, X_2) + \varepsilon \tag{21}$$

where ε represents the error in the response y, i.e. Yield (Y) or Productivity (P). If the expected response is denoted by $E(y) = f(X_1, X_2) = \eta$, then the surface represented by $\eta = f(X_1, X_2)$ is called a response surface.

$$\text{Therefore, } y = \begin{bmatrix} Y \\ P \end{bmatrix} = \begin{bmatrix} f_1(X_1, X_2) \\ f_2(X_1, X_2) \end{bmatrix} + \begin{bmatrix} \varepsilon_1 \\ \varepsilon_2 \end{bmatrix} \tag{22}$$

As a first approximation, a quadratic model (see Equation 23) is used to fit the experimental data, whereby β_0, β_1 and β_2 values are to be generated based on experimental data. β's are estimated in such that the sum of the squares of the errors (the ε's) are minimized. Thus, predicted yield and productivity as well as optimum aeration rate and stirrer speed are obtained.

The quadratic model as a first approximation utilized as follows:

$$y = \beta_0 + \beta_1 x + \beta_2 x^2 + \varepsilon \tag{23}$$

where β_0, β_1 and β_2 are unknown parameters to be estimated and ε is a random error term.

$$\text{Thus, } y = \left[\frac{\beta_0^{\,Y}}{\beta_0^{\,P}}\right] + \left[\frac{\beta_1^{\,Y}}{\beta_1^{\,P}}\right] x + \left[\frac{\beta_2^{\,Y}}{\beta_2^{\,P}}\right] x^2 + \varepsilon \tag{24}$$

Full factorial design experiments are conducted and experimental results are to be proven to be significant with the kinetic parameters. In order to proceed to optimization, statistical analysis is required to be conducted by using Analysis of Variance (ANOVA). Results must be significant along with the analysis of curvature. Curvature analysis is vital to indicate whether the experimental results could fit well into the proposed model. If the curvature is significant, i.e. the curvature lies in the region of the desired optimum response, thus optimization could proceed. If the curvature is insignificant, optimization could not be preceded yet. Path of Steepest Ascent (POA) is required to be done and curvature is to be indicated again until curvature is shown to be helpful or significant. Once results and curvature analysis are significant, augmentation or further analysis is required in order to proceed to RSM for optimization stage.

4.3.2 Kinetics Hybrid Model

In the kinetics hybrid model development, experimental data of substrate, product and biomass concentrations for different conditions of aeration rate and stirrer speed is used to predict kinetics parameters, k_1, k_2, ..., k_6 using the Herbert's concept of endogenous metabolism. In our study, the byproduct concentration is not included in order to concentrate in the study of aeration rate and stirrer speed towards substrate, product and biomass concentrations and finally yield and productivity predictions. Herbert's concept is chosen in our study since it has been used in numerous studies to describe the kinetics of ethanolic fermentation [75]. An optimization approach is formulated for the identification of the kinetic parameters.

Equation 25 below expresses the linear regression model, which is then utilized for a set of identified kinetics parameter data for different conditions of aeration rate and stirrer speed:

$$Variable = \beta_1 + \beta_2 \frac{(r - \bar{r})}{\Delta r} + \beta_3 \frac{(R - \bar{R})}{\Delta R} \tag{25}$$

whereby *Variable* represents predicted k_1 to k_6, r represents aeration rate (*AR*), R denotes stirrer speed (*SS*), whereas $\bar{r}$ and $\bar{R}$ represent the baseline values for aeration rate (*AR*) and stirrer speed (*SS*). β_1, β_2 and β_3 values will be obtained through least squares optimization.

The Herbert's kinetics model embedded with the linear regression model is then combined with the macro-scale bioreactor model to produce the so-called kinetics hybrid model as depicted in Figure 4-3. Clearly in this approach, mixing is integrated by including both aeration rate and stirrer speed in the model development.

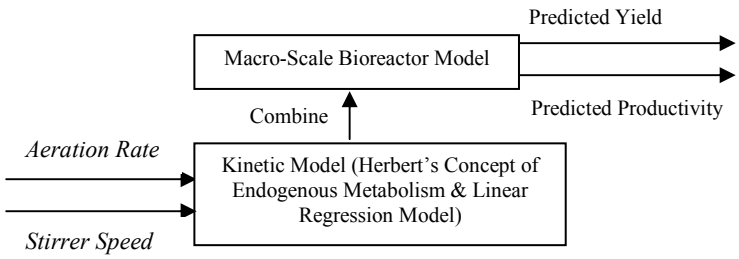


Figure 4-3 Schematic Diagram of Ethanolic Fermentation Kinetics Hybrid Model

4.3.2.1 Herbert's Kinetics Model

The kinetics parameter data is estimated using the experimental data of substrate, product and biomass concentrations for different aeration rate and stirrer speed conditions. For this purpose, the Herbert's concept is applied as follows: It is assumed that the observed rate of biomass formation comprised of the growth rate and the rate of endogenous metabolism:

$$r_x = (r_x)_{growth} + (r_x)_{end} \tag{26}$$

where

$$(r_x)_{growth} = \frac{k_1 XS}{k_2 + S} \exp(-k_5 P) \tag{27}$$

It is also assumed that the rates of substrate consumption and product formation are proportional to the biomass growth rate:

$$r_s = (r_s)_{growth} = -k_3 (r_x)_{growth} \tag{28}$$

$$r_p = (r_p)_{growth} = k_4 (r_x)_{growth} \tag{29}$$

The rate of growth due to endogenous metabolism by a linear dependence is shown below:

$$(r_x)_{end} = -k_6 X \tag{30}$$

Given the initial values of the kinetic parameters obtained from the literature data, an optimization problem is formulated to predict the kinetic parameters for each aeration rate and stirrer speed conditions. The obtained kinetic parameters are then used to obtain the linear regression model.

4.3.2.2 Macro-scale Bioreactor Model

A macro-scale bioreactor model is formulated to compare the prediction of yield and productivity using the kinetics hybrid model in Figure 4-3 with experimental data:

Biomass formation: $dX / dt = r_x$ (31)

Substrate consumption: $dS / dt = r_s$ (32)

Product formation: $dP / dt = r_p$ (33)

$$\text{Yield} = \frac{P}{S_0 - S} \times 100\% \tag{34}$$

$$\text{Productivity} = \frac{P}{BT} \tag{35}$$

where S_0 is the initial substrate concentration (g/L) of the medium and BT is the batch time (hrs) allocated for the fermentation process.

4.3.3 Kinetics Multi-Scale Model

The kinetics multi-scale bioreactor model is developed using a slightly different approach from the kinetics hybrid model. The developed Herbert's kinetics model (*Section 4.3.2.1*), macro-scale bioreactor model (*Section 4.3.2.2*) and mixing model are combined, which is called the kinetics multi-scale model. The mixing model is proposed based on the k-ε turbulence model, Navier-Stokes equations and general balance over an element of reactor volume. The resulted model is illustrated in Figure 4-4.

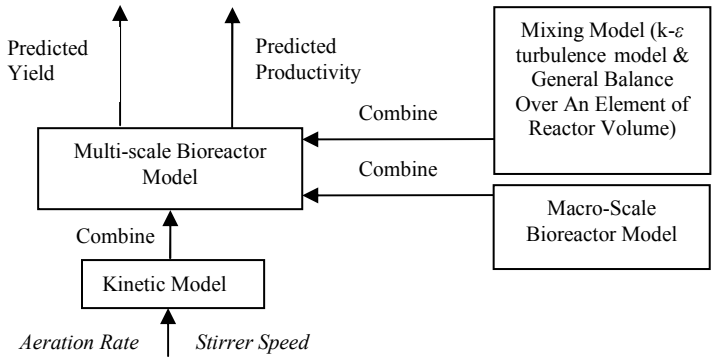


Figure 4-4 Schematic Diagram of Ethanolic Fermentation Kinetics Multi-Scale Model

4.3.3.1 k-ε turbulence model

One of the models used to develop the mixing model is the k-ε turbulence model (governing turbulence). The standard k-ε turbulence model is used since it is proven to be most successful in past works [76]. The k-ε turbulence model is normally used

to describe the mixing behaviour and to compute turbulence in the bioreactor. The following is the standard k-ε turbulence model:

The energy dissipation can be expressed as:

$$\varepsilon = (\Delta pFu)/m = (\Delta pu)/(x\rho) \tag{36}$$

where Δp denotes the pressure drop, m the mass, F the tube cross-section and x the axial coordinate.

The fluid flow equations to be solved for a constant density fluid are [58]:

$$div(\rho u) = 0 \text{ (Continuity Equation)} \tag{37}$$

$$div(\rho uk) = div(\frac{\mu_{eff}}{\sigma_k} grad\_k) + G - \rho\varepsilon \text{ (Transport Equation)} \tag{38}$$

$$div(\rho u\varepsilon) = div(\frac{\mu_{eff}}{\sigma_\varepsilon} grad\_\varepsilon) + (C_1 G - C_2\rho\varepsilon)\frac{\varepsilon}{k} \text{ (Transport Equation)} \tag{39}$$

$$u_T = C_\mu \rho \frac{k^2}{\varepsilon} \text{ (Eddy Viscosity)} \tag{40}$$

whereby G is the dissipation function $\tau_{ij}\tau_{ij}/(2\mu_{eff})$; C_μ = 0.09; C_1 = 1.44; C_2 = 1.92; σ_k = 1.0; σ_T = 1.3.

4.3.3.2 Navier-Stokes Equation

The Navier-Stokes equation is the most commonly used flow equations in describing the instantaneous behaviours of turbulent liquid flow in ethanolic fermentation process [1]. The resulting Reynolds equations and the continuity equation are summarized below:

$$\frac{\partial(\rho u_i)}{\partial t} + \frac{\partial(\rho u_i u_j)}{\partial x_i} = -\frac{\partial}{\partial x_i}(\tau_{ij} + \rho u_i' u_j') - \frac{\partial p}{\partial x_i} + \rho g_i \tag{41}$$

$$\frac{\partial \rho}{\partial t} + \frac{\partial}{\partial x_i}(\rho u_i) = 0 \tag{42}$$

For model accuracy and computational expense, a reasonable compromise are eddy viscosity models relating the individual Reynolds stresses to mean flow gradients:

$$\rho u_i^{'} u_j^{'} = -\rho v_{turb}(\frac{\partial u_i}{\partial x_j} + \frac{\partial u_j}{\partial x_i}) + \frac{2}{3}\rho \partial_{ij} k \tag{43}$$

where v_{turb} is the turbulent eddy viscosity. The transport of momentum which is related to turbulence, is thought of as turbulent eddies, which like molecules, collide and exchange momentum.

4.3.3.3 General Balance Over an Element of Reactor Volume Model

Another model used in developing the mixing model is the general balance over an element of reactor volume model [46], which is adopted as a reactor model as follows:

$$\frac{\partial(\rho\phi)}{\partial t} + \frac{\partial(\rho U_i \phi)}{\partial x_i} = \frac{\partial}{\partial x_i}(\Gamma_\phi \frac{\partial \phi}{\partial x_i}) + S_\phi \tag{44}$$

where ρ is the density of fluid, $ø$ is the concentration of any component, U_i is the local velocity in the x_i direction, $\Gamma_ø$ is the effective diffusivity of $ø$ and $S_ø$ is a volumetric source term (rate of production of $ø$ per unit volume) of $ø$.

The source term will be equal to the rate based on intrinsic kinetics, i.e. there are no concentrations or temperature gradients within the volume element under consideration. Due to the complexity of the mixing model, Computational Fluid Dynamics (CFD) software aided in the prediction of yield and productivity since macro-scale model, k-ε turbulence model, Navier-Stokes equations and the general balance over an element reactor volume model are already embedded in the CFD software, by solving Equations 31-44. Thus, data such as ε and u_Γ are obtained. Along with the multi-scale model, the rates of substrate consumption, product formation and biomass formation which are predicted from the kinetics model are

substituted into Equations 26-30, for yield and productivity prediction. Predicted results of yield and productivity are then be used to predict the optimum aeration rate and stirrer speed.

4.4 CASE STUDIES

Two case studies are performed to develop the kinetics model based on the three modeling approaches. Experimental data obtained from glucose and cassava substrates are utilized to develop the data-based, kinetics hybrid and kinetics multi-scale models. The data-based and kinetics hybrid models are developed for both glucose and cassava substrates. However, the kinetics multi-scale model is only developed for glucose substrate. This is due to the complexity of the cassava structure, whereby more information, such as density, molecular weight is required to be imbedded into CFD. Therefore, for the development of the kinetics multi-scale model, only glucose substrate is considered. Statistical analysis is conducted for validation purposes.

4.5 RESULTS AND DISCUSSION

4.5.1 Model Development

The data-based, kinetics hybrid and kinetics multi-scale models are developed by using different approaches, as outlined in Section 4.3. In this section, the model development of each model is presented.

4.5.1.1 Data-Based Model

To develop the data-based models for both case studies, it is important to conduct factorial design analysis at the initial stage. This is to perform preliminary screening analysis, to investigate whether there is interaction between aeration rate and stirrer speed on bioreactor performance. If interaction is available, therefore both aeration rate and stirrer speed have significant effects on the bioreactor performance.

To aid with the design analysis, experimental data collected are analyzed by following the steps as follows [77]:

1. Choose a transformation if desired. Otherwise, leave the option at "None".
2. Perform ANOVA for analysis of residuals and outlier detection.
3. Inspect various diagnostic plots to statistically validate the model.
4. Generate model graphs for interpretation if the model looks good. The analysis and inspection performed in steps (3) and (4) above will show whether the model is good or otherwise. A good model must be significant and curvature must be significant too. The various coefficient of determination, R^2 values should be close to 1.

By following the guidelines stated above, this will assist in quantifying the relationships between the output variables (yield and productivity) and the input variables (aeration rate and stirrer speed). Data must be collected and analyzed in a statistically sound manner using regression in order to determine if there exist a relationship between the factors and the response variables.

Therefore, based on the experimental data collected for both glucose and cassava substrates (Refer to Table 3-4 and Table 3-5), the data are subjected to be analyzed statistically by following the above guidelines before the data-based models for both substrates are developed.

Based on statistical results, the data-based models for both glucose and cassava substrates proposed are as follows:

Glucose Substrate:

$$\hat{Yield} = 33.098 - 18.785 * AR - 0.143 * SS + 0.147 * AR * SS \quad (45)$$

$$\hat{\text{Productivity}} = 0.234 - 0.139 * AR - 9.900E-4 * SS + 1.02E-3 AR * SS \quad (46)$$

Cassava Substrate:

$$\hat{Yield} = -3.346 - 14.360 * AR + 0.342 * SS - 0.093 * AR * SS \quad (47)$$

$$\text{Pr}\,\hat{oductivity} = -0.355 + 3.000E-3 * AR + 6.230E-3 * SS - 2.020E-3 AR * SS \quad (48)$$

Both data-based models for glucose and cassava substrates are proposed based on satisfactory statistical results (Refer to Appendix C.1 for statistical results). For glucose substrate, the proposed data-based models for both yield and productivity are significant, which indicates that both aeration rate and stirrer speed have significant effect on yield and productivity. The curvature is significant as well. This is desirable as it is important to ensure that the model fits before proceeding to optimization. The R^2 value for yield is 0.9973, i.e. close to 1, which is desirable. Same goes to productivity, whereby the R^2 value is 0.9950. Results show that the proposed data-based models are precisely accurate.

For cassava substrate, it is also indicated that the proposed data-based models for yield and productivity are significant. On the other hand, the curvature is significant too. Additionally, the R^2 values for yield and productivity are 0.9997 and 0.9852 respectively.

For detailed information on the significance of the data-based models for both glucose and cassava substrates, model graphs for each data-based model can be observed in Appendix C.1.

4.5.1.2 Kinetics Hybrid Model

The analysis of kinetics hybrid model is slightly different than data-based model. By using linear regression, model fitting is conducted in order to check whether the experimental data fit into the proposed kinetics model, i.e. Herbert's kinetics model. This kinetics model is developed to capture the kinetics in the batch bioreactor. Finally, statistical analysis is conducted to check the adequacy of the proposed kinetics model.

Based on the Herbert's model, experimental data of biomass (X), substrate (S) and product (P) concentrations are required. All data is used to generate a set of kinetics parameter data, i.e. $k_1, k_2, \ldots, k_6$. Linear regression analysis is then performed.

Experiments are conducted under different conditions of aeration rate (*AR*) and stirrer speed (*SS*) for both glucose and cassava substrates. Table 4-1 and Table 4-2 summarize the experimental data for glucose and cassava substrates respectively.

Table 4-1 Summary of Experimental Data at Different Aeration Rate and Stirrer Speed Conditions (Glucose Substrate)

Run Order	X_1: **Aeration Rate (LPM)**	X_2: **Stirrer Speed (rpm)**	X **(g/L)**	S **(g/L)**	P **(g/L)**
1	1.25	200	37.0	4.75	9.10
2	1.0	150	30.0	4.67	6.33
3	1.25	200	37.5	4.85	9.20
4	1.25	200	36.5	4.50	9.30
5	1.0	250	39.9	2.59	6.39
6	1.5	250	34.3	1.99	9.91
7	1.5	150	37.4	4.32	6.80

Table 4-2 Summary of Experimental Data at Different Aeration Rate and Stirrer Speed Conditions (Cassava Substrate)

Run Order	X_1: **Aeration Rate (LPM)**	X_2: **Stirrer Speed (rpm)**	X **(g/L)**	S **(g/L)**	P **(g/L)**
1	1.25	200	36.5	5.61	45.49
2	1.0	150	39.0	8.64	18.43
3	1.25	200	39.7	6.79	47.98
4	1.25	200	36.4	4.85	35.67
5	1.0	250	34.0	4.66	41.06
6	1.5	250	32.9	6.91	24.76
7	1.5	150	37.5	5.18	5.18

Based on the experimental data as presented in Table 4-1 and Table 4-2, the linear regression results are presented for both glucose and cassava substrate respectively.

The linear regression results for glucose substrate are given as:

$$\hat{k}_1 = 1.4085 - 0.2852X_1 + 0.3692X_2 \quad (49)$$

$$\hat{k}_2 = 0.0010 \quad (50)$$

$$\hat{k}_3 = 0.6631 - 0.0148X_1 + 0.0220X_2 \quad (51)$$

$$\hat{k}_4 = 0.1040 + 0.0142X_1 + 0.0128X_2 \quad (52)$$

$$\hat{k}_5 = 0.7558 - 0.1019X_1 - 0.0211X_2 \quad (53)$$

$$\hat{k}_6 = 0.0143 - 0.0001X_1 - 0.0019X_2 \quad (54)$$

where $X_1 = \frac{(AR - 1.25)}{0.25}$ and $X_2 = \frac{(SS - 200)}{50}$.

On the other hand, the linear regression results for cassava substrate are given as:

$$\hat{k}_1 = 0.9950 - 0.5218X_1 + 0.6978X_2 \quad (55)$$

$$\hat{k}_2 = 0.0010 \quad (56)$$

$$\hat{k}_3 = 1.3825 - 0.1276X_1 + 0.2098X_2 \quad (57)$$

$$\hat{k}_4 = 0.3434 + 0.5722X_1 + 0.1745X_2 \quad (58)$$

$$\hat{k}_5 = 0.3906 - 0.2093X_1 - 0.1123X_2 \quad (59)$$

$$\hat{k}_6 = 0.0149 - 0.0005X_1 - 0.0185X_2 \quad (60)$$

where $X_1 = \frac{(AR - 1.25)}{0.25}$ and $X_2 = \frac{(SS - 200)}{50}$.

With the utilization of the developed kinetics model which is based on Equations 49-54 (glucose substrate) and Equations 56-60 (cassava substrate), the kinetics parameters, $k_1, k_2, \ldots, k_6$ are predicted with the implementation of different conditions of aeration rate (*AR*) and stirrer speed (*SS*). Table 4-3 and Table 4-4 show the

summary of predicted kinetics parameters for different aeration rate (*AR*) and stirrer speed (*SS*) conditions calculated based on the developed kinetics model for glucose and cassava substrates respectively.

Table 4-3 Summary of Predicted Kinetics Parameters (Glucose Substrate)

Run Order	***X*₁: Aeration Rate (LPM)**	***X*₂: Stirrer Speed (rpm)**	$\hat{k}_1$	$\hat{k}_2$	$\hat{k}_3$	$\hat{k}_4$	$\hat{k}_5$	$\hat{k}_6$
1	1.25	200	1.4085	0.0010	0.6631	0.1040	0.7558	0.0143
2	1.0	150	1.3245	0.0010	0.6559	0.0770	0.8788	0.0163
3	1.25	200	1.1257	0.0010	0.6533	0.0909	0.7252	0.0173
4	1.25	200	1.2591	0.0010	0.6731	0.0879	0.7127	0.0179
5	1.0	250	2.0629	0.0010	0.6999	0.1026	0.8366	0.0125
6	1.5	250	1.4925	0.0010	0.6703	0.1310	0.6328	0.0123
7	1.5	150	0.7541	0.0010	0.6263	0.1054	0.6750	0.0161

Table 4-4 Summary of Predicted Kinetic Parameters (Cassava Substrate)

Run Order	***X*₁: Aeration Rate (LPM)**	***X*₂: Stirrer Speed (rpm)**	$\hat{k}_1$	$\hat{k}_2$	$\hat{k}_3$	$\hat{k}_4$	$\hat{k}_5$	$\hat{k}_6$
1	1.25	200	1.5934	0.0010	0.9362	0.3854	0.9347	0.0093
2	1.0	150	1.2870	0.0010	1.5745	0.3130	0.3974	0.0034
3	1.25	200	1.6921	0.0010	0.9127	0.2589	0.9014	0.0059
4	1.25	200	1.1359	0.0010	0.8536	0.2479	0.8217	0.0104
5	1.0	250	0.9272	0.0010	1.5371	0.6936	0.1253	0.0101
6	1.5	250	0.4467	0.0010	1.0329	0.3010	0.1429	0.0256
7	1.5	150	1.3191	0.0010	1.3854	0.0658	0.8968	0.0205

4.5.1.3 Kinetics Multi-Scale Model

As explained in Section 4.3.3, the kinetics multi-scale model is developed with the combination of the developed Herbert's kinetics model (*Section 4.3.2.1*), macro-scale bioreactor model (*Section 4.3.2.2*) and mixing model. The mixing model is proposed based on the k-ε turbulence model, Navier-Stokes equations and general balance over an element of reactor volume. Due to the complexity of the kinetics multi-scale model, this model is implemented into CFD to aid in solving this model and also to observe the mixing profile of the batch bioreactor under different conditions of aeration rate and stirrer speed. Table 4-5 shows the summary results for both experimental and CFD simulated data for yield under different conditions of aeration rate (*AR*) and stirrer speed (*SS*).

Table 4-5 Summary of Experimental and CFD Simulated Yield (%) (Glucose Substrate)

				Yield (%)		
Standard Order	**Run Order**	X_1**: Aeration Rate (LPM)**	X_2**: Stirrer Speed (rpm)**	**Experimental Data**	**CFD Simulated Data**	**% Difference**
7	1	1.25	200	21.500	21.700	0.922
1	2	1.0	150	14.788	18.600	20.494
5	3	1.25	200	21.050	21.250	0.941
6	4	1.25	200	21.250	21.500	1.163
3	5	1.0	250	15.105	15.900	5.000
4	6	1.5	250	24.040	23.100	3.910
2	7	1.5	150	16.392	17.000	3.576

Results show that within the experimental range, the kinetics multi-scale model is capable in predicting yield within 20% error. Despite of the complexity of the kinetics multi-scale model, CFD is able to predict yield under different conditions of aeration rate and stirrer speed. Next, the CFD mixing profile in terms of yield for each experiment based on experimental conditions from Table 4-5 is shown in Figure 4-5 to Figure 4-14. Samples are taken at the sampling point as shown in each figure,

whereby the sampling point is similar for each experiment for consistency.

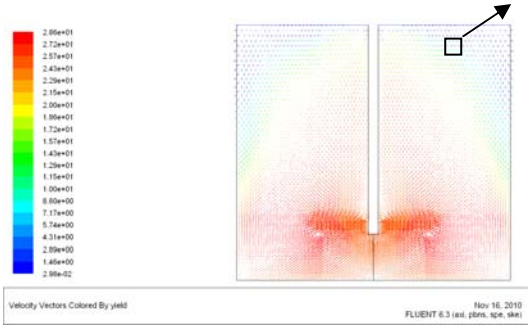


Figure 4-5 Velocity Vectors of Yield (1.0LPM *AR*, 150rpm *SS*)

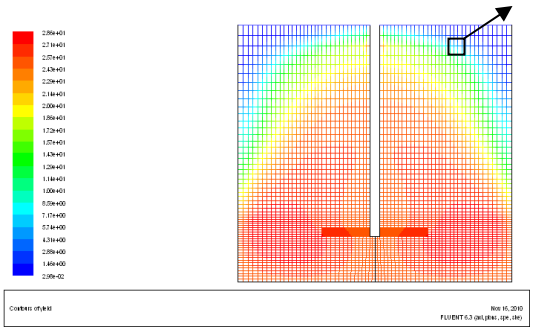


Figure 4-6 Contours of Yield (1.0LPM *AR*, 150rpm *SS*)

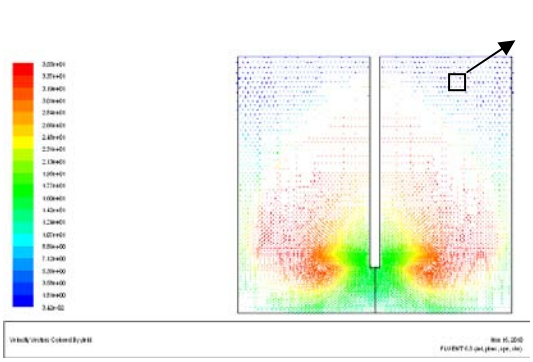


Figure 4-7 Velocity Vectors of Yield (1.5LPM *AR*, 250rpm *SS*)

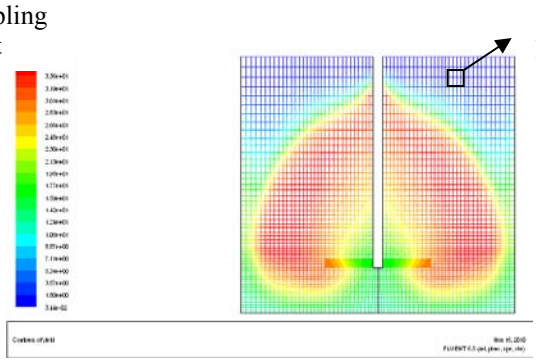


Figure 4-8 Contours of Yield (1.5LPM *AR*, 250rpm *SS*)

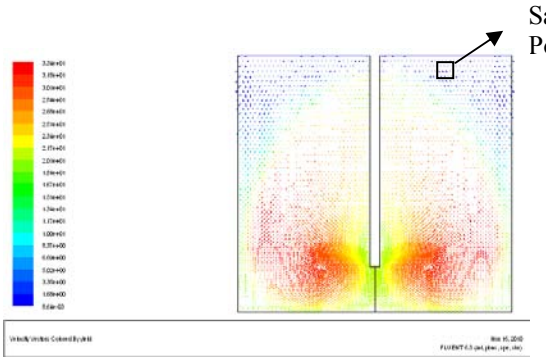


Figure 4-9 Velocity Vectors of Yield (1.25LPM *AR*, 200rpm *SS*)

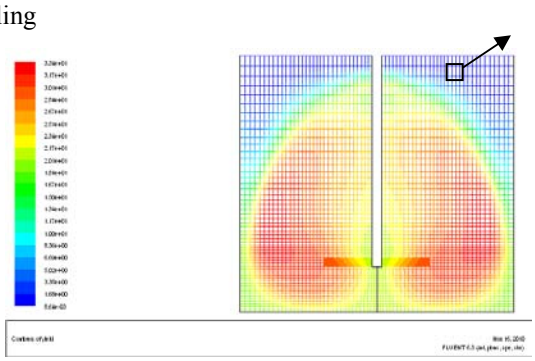


Figure 4-10 Contours of Yield (1.25LPM *AR*, 200rpm *SS*)

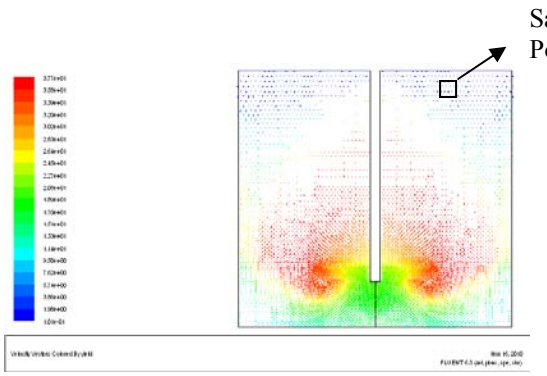


Figure 4-11 Velocity Vectors of Yield (1.5LPM *AR*, 150rpm *SS*)

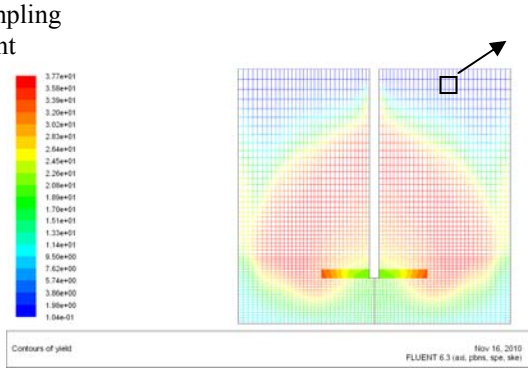


Figure 4-12 Contours of Yield (1.5LPM *AR*, 150rpm *SS*)

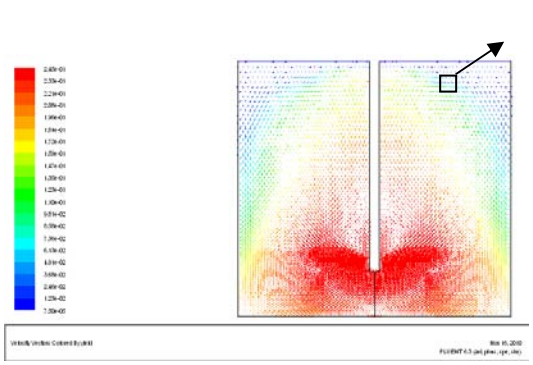


Figure 4-13 Velocity Vectors of Yield (1.0LPM *AR*, 250rpm *SS*)

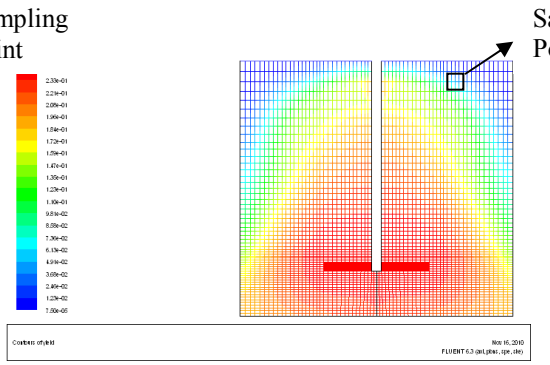


Figure 4-14 Contours of Yield (1.0LPM *AR*, 250rpm *SS*)

Each profile demonstrates different mixing behaviours, especially for experiment under aeration rate (*AR*) of 1.0LPM and stirrer speed (*SS*) of 150rpm. As shown in Figure 4-5 and Figure 4-6, yield is concentrated around the stirrer blades and at the bottom of the bioreactor vessel. These show that mixing is concentrated around the stirrer blades and beneath the bioreactor vessel. Thus, aeration rate (*AR*) and stirrer speed (*SS*) play an important role in the mixing mechanism within the bioreactor, and also the bioreactor performance.

Comparing to experiments under aeration rate (*AR*) of 1.25LPM and stirrer speed (*SS*) of 200rpm as well as aeration rate (*AR*) of 1.5LPM and stirrer speed (*SS*) of 150rpm, the profiles are slightly different, whereby yield is concentrated by the sides of the impeller blades too but not around the bottom of the bioreactor vessel. From Figure 4-9 to Figure 4-12, the mixing profiles are comparably similar. The difference is that yield is more concentrated around the stirrer blades for aeration rate (*AR*) of

1.25LPM and stirrer speed (*SS*) of 200rpm as compared to aeration rate (*AR*) of 1.5LPM and stirrer speed (*SS*) of 150rpm. These show that at lower aeration rate (*AR*) and higher stirrer speed (*SS*), yield is more concentrated around the impeller blades and towards the bottom of the bioreactor vessel, but with lower value of yield. It is suggested that, high percentage yield could be obtained at aeration rate (*AR*) of 1.25LPM and stirrer speed (*SS*) of 200rpm, which is the baseline of the experimental range. The difference between experimental and simulated yield for this condition is also the lowest, thus it is suitable to predict yield under this condition.

As observed from each figure, the glucose substrate flows upwards and downwards repeatedly and forms a circular flow pattern throughout the bioreactor. The flow concentrates at the sides of the impeller blades, whereby yield is highest at the impeller blades surrounding regions. On the other hand, all figures show the potential strength of CFD, whereby the internal distribution of the medium inside bioreactor could be calculated. Thus, the model utilized for simulation is capable of predicting the essential features of this particular flow regime: high concentration beneath the impeller; concentration of ethanol in the upper part of the stirred vessel; and the accumulation of ethanol at the middle and lower part of the vessel near the vessel wall. The impeller causes circulation flow patterns in the bioreactor below and above the impeller plane if the liquid rotation is hindered by baffles.

At the same time, bubbles are dispersed and re-dispersed by the impeller. Large bubbles quickly escape from the bioreactor, and small bubbles are dragged along with the liquid and recirculated. Oxygen is gradually exhausted in the bubbles along with the recirculation loop at high cell concentrations when the oxygen consumption is high. These mixing phenomena are expected as in the impeller region, whereby the oxygen transfer rate is high. Oxygen is consumed along the passage of the liquid elements, but only a low amount of oxygen is transferred from the gas phase into the liquid during this passage. The nutrient has to be quickly distributed to avoid local growth inhibitions and limitations [1]. All contours of yield and productivity show similar flow patterns, despite having differences in their respective phases and measurement values. These results show that all have mixing effects within the bioreactor which is embedded with impeller, as all show similar flow patterns. Thus, the integration of the kinetics multi-scale model is effective in describing the mixing

behaviour of ethanolic fermentation process, despite of the complexity of the fermentation process. Both aeration rate and stirrer speed show effects on the bioreactor performance.

Besides yield predictions, productivity is also predicted based on the kinetics multi-scale model. Similar to yield, both experimental and CFD simulated results for productivity are compared. Table 4-6 shows the summary results for both experimental and CFD simulated data for productivity under the same experimental conditions for yield.

Table 4-6 Summary of Experimental and CFD Simulated Productivity (g/L.hr) (Glucose Substrate)

				Productivity (g/L.hr)		
Standard Order	**Run Order**	***X_1*: Aeration Rate (LPM)**	***X_2*: Stirrer Speed (rpm)**	**Experimental Data**	**CFD Simulated Data**	**% Difference**
7	1	1.25	200	0.180	0.203	11.330
1	2	1.0	150	0.099	0.139	28.777
5	3	1.25	200	0.176	0.199	11.558
6	4	1.25	200	0.178	0.202	11.881
3	5	1.0	250	0.102	0.113	9.735
4	6	1.5	250	0.160	0.153	4.375
2	7	1.5	150	0.106	0.110	3.636

The percentage differences observed are higher than yield, thus this model is more precise in predicting yield. The integration of both aeration rate (*AR*) and stirrer speed (*SS*) into the kinetics multi-scale model improve the classical kinetics model as the non-ideally mixed behaviour of the bioreactor is described. This proves that the integration of aeration rate and stirrer speed into kinetics modeling is important and could further improve the reliability of predictions.

Figure 4-15 to Figure 4-24 represent the CFD mixing profile in terms of productivity for each experiment based on experimental conditions from Table 4-2. Similarly, samples are taken at the sampling point as shown in each figure, whereby the sampling point is similar for each experiment for consistency.

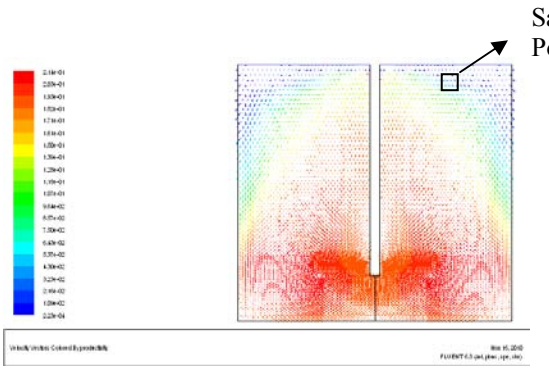


Figure 4-15 Velocity Vectors of Productivity (1.0LPM *AR*, 150rpm *SS*)

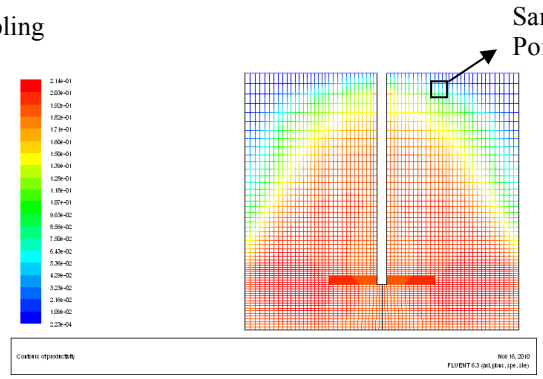


Figure 4-16 Contours of Productivity (1.0LPM *AR*, 150rpm *SS*)

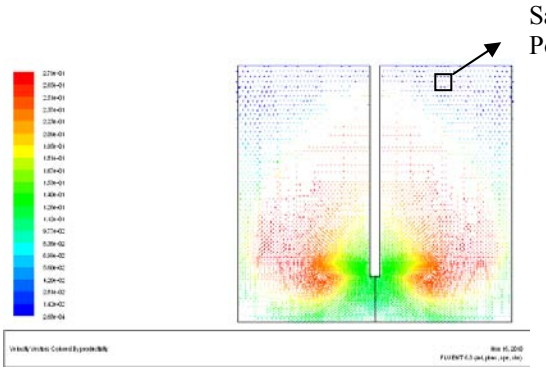


Figure 4-17 Velocity Vectors of Productivity (1.5LPM *AR*, 250rpm *SS*)

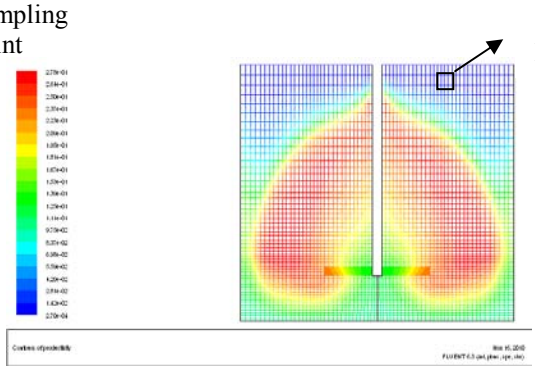


Figure 4-18 Contours of Productivity (1.5LPM *AR*, 250rpm *SS*)

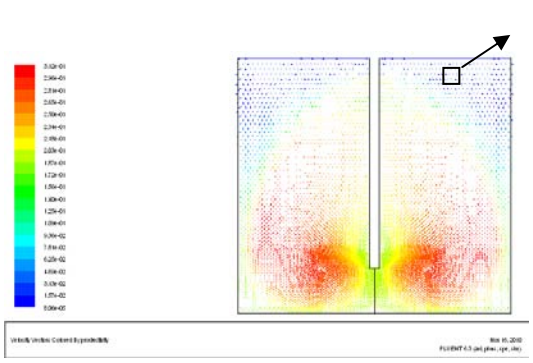


Figure 4-19 Velocity Vectors of Productivity (1.25LPM *AR*, 200rpm *SS*)

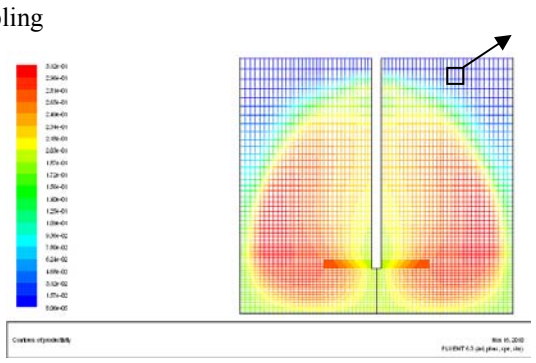


Figure 4-20 Contours of Productivity (1.25LPM *AR*, 200rpm *SS*)

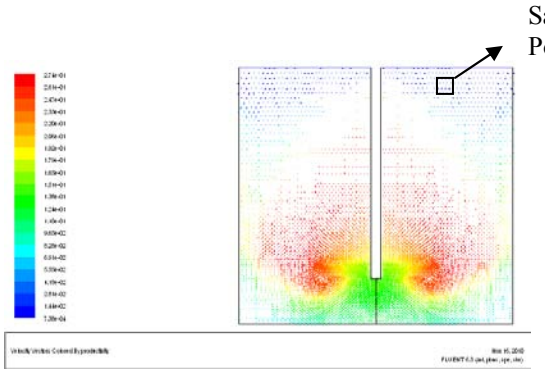


Figure 4-21 Velocity Vectors of Productivity (1.5LPM *AR*, 150rpm *SS*)

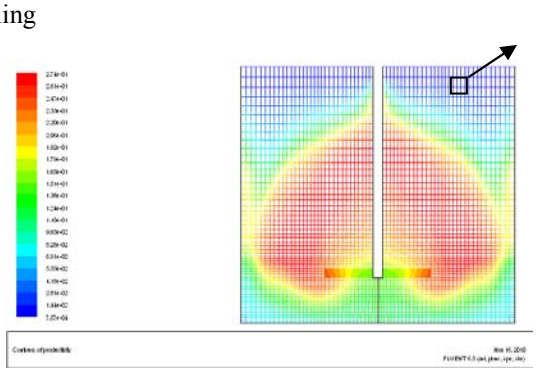


Figure 4-22 Contours of Productivity (1.5LPM *AR*, 150rpm *SS*)

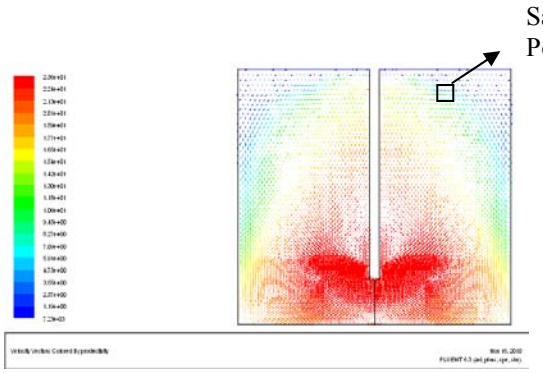


Figure 4-23 Velocity Vectors of Productivity (1.0LPM *AR*, 250rpm *SS*)

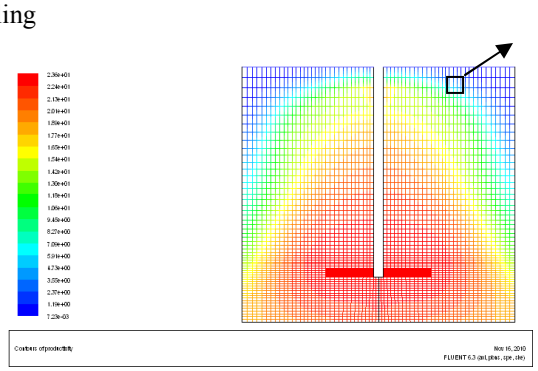


Figure 4-24 Contours of Productivity (1.0LPM *AR*, 250rpm *SS*)

Results show that the kinetics multi-scale model is able to predict the productivity of the fermentation process within 29% error. These show that both aeration rate (*AR*) and stirrer speed (*SS*) are vital parameters in the prediction of productivity. It is observed from the mixing profiles that the impeller causes circulation flow patterns

in the bioreactor below and above the impeller plane if the liquid rotation is hindered by baffles. At the same time, bubbles are dispersed and re-dispersed by the impeller. Large bubbles quickly escape from the bioreactor, and small bubbles are dragged along with the liquid and recirculated. Oxygen is gradually exhausted in the bubbles along with the recirculation loop at high cell concentrations when the oxygen consumption is high. These mixing phenomena are expected as in the impeller region, the oxygen transfer rate is high. Oxygen is consumed along the passage of the liquid elements, but only a low amount of oxygen is transferred from the gas phase into the liquid during this passage. The nutrient has to be quickly distributed to avoid local growth inhibitions and limitations [1]. Thus, with the integration of both aeration rate (*AR*) and stirrer speed (*SS*) into the kinetics multi-scale model, the bioreactor performance is able to be predicted and could decrease experimental and computational burden. This is further investigated statistically, whereby the kinetics multi-scale model is proven to be significant (refer to Appendix C.3 for statistical results).

4.5.3 Model Validations

Model validation is important to ensure that the developed model is able to predict the bioreactor performance accurately. Each model is validated by undergoing confirmation run experiments.

4.5.3.1 Data-Based Model

For data-based model, confirmation run experiments are performed and results are tabulated in Table 4-7 and Table 4-8 for glucose substrate. For cassava substrate, results are tabulated in Table 4-9 and Table 4-10.

Table 4-7 Summary of Confirmation Experimental Data and Model Predicted Yield Data (Glucose Substrate)

				Yield (%)		
Standard Order	**Run Order**	**X_1: Aeration Rate (LPM)**	**X_2: Stirrer Speed (rpm)**	**Experimental Data**	**Model Predicted Data**	**Error (%)**
7	1	1.25	200	22.500	17.767	21.036
1	2	1.0	150	15.900	14.913	6.208
5	3	1.25	200	22.050	17.767	19.424
6	4	1.25	200	21.750	17.767	18.313
3	5	1.0	250	16.115	15.313	4.977
4	6	1.5	250	24.658	24.296	1.468
2	7	1.5	150	17.214	16.546	3.881

Table 4-8 Summary of Confirmation Experimental Data and Model Predicted Productivity Data (Glucose Substrate)

				Productivity (g/L.hr)		
Standard Order	**Run Order**	**X_1: Aeration Rate (LPM)**	**X_2: Stirrer Speed (rpm)**	**Experimental Data**	**Model Predicted Data**	**Error (%)**
7	1	1.25	200	0.185	0.117	36.757
1	2	1.0	150	0.105	0.100	4.762
5	3	1.25	200	0.187	0.117	37.433
6	4	1.25	200	0.170	0.117	31.176
3	5	1.0	250	0.110	0.103	6.364
4	6	1.5	250	0.165	0.161	2.424
2	7	1.5	150	0.114	0.107	6.140

Table 4-9 Summary of Experimental and Model Predicted Yield Data (Cassava Substrate)

				Yield (%)		
Standard Order	**Run Order**	**X_1: Aeration Rate (LPM)**	**X_2: Stirrer Speed (rpm)**	**Experimental Data**	**Model Predicted Data**	**Error (%)**
7	1	1.25	200	48.962	23.854	51.281
1	2	1.0	150	19.961	19.644	1.588
5	3	1.25	200	48.550	23.854	50.867
6	4	1.25	200	47.921	23.854	50.222
3	5	1.0	250	44.971	44.544	0.950
4	6	1.5	250	26.357	25.739	2.345
2	7	1.5	150	5.917	5.489	7.233

Table 4-10 Summary of Experimental and Model Predicted Productivity Data (Cassava Substrate)

				Productivity (g/L.hr)		
Standard Order	**Run Order**	**X_1: Aeration Rate (LPM)**	**X_2: Stirrer Speed (rpm)**	**Experimental Data**	**Model Predicted Data**	**Error (%)**
7	1	1.25	200	0.955	0.336	64.817
1	2	1.0	150	0.293	0.269	8.191
5	3	1.25	200	1.045	0.336	67.847
6	4	1.25	200	0.995	0.336	66.231
3	5	1.0	250	0.751	0.601	19.973
4	6	1.5	250	0.496	0.362	27.016
2	7	1.5	150	0.127	0.112	11.811

As observed, the percentage error is as high as 68%. Therefore, to investigate the model accuracy and to determine whether such errors are acceptable for data-based model, statistical analysis is conducted for both data-based models for glucose and cassava substrates. Statistical results indicate that both the data-based models are significant (See Appendix C). Therefore, there are significant effects of both aeration rate and stirrer speed on the bioreactor performance for both glucose and cassava substrates.

4.5.3.2 Kinetics Hybrid Model

Based on the predicted kinetic parameters, predicted rates of substrate consumption, product formation and biomass formation as well as the predicted yield and productivity are calculated. Table 4-11 and Table 4-12 summarize the experimental and predicted results for both yield and productivity for glucose substrate. On the other hand, Table 4-13 and Table 4-14 summarize the experimental and predicted yield and productivity values for cassava substrate.

Table 4-11 Summary of Experimental and Model Predicted Yield Data (Glucose Substrate)

				Yield (%)		
Standard Order	**Run Order**	***X_1*: Aeration Rate (LPM)**	***X_2*: Stirrer Speed (rpm)**	**Experimental Data**	**Model Predicted Data**	**Error (%)**
7	1	1.25	200	21.500	13.270	38.279
1	2	1.0	150	14.788	12.006	18.813
5	3	1.25	200	21.050	13.050	38.005
6	4	1.25	200	21.250	13.100	38.353
3	5	1.0	250	15.105	17.214	12.252
4	6	1.5	250	24.040	21.099	12.234
2	7	1.5	150	16.392	13.011	20.626

Table 4-12 Summary of Experimental and Model Predicted Productivity Data (Glucose Substrate)

				Productivity (g/L.hr)		
Standard Order	**Run Order**	X_1: **Aeration Rate (LPM)**	X_2: **Stirrer Speed (rpm)**	**Experimental Data**	**Model Predicted Data**	**Error (%)**
7	1	1.25	200	0.180	0.121	32.778
1	2	1.0	150	0.099	0.083	16.162
5	3	1.25	200	0.176	0.115	34.659
6	4	1.25	200	0.178	0.119	33.146
3	5	1.0	250	0.102	0.092	9.804
4	6	1.5	250	0.160	0.144	10.000
2	7	1.5	150	0.106	0.086	18.868

Table 4-13 Summary of Experimental and Model Predicted Yield Data (Cassava Substrate)

				Yield (%)		
Standard Order	**Run Order**	X_1: **Aeration Rate (LPM)**	X_2: **Stirrer Speed (rpm)**	**Experimental Data**	**Model Predicted Data**	**Error (%)**
7	1	1.25	200	48.322	19.040	60.598
1	2	1.0	150	19.577	16.220	20.369
5	3	1.25	200	48.500	18.790	61.258
6	4	1.25	200	48.952	19.917	59.314
3	5	1.0	250	44.432	37.603	15.369
4	6	1.5	250	25.597	20.383	20.369
2	7	1.5	150	5.404	3.490	35.415

Table 4-14 Summary of Experimental and Predicted Productivity (g/L.hr) (Cassava Substrate)

				Productivity (g/L.hr)		
Standard Order	**Run Order**	X_1: **Aeration Rate (LPM)**	X_2: **Stirrer Speed (rpm)**	**Experimental Data**	**Model Predicted Data**	**Error (%)**
7	1	1.25	200	0.922	0.454	50.741
1	2	1.0	150	0.280	0.212	24.361
5	3	1.25	200	0.980	0.477	51.347
6	4	1.25	200	0.990	0.466	52.951
3	5	1.0	250	0.701	0.614	12.392
4	6	1.5	250	0.450	0.337	25.123
2	7	1.5	150	0.130	0.090	30.961

Similar to data-based model for glucose and cassava substrates, results show that the percentage error is as high as 61%. The percentage error for kinetics hybrid model is slightly lower than data-based model, which show better predictions. To investigate the model accuracy and to determine whether such errors are acceptable for kinetics hybrid model, model fitting is conducted to investigate the kinetic dynamics of glucose, ethanol and glycerol concentrations.

Therefore, with the combined analysis of the linear regression results (see Equations 49-54 for glucose substrate; Equations 55-60 for cassava substrate), Herbert's kinetics model (see Equations 26-30) as well as the macro-scale bioreactor model (see Equations 31-35), glucose and ethanol concentrations are predicted. These predictions are then validated against the experimental data of another set of aeration rate (*AR*) and stirrer speed (*SS*) conditions, whereby the conditions chosen are within experimental range. This is to investigate the capability of the proposed kinetics model in predicting other conditions within experimental range, since the kinetics model is more complicated than the data-based model. Thus, experimental conditions of aeration rate of 1.2LPM and stirrer speed of 175rpm are chosen as these conditions are within the experimental range.

Figures 4-25 to 4-27 show the model fitting analysis based on experimental data for glucose substrate. As observed, the kinetics hybrid model reasonably fit the experimental data of aeration rate of 1.2LPM and stirrer speed of 175rpm. These prove that the linear regression results (see Equations 49-54) and Herbert's kinetics model (see Equations 26-30) can be used to describe the kinetics of the fermentation process and predict both yield and productivity.

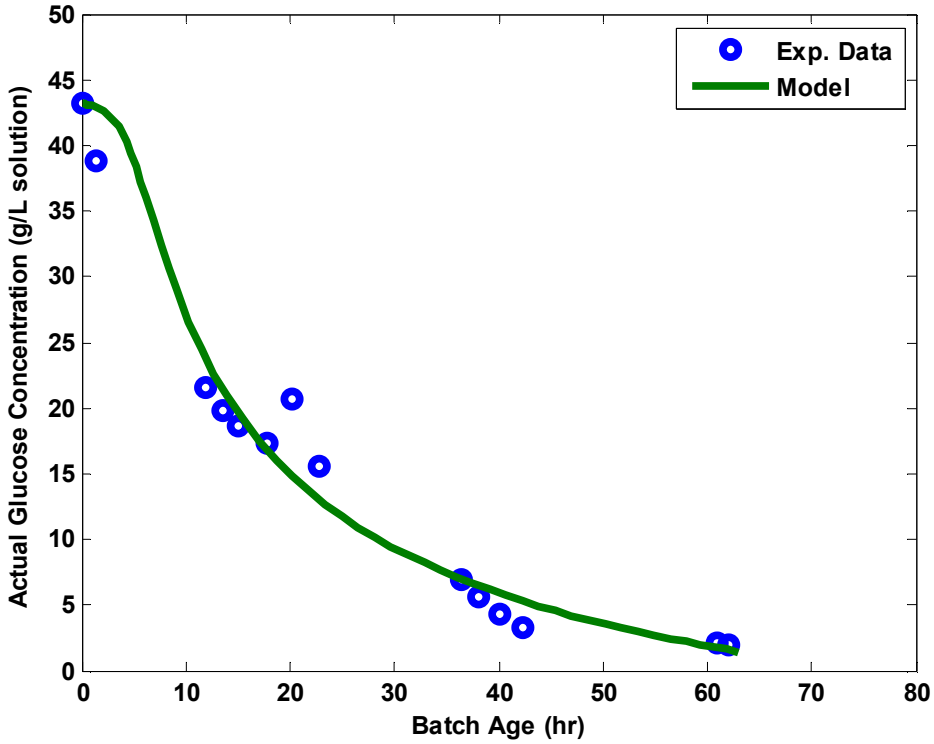


Figure 4-25 Model Fitting for Actual Glucose Concentration (g/L solution) (Glucose Substrate)

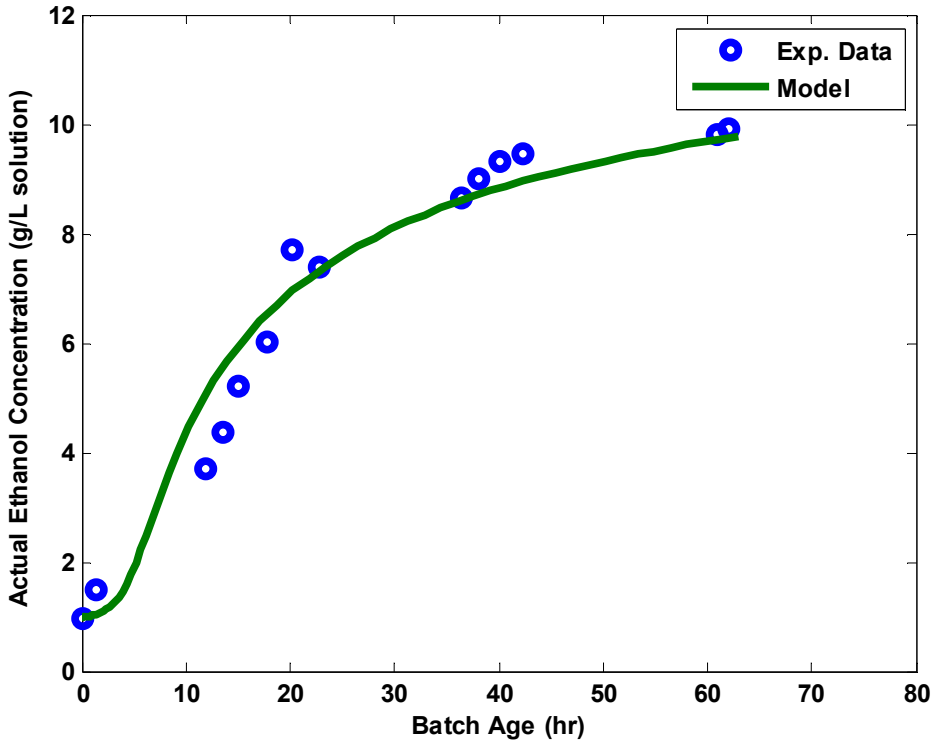


Figure 4-26 Model Fitting for Actual Ethanol Concentration (g/L solution) (Glucose Substrate)

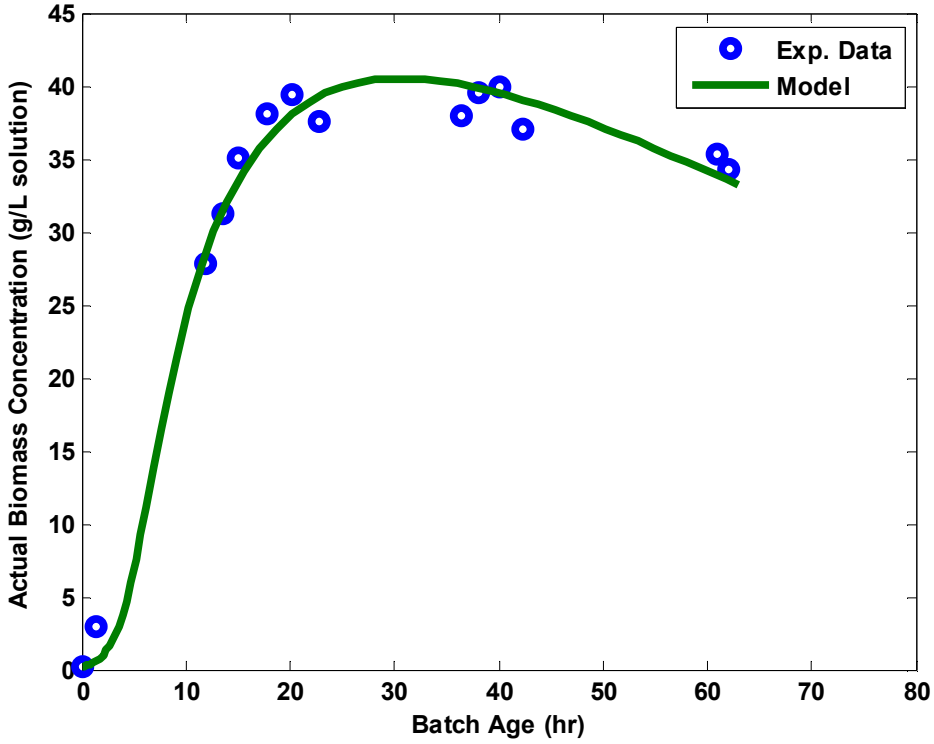


Figure 4-27 Model Fitting for Actual Biomass Concentration (g/L solution) (Glucose Substrate)

Based on the model fitting analysis, it is predicted that the kinetics hybrid model for glucose substrate is able to predict the yield and productivity adequately. It is indicated statistically that the kinetics hybrid model is significant, whereby the value of "Prob > F" is less than 0.05 which is desirable. This indicates that the terms in this model, i.e. aeration rate and stirrer speed, have a significant effect on the response, i.e. yield and productivity. Further, the Lack of Fit is insignificant. Thus, statistical results indicate that the kinetics hybrid model is suitable to be used for prediction and thus, optimization could proceed. More detailed statistical results are subject to view at Appendix C.2.

On the other hand, for cassava substrate, by using the linear regression results (see Equations 55-60), the Herbert's kinetics model (see Equations 26-30) as well as the macro-scale bioreactor model (see Equations 31-35), the glucose and ethanol concentrations are predicted. These predictions are then validated against the experimental data for another set of aeration rate (*AR*) and stirrer speed (*SS*) conditions. Same conditions, i.e. aeration rate of 1.2LPM and stirrer speed of 175rpm are utilized as glucose substrate in order to compare the kinetics for both glucose and cassava substrate. Figures 4-28 to 4-30 show the model fitting analysis.

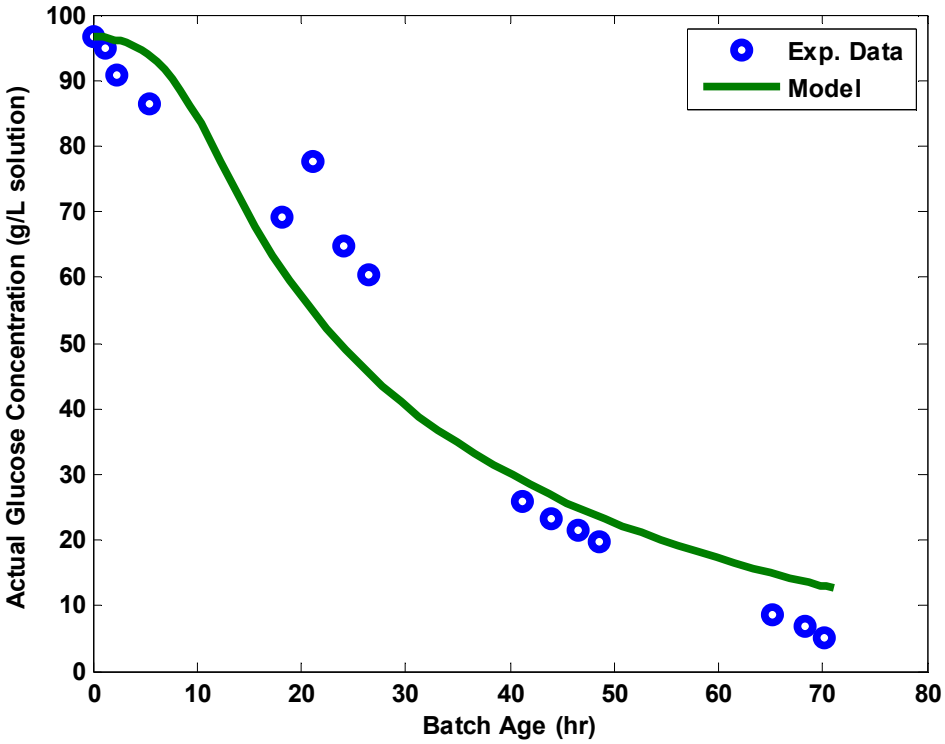


Figure 4-28 Model Fitting for Actual Glucose Concentration (g/L solution) (Cassava Substrate)

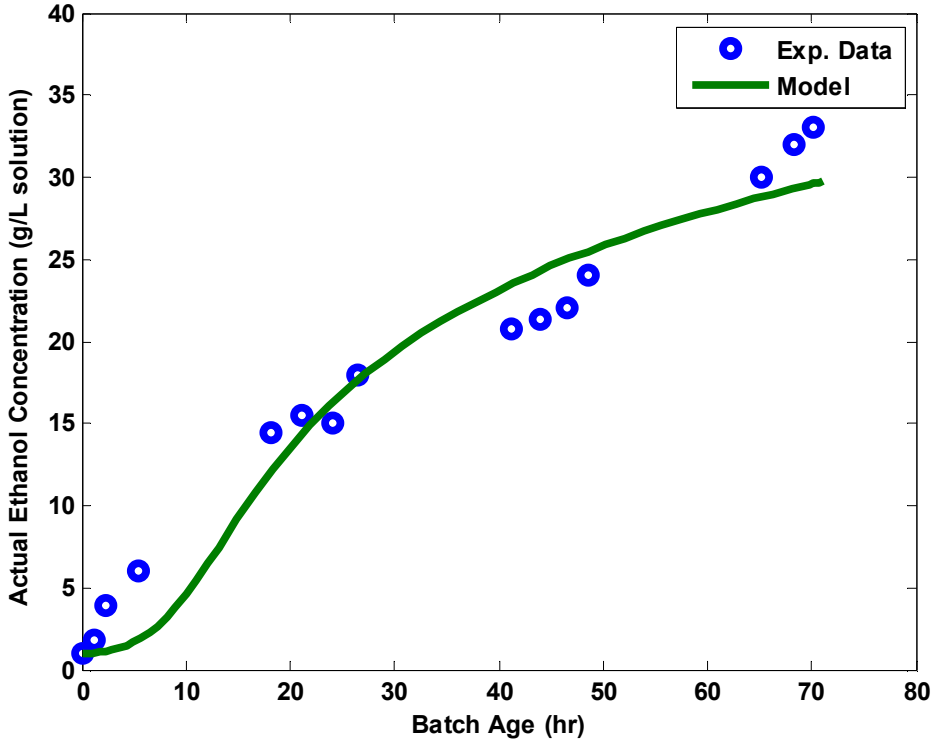


Figure 4-29 Model Fitting for Actual Ethanol Concentration (g/L solution) (Cassava Substrate)

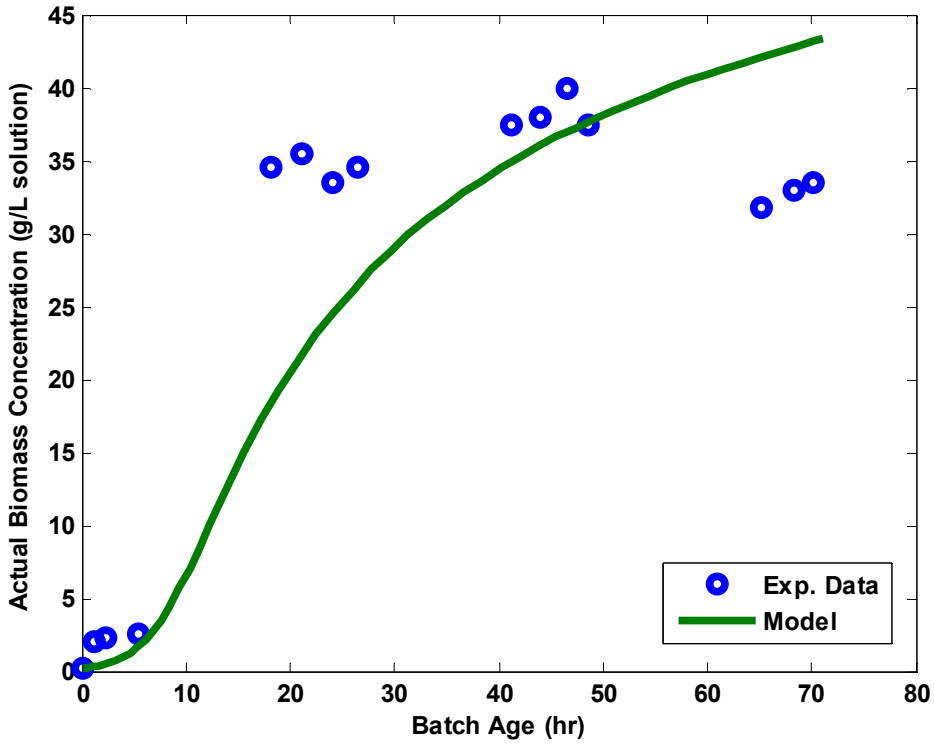


Figure 4-30 Model Fitting for Actual Biomass Concentration (g/L solution) (Cassava Substrate)

As observed in Figures 4-28 to 4-30, the kinetics hybrid model for cassava substrate does not reasonably fit the experimental data of aeration rate of 1.2LPM and stirrer speed of 175rpm. These proved that the linear regression results (see Equations 55-60) and Herbert's kinetics model (see Equations 26-30) could not be used to describe the kinetics of the fermentation process of cassava accurately. Furthermore, from each figures, the kinetics model could not predict well, especially during the exponential phase of the process. The predictions deviate a lot from the experimental data until towards the end of the fermentation process. These results show that the microbial activities during the exponential phase are very complex. Since mixing is engaged in the fermentation process, there is a need to describe the microbial activities, whether the cells are growing at a constant or maximum rate. All these activities are influenced by the mixing mechanism in the bioreactor. Thus, both aeration rate and stirrer speed have significant effects on the bioreactor performance. Glucose and cassava are both very different substrates, therefore the Herbert's kinetics model is suggested to be suitable for glucose substrate, but not for cassava substrate.

To further interpret whether the kinetics hybrid model is suitable for cassava, statistical analysis is conducted. Statistical results show that the kinetics hybrid model is not significant and the Lack of Fit is significant. Thus, these show that the kinetics hybrid model is not suitable for cassava. Therefore, only glucose substrate will be considered for the proposed multi-scale kinetics model since the kinetics hybrid model is suitable for glucose substrate but not cassava substrate.

4.6 MODEL APPLICATIONS

Based on results and discussions presented, the data-based, kinetics hybrid and kinetics multi-scale models are able to predict the bioreactor performance. The data-based model is able to predict the bioreactor performance for glucose and cassava substrates. However, it is not suitable to use the kinetics hybrid and kinetics multi-scale models for bioreactor performance predictions for cassava substrate. Therefore, all three modeling approaches are suitable in predicting the optimum conditions of aeration rate and stirrer speed for glucose substrate and to be used for control strategy purposes.

4.7 CONCLUSIONS

Three modeling approaches are proposed, i.e. data-based, kinetics hybrid and kinetics multi-scale models for the prediction of bioreactor performance by varying both aeration rate and stirrer speed. Statistical analysis is conducted for each modeling approach to ensure that the developed models are accurate in prediction of bioreactor performance.

It is concluded that:

- The data-based model is the simplest model to be developed. This model is able to predict the bioreactor performance for both glucose and cassava substrates. Statistical analysis show that this model does not predict the best among all the three modeling approaches, i.e. around 67% difference.
- The kinetics hybrid model is developed based on Herbert's kinetics. Model fitting is required to predict the kinetics parameters. This model is able to predict the bioreactor performance for glucose substrate. However, it is not

suitable to predict the bioreactor performance for cassava substrate. The experimental data for cassava substrate do not fit into the kinetics hybrid model. Statistical analysis show that this model predicts better than the data-based model, i.e. around 61% difference.

- The kinetics multi-scale model is the most complicated model, whereby more complex models are taken into account, i.e. the mixing model. Due to its complexity, CFD is required to aid in the prediction of bioreactor performance. CFD simulations demonstrate the mixing behaviour under different conditions of aeration rate and stirrer speed. Thus, this shows the effects of aeration rate and stirrer speed on bioreactor performance. Statistical analysis shows that this model predicts best bioreactor performance, i.e. around 28% difference. Thus, the kinetics multi-scale model is suitable to predict the bioreactor performance for glucose substrate. This model has potential in predicting the optimum operating conditions of aeration rate and stirrer speed for control.

In the next chapter, the optimization approach is analyzed and the optimum points obtained will then be used as a basis for control strategy development. Only glucose substrate will be considered for optimization since the kinetics hybrid model and kinetics multi-scale model are not competent to cassava substrate.

Chapter 5 Optimization of Batch Bioreactor Using Response Surface Methodology (RSM)

5.1 INTRODUCTION

The objective of this chapter is to obtain the optimum conditions of aeration rate and stirrer speed of a batch bioreactor to achieve maximum bioreactor performance. The optimum conditions obtained will be used for control strategy purpose. This chapter outlines the optimization approach from which the optimum conditions of both aeration rate and stirrer speed on the batch bioreactor are obtained.

In this study, only glucose substrate will be considered for optimization analysis. Central Composite Design (CCD) is considered as the optimization approach in this study since it is the most commonly method used in the optimization of fermentation processes.

This chapter is organized as follows. Section 5.2 presents an approach for designing and analyzing the optimization problem. The case studies involve in the optimization process is also outlined in this section. In Section 5.3, the optimization results obtained are presented and discussed. Finally, concluding remarks are presented in Section 5.4.

5.2 APPLICATION AND CASE STUDIES

In fermentation studies, the most commonly used Response Surface Method (RSM) is Central Composite Design (CCD) since this design provides a solid foundation for

the generation of a response surface map [78]. Thus, CCD is suggested to be utilized in our study.

In our study, glucose substrate is considered as case study for optimization. Cassava substrate is not considered due to the incompatibility of the model fitting (See Figures 4-28 to 4-30). It is important to ensure that the optimum conditions obtained are compatible with the kinetics model so that the optimum conditions could be used for control strategy purpose adequately.

To create a CCD, it is important to locate new points along the axes of the factor space. For maximum efficiency, the axial or star points are to be located a specific distance outside the original factor range. The factorial design displayed in Table 3-3 is augmented, whereby additional centre points provide a link between the blocks and added more power to the estimation of second-order effects needed to characterize curvature.

Table 5-1 shows the augmented design, whereby the new points are designated as block 2. Additional experiments are conducted based on the new points. Results are then analyzed via ANOVA analysis.

Table 5-1 CCD Matrix Employed for Two Independent Variables, i.e. Aeration Rate and Stirrer Speed

Standard Order	**Run Order**	**Block**	X_1: **Aeration Rate (LPM)**	X_2: **Stirrer Speed (rpm)**	Y_1: **Yield (%)**	Y_2: **Productivity (g/L.hr)**
7	1	1	1.25	200	21.500	0.180
1	2	1	1.0	150	14.788	0.099
5	3	1	1.25	200	21.050	0.176
6	4	1	1.25	200	21.250	0.178
3	5	1	1.0	250	15.105	0.102
4	6	1	1.5	250	24.040	0.160
2	7	1	1.5	150	16.392	0.106
13	8	2	1.25	200	24.000	0.230
12	9	2	1.25	200	23.500	0.200
14	10	2	1.25	200	22.000	0.190
10	11	2	1.25	129.29	18.511	0.115
9	12	2	1.60	200	22.250	0.195
11	13	2	1.25	270.71	23.500	0.210
8	14	2	0.90	200	20.500	0.165

As shown in Table 5-1, the experimental tests involve fourteen trials and the response variables measured are yield and productivity. For each experimental trial, new conditions of aeration rate and stirrer speed are utilized. These results are subjected for further analysis following the steps outlined as follows [77]:

1. Choose a transformation if desired. Otherwise, leave the option at “None”.
2. Select the appropriate model to be used. The Fit Summary button displays the sequential F-tests, Lack of Fit tests and other adequacy measures that could be used to assist in selecting the appropriate model.
3. Perform ANOVA for analysis of residuals and outlier detection.
4. Inspect various diagnostic plots to statistically validate the model.

5. Generate model graphs for interpretation if the model looks good. The analysis and inspection performed in steps (3) and (4) above will show whether the model is good or otherwise. A good model must be significant and curvature must be significant too. The various coefficient of determination, R^2 values should be close to 1.

5.3 RESULTS AND DISCUSSION

5.3.1 Data-Based Model

5.3.1.1 Statistical Analysis

In order to obtain the optimum conditions of both aeration rate and stirrer speed by using the data-based model, ANOVA analysis is conducted by utilizing the experimental results as shown in Table 5-1. The ANOVA results of the response surface model are shown in Table 5-2 for yield, whereby *AR* indicates aeration rate and *SS* indicates stirrer speed.

Table 5-2 ANOVA Results for CCD on Yield

Source	**Sum of Squares**	**Degree of Freedom**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	90.10	5	18.02	9.73	0.0047	Significant
$A - AR$	21.17	1	21.17	11.44	0.0117	
$B - SS$	28.20	1	28.20	15.24	0.0059	
AB	13.44	1	13.44	7.26	0.0309	
A^2	12.76	1	12.76	6.90	0.0341	
B^2	16.60	1	16.60	8.97	0.0201	
Residual	12.96	7	1.85			
Lack of Fit	10.64	3	3.55	6.12	0.0563	Not Significant
Pure Error	2.32	4	0.58			
Cor Total	132.31	13				

As shown in Table 5-2, all terms are significant with "Prob > F", which is less than 0.05. The smaller the "Prob > F", the more significant is the corresponding coefficient [56]. This implies that the quadratic effects of both aeration rate (*AR*) and stirrer speed (*SS*) are highly significant, as is evident from their respective "Prob > F" values. The coefficient of *AB* ("Prob > F" = 0.0309) indicates that both aeration rate (*AR*) and stirrer speed (*SS*) have significant effects on yield since the "Prob > F" is less than 0.05. The Fisher variance ratio, *F* value, is a statistically valid measure of how well the factors describe the variation in the data about its mean. The greater the *F* value from unity, the more certain it is that the factors explain adequately the variation in the data about its mean and the estimated factor effects are real [56]. ANOVA results of the model demonstrate that the model is highly significant, as is evident from the Fisher's *F* test (F_{model} = 9.73) and a low "Prob > F" value ($P_{model} > F$ = 0.0047).

On the other hand, the Lack of Fit is not significant relative to the pure error when "Prob > F" = 0.0563 > 0.05, also supports the fitness of the model. In addition, the goodness of the fit of the model is also checked by the determination coefficient (R^2). In this case, the value of the determination coefficient (R^2 = 0.8743) indicates that 87.43% of the sample variation in yield is attributed to the independent variables. The R^2 value is higher than 0.80, indicating that the regression model explains the experiment well. The fit degree of the model is high enough to explain 87.43% of yield, thus this model is statistically adequate to be applied to predict the yield within the experimental setting range. To further investigate the adequacy of the developed model, a check of the response surface plots are available at Appendix D.1.

Next, the interpretation of ANOVA results for productivity is conducted as well. Table 5-3 shows the ANOVA for productivity, whereby the ANOVA of the model demonstrate that the model is highly significant, and is evident from the Fisher's *F* test (F_{model} = 9.30) and a low "Prob > F" value ($P_{model} > F$ = 0.0054).

Table 5-3 ANOVA Results for CCD on Productivity

Source	**Sum of Squares**	**Degree of Freedom**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	0.015	5	2.986E-3	9.30	0.0054	Significant
A – AR	1.443E-3	1	1.443E-3	4.49	0.0718	
B – SS	4.577E-3	1	4.577E-3	14.25	0.0069	
AB	6.503E-4	1	6.503E-4	2.02	0.0780	
A^2	2.868E-3	1	2.868E-3	8.93	0.0203	
B^2	5.981E-3	1	5.981E-3	18.62	0.0035	
Residual	2.248E-3	7	3.212E-4			
Lack of Fit	1.369E-3	3	4.563E-4	2.08	0.2461	Not Significant
Pure Error	8.793E-4	4	2.198E-4			
Cor Total	0.024	13				

Based on the ANOVA results, the Lack of Fit is not significant relative to the pure error when p-value = 0.2461 > 0.05, also supports the fitness of the model. The coefficient of *AB* ("Prob > F" = 0.0780) indicates that both aeration rate (*AR*) and stirrer speed (*SS*) have significant effects on yield since the "Prob > F" is less than 0.05. The value of the determination coefficient (R^2 = 0.8691) which indicates that 86.91% of the sample variation in productivity is attributed to the independent variables. Thus, this model is statistically adequate to be applied to predict the productivity within the experimental setting range. Thus, the optimum conditions for both aeration rate and stirrer speed could be determined to achieve maximum yield and productivity.

5.3.1.2 Analysis of Response Surfaces

Based on the ANOVA results, optimization is preceded and is represented in 3D response surface plots. The response surface plots are made as a function of aeration rate (*AR*) and stirrer speed (*SS*). The effect of the two variables on yield and productivity are illustrated in Figure 5-1 and Figure 5-2 respectively.

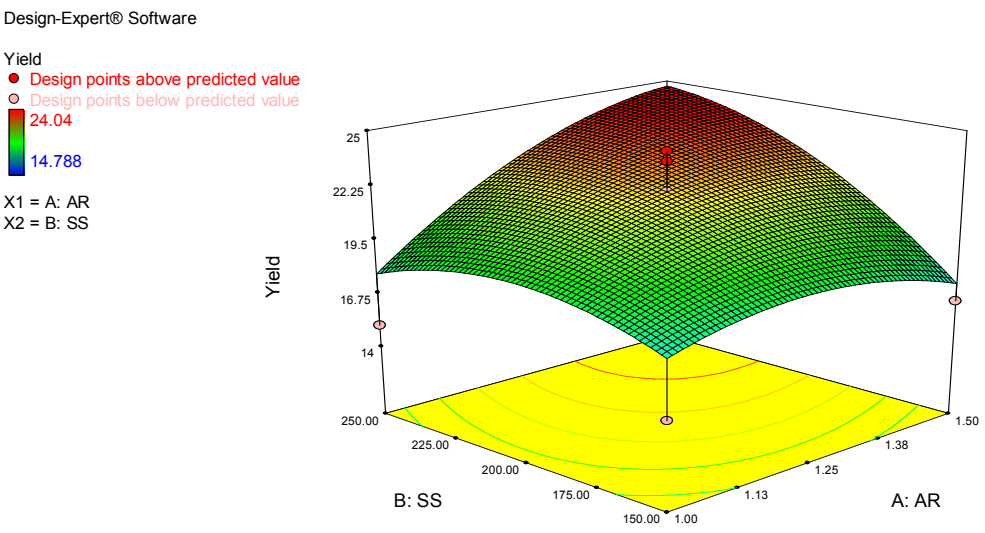


Figure 5-1 Response Surface Plot for the Effect of Aeration Rate (*AR*) and Stirrer Speed (*SS*) on Yield

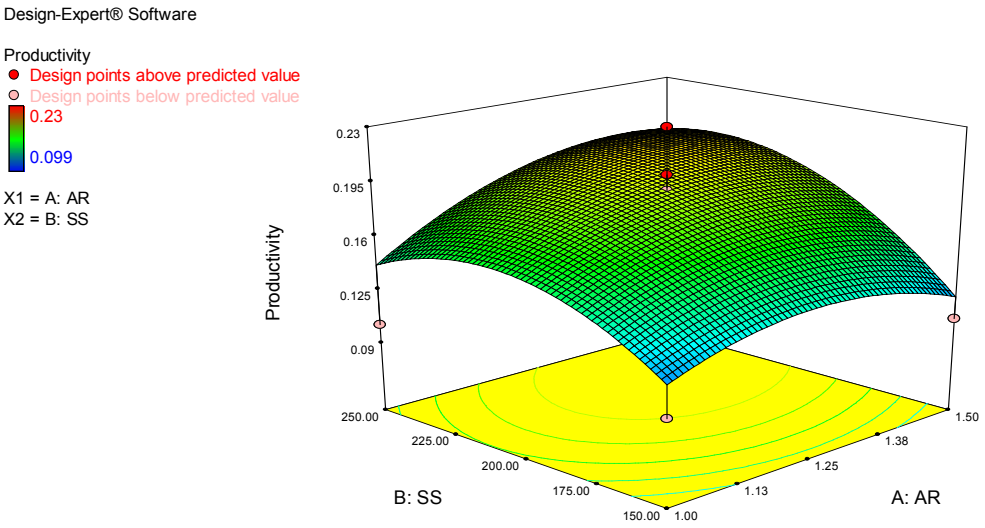


Figure 5-2 Response Surface Plot for the Effect of Aeration Rate (*AR*) and Stirrer Speed (*SS*) on Productivity

Based on Figure 5-1 and Figure 5-2, both aeration rate (*AR*) and stirrer speed (*SS*) demonstrate a quadratic effect on the response surface. Within the experimental range, the suggested optimum value of aeration rate (*AR*) and stirrer speed (*SS*) to maximize yield is **aeration rate (*AR*) of 1.47LPM and stirrer speed (*SS*) of 242rpm**. Under these conditions, it is expected to **yield a maximum of 24.5%**

ethanol and 0.2g/L.hr maximum productivity. It is indicated from the ANOVA analysis that 97.8% desirable that these optimum values would be able to yield and produce maximum amount of ethanol, i.e. well above the 80% satisfactory limit.

On the other hand, the response, yield, is found to increase with the increase in stirrer speed (*SS*) from 150rpm and reached its peak at 242rpm. There is a significant increase in yield with increase in aeration rate (*AR*) too. It reaches optimum at aeration rate (*AR*) of 1.47LPM, showing less significant difference in yield thereafter. Based on the results obtained, both aeration rate (*AR*) and stirrer speed (*SS*) contribute to the mixing mechanism of the bioreactor, as both yield and productivity differ with different aeration rate (*AR*) and stirrer speed (*SS*) conditions. Thus, both aeration rate (*AR*) and stirrer speed (*SS*) have significant effect on the batch bioreactor performance. Therefore, the data-based model demonstrates good predictions of yield and productivity by considering both aeration rate (*AR*) and stirrer speed (*SS*) in the data-based model.

5.3.2 Kinetics Hybrid Model

5.3.2.1 Statistical Analysis

The analysis of yield and productivity based on the proposed kinetics hybrid model is analyzed. Similar to the analysis of the data-based model, without performing any transformation on the responses, examination of the Fit Summary output reveals that the kinetics hybrid model is statistically significant. The ANOVA results for yield are shown at Table 5-4 below.

Table 5-4 ANOVA Results for CCD on Yield

Source	**Sum of Squares**	**Degree of Freedom**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	44.40	2	22.20	7.20	0.0115	Significant
Residual	30.81	10	3.08			
Lack of Fit	27.76	6	4.63	6.06	0.0514	Not Significant
Pure Error	3.05	4	0.76			

As shown in Table 5-4, ANOVA results of the model demonstrate that the model is highly significant, as it is evident from the Fisher's *F* test (F_{model} = 9.73) and a low "Prob > F" value ($P_{model} > F$ = 0.0047). This analysis indicates that both aeration rate (*AR*) and stirrer speed (*SS*) have significant effects on yield since the "Prob > F" is less than 0.05. On the other hand, the Lack of Fit is not significant relative to the pure error when "Prob > F" = 0.0514 > 0.05, also supports the fitness of the model. The value of the determination coefficient (R^2 = 0.8591) indicates that 85.91% of the sample variation in yield is attributed to the independent variables. Thus, this model is statistically adequate to be applied to predict yield within the experimental setting range. To further investigate the adequacy of the developed kinetics hybrid model, a check of the response surface plots can be done which is available at Appendix D.2.

Next, the interpretation of ANOVA results for productivity is vital as well. Table 5-5 shows the ANOVA for productivity, whereby the ANOVA of the model demonstrate that the model is highly significant, and is evident from the Fisher's *F* test (F_{model} = 11.97) and a low "Prob > F" values ($P_{model} > F$ = 0.0022).

Table 5-5 ANOVA Results for CCD on Productivity

Source	**Sum of Squares**	**Degree of Freedom**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	8.180E-3	2	4.090E-3	11.97	0.0022	Significant
Residual	3.417E-3	10	3.417E-4			
Lack of Fit	2.974E-3	6	4.956E-4	4.47	0.0844	Not Significant
Pure Error	4.433E-4	4	1.108E-4			

The Lack of Fit is not significant relative to the pure error when "Prob > F" = 0.0814 > 0.05, also supports the fitness of the model. This analysis indicates that both aeration rate (*AR*) and stirrer speed (*SS*) have significant effects on productivity since the "Prob > F" is less than 0.05. The value of the determination coefficient (R^2 = 0.8754) indicates that 87.54% of the sample variation in productivity is attributed to the independent variables. Thus, this model can be used to predict the productivity within the experimental setting range. Overall, the developed kinetics hybrid model

is significant and the optimum conditions of aeration rate (*AR*) and stirrer speed (*SS*) could be determined.

5.3.2.2 Analysis of Response Surfaces

Figure 5-3 and Figure 5-4 show the response surface plots for both yield and productivity as a function of both aeration rate (*AR*) and stirrer speed (*SS*).

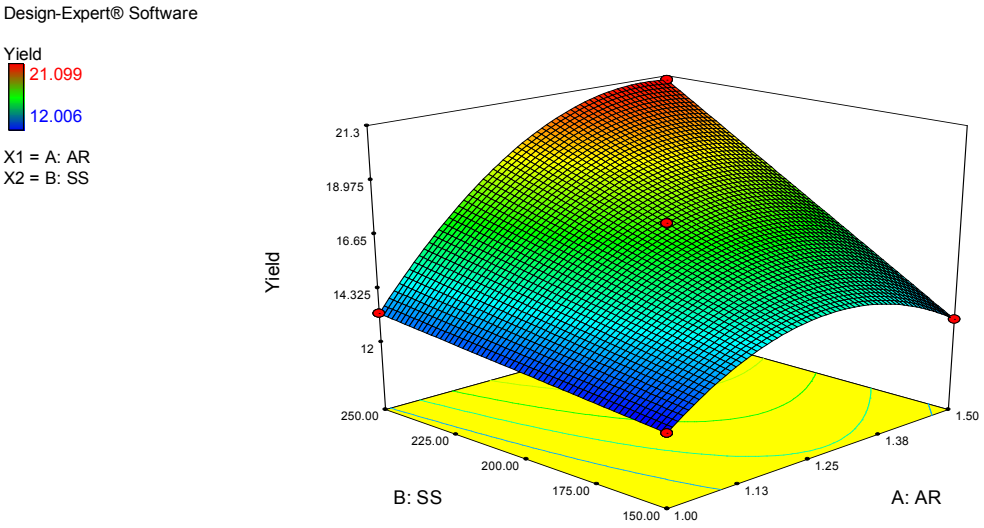


Figure 5-3 Response Surface Plot for the Effect of Aeration Rate (*AR*) and Stirrer Speed (*SS*) on Yield

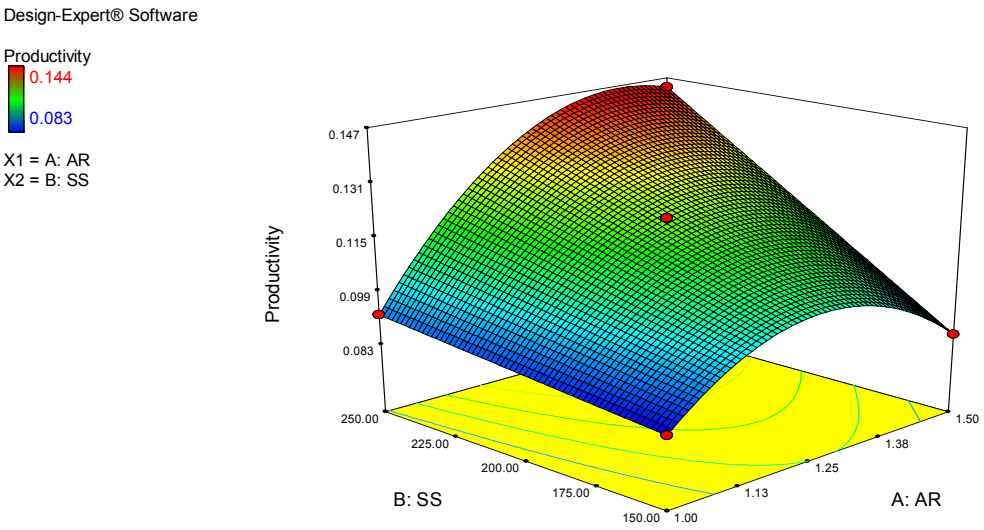


Figure 5-4 Response Surface Plot for the Effect of Aeration Rate (*AR*) and Stirrer Speed (*SS*) on Productivity

The 3D surface plots of yield and productivity show that both yield and productivity are affected by aeration rate (*AR*) and stirrer speed (*SS*) respectively. Based on the surface plots, yield and productivity increase with an increase in aeration rate (*AR*) and stirrer speed (*SS*). Thus, both aeration rate (*AR*) and stirrer speed (*SS*) influence the level of both yield and productivity of the batch fermentation process. Yield increases when aeration rate (*AR*) increases from 1.0LPM and peaks at 1.43LPM, whereas stirrer speed (*SS*) increases from 150rpm and hits the highest point at 250rpm.

In order to obtain maximum yield and productivity, the optimum conditions for both aeration rate (*AR*) and stirrer speed (*SS*) are suggested at **1.43LPM** and **250rpm**. With these optimum conditions, it is suggested that the **maximum yield is 21.150% and maximum productivity is around 0.150g/L.hr**. It is indicated that 96.6% desirable that these optimum values could achieve maximum yield and productivity. Thus, these optimum values of aeration rate (*AR*) and stirrer speed (*SS*) are recommended to achieve maximum yield and productivity. Based on the results obtained, both aeration rate (*AR*) and stirrer speed (*SS*) have significant effects on the bioreactor performance. Thus, the kinetics hybrid model demonstrates good predictions of yield and productivity by considering both aeration rate (*AR*) and stirrer speed (*SS*) in the kinetics hybrid model.

5.3.3 Kinetics Multi-Scale Model

5.3.3.1 Statistical Analysis

The statistical analysis of the kinetics multi-scale model is done predict the optimum conditions of both aeration rate and stirrer speed to achieve maximum yield and productivity. The ANOVA results of the model fitting are shown in Table 5-6 for yield.

Table 5-6 ANOVA Results for CCD on Yield

Source	**Sum of Squares**	**Degree of Freedom**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	52.65	5	10.53	7.10	0.0115	Significant
Residual	10.38	7	1.48			
Lack of Fit	6.75	3	2.25	2.48	0.2003	Not Significant
Pure Error	3.63	4	0.91			

As shown in Table 5-6, it is indicated that the kinetics multi-scale model is highly significant, as it is evident from the Fisher's *F* test (F_{model} = 7.10) and a low "Prob > F" value ($P_{model} > F$ = 0.0115). This analysis indicates that both aeration rate (*AR*) and stirrer speed (*SS*) have significant effects on yield since the "Prob > F" is less than 0.05. On the other hand, the Lack of Fit is not significant relative to the pure error when "Prob > F" = 0.0115 > 0.05, also supports the fitness of the model. The value of the determination coefficient (R^2 = 0.8353) indicates that 83.53% of the sample variation in yield is attributed to the independent variables. Thus, this model is statistically ample for the prediction of yield within the experimental setting range.

Next, the interpretation of ANOVA results for productivity is vital as well. Table 5-7 shows the ANOVA for productivity, whereby the ANOVA of the model demonstrate that the model is highly significant, and is evident from the Fisher's *F* test (F_{model} = 4.74) and a low "Prob > F" values ($P_{model} > F$ = 0.0328).

Table 5-7 ANOVA Results for CCD on Productivity

Source	**Sum of Squares**	**Degree of Freedom**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	0.016	5	3.144E-3	4.74	0.0328	Significant
Residual	4.641E-3	7	6.630E-4			
Lack of Fit	3.159E-3	3	1.053E-3	2.84	0.1693	Not Significant
Pure Error	1.481E-4	4	3.703E-4			

The Lack of Fit is not significant relative to the pure error when "Prob > F" = 0.1693 > 0.05, also supports the fitness of the model. This analysis indicates that both aeration rate (*AR*) and stirrer speed (*SS*) have significant effects on productivity since the "Prob > F" is less than 0.05. The value of the determination coefficient (R^2 = 0.8021) indicates that the fit degree of the model is high. Thus, this model can be applied to predict the productivity within the experimental setting range. Therefore, the model is statistically adequate for productivity prediction. A check of the response surface plots can be done which is available at Appendix D.3, to further investigate the adequacy of the developed kinetics multi-scale model.

5.3.3.2 Analysis of Response Surfaces

In the analysis of the response surfaces of both yield and productivity, both 3D surface plots of yield and productivity are made as a function of aeration rate (*AR*) and stirrer speed (*SS*). The effect of both variables is illustrated in Figure 5-5 and Figure 5-6.

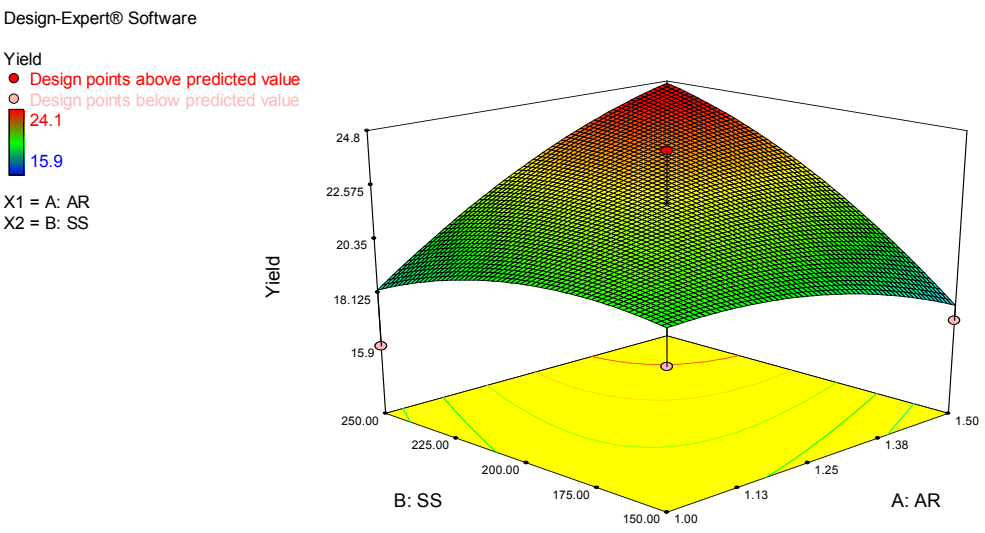


Figure 5-5 Response Surface Plot for the Effect of Aeration Rate (*AR*) and Stirrer Speed (*SS*) on Yield

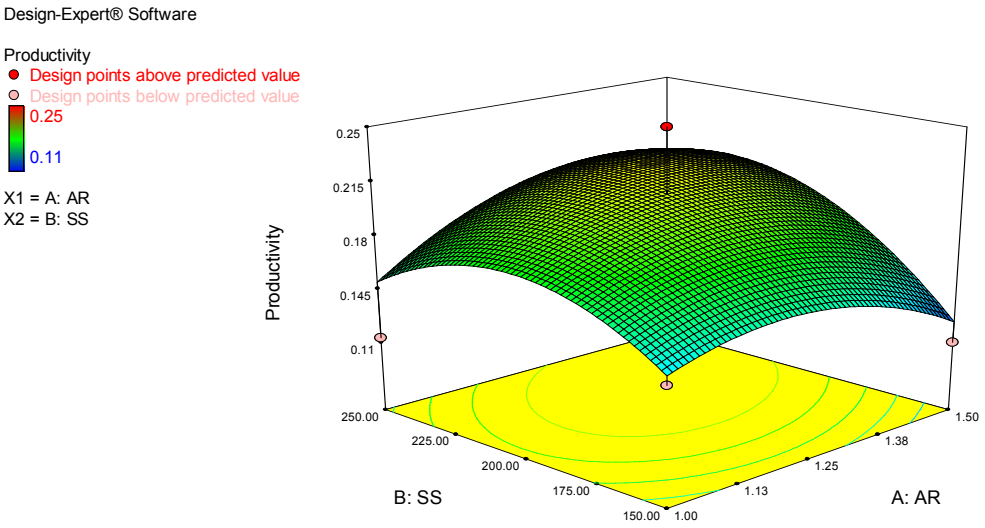


Figure 5-6 Response Surface Plot for the Effect of Aeration Rate (*AR*) and Stirrer Speed (*SS*) on Productivity

The responses show that both yield and productivity are affected by both aeration rate (*AR*) and stirrer speed (*SS*) respectively. Based on the surface plots, yield and productivity increase with an increase in aeration rate (*AR*) and stirrer speed (*SS*). Thus, both aeration rate (*AR*) and stirrer speed (*SS*) influence the yield and productivity of the fermentation process. Within the experimental range, the

suggested optimum values of aeration rate (*AR*) and stirrer speed (*SS*) to maximize yield are **1.45LPM *AR*** and **240rpm *SS***. Under these conditions, it is expected to **yield a maximum of 24.128%** ethanol and **produce a maximum of 0.207g/L.hr** ethanol. It is indicated that 81.3% desirable that these optimum values could be able to achieve maximum yield and productivity.

Yield is found to increase with the increase in stirrer speed (*SS*) from 150rpm and reach its peak at 240rpm. On the other hand, there is a significant increase in yield with increase in aeration rate (*AR*). It reaches optimum at 1.45LPM showing less significant difference in yield thereafter. The trend of the 3D mesh generated for productivity is found to be different to that of yield. The productivity is found to increase with the increase in aeration rate (*AR*) and stirrer speed (*SS*) but there is a drop in activity with further increment in aeration rate (*AR*) and stirrer speed (*SS*), i.e. to 1.5LPM and 250rpm. It reaches optimum showing less significant difference in productivity. Based on the results obtained, both aeration rate (*AR*) and stirrer speed (*SS*) have significant effects on the bioreactor performance. Thus, the kinetics multi-scale model demonstrates good predictions of yield and productivity by considering both aeration rate (*AR*) and stirrer speed (*SS*) in the model.

5.3.4 Model Validation

Model validation is important to be performed once optimization has been conducted. The goal is to check the results of the response surface experimentally, in order to ensure that the suggested optimum conditions are valid. The methods and experimental procedures conducted are similar to previous experiments presented in Chapter 3 for consistency.

Table 5-8 and Table 5-9 show the summary results of the optimum aeration rate (*AR*) and stirrer speed (*SS*) as well as the maximum predicted yield and productivity for each model.

Table 5-8 Summary of Model Predicted and Experimental Verified Results for Yield

Model	**Optimum Aeration Rate (LPM)**	**Optimum Stirrer Speed (rpm)**	**Model Predicted Maximum Yield (%)**	**Experimental Verified Maximum Yield (%)**	**Error (%)**
Data-Based	1.47	242	24.495	23.720	3.164
Kinetics Hybrid	1.43	250	21.150	20.950	0.946
Kinetics Multi-Scale	1.45	240	24.128	24.570	1.799

Table 5-9 Summary of Model Predicted and Experimental Verified Results for Productivity

Model	**Optimum Aeration Rate (LPM)**	**Optimum Stirrer Speed (rpm)**	**Model Predicted Maximum Productivity (g/L.hr)**	**Experimental Verified Maximum Productivity (g/L.hr)**	**Error (%)**
Data-Based	1.47	242	0.198	0.185	6.566
Kinetics Hybrid	1.43	250	0.150	0.148	1.333
Kinetics Multi-Scale	1.45	240	0.207	0.210	1.429

Based on the summarized results tabulated above, the kinetics hybrid model predicts the least amount of yield and productivity despite having the lowest percentage error. Interestingly, the kinetics multi-scale model could predict the maximum yield and productivity although higher percentage error is measured for both experimental and model predicted data. Therefore, further analysis is to be conducted statistically in order to decide which kinetics model is suitable to be utilized for maximum yield and productivity prediction. Table 5-10 presents the summary of the Prob > F values for all the three models.

Table 5-10 Summary of "Prob > F": Values for Yield and Productivity

Model	**Yield**	**Productivity**
Data-Based	0.0047	0.0054
Kinetics Hybrid	0.0115	0.0022
Kinetics Multi-Scale	0.0115	0.0328

Based on the statistical results obtained, the kinetics hybrid measures lower "Prob > F" value for both yield and productivity as compared to that of the kinetics multi-scale model. These predictions show that the kinetics hybrid model offer better predictions compared to kinetics multi-scale model. Even though the "Prob > F" value for data-based model is lowest among the three models, however it would be more practical to utilize the kinetics model. This is because the kinetics model describes the combined mechanistic information about the fermentation process in the form of general mass balance expressions and kinetics obtained from experiments.

From this point of view, the kinetics Monod expression would be a preferable choice for optimization. The input and output relationships are formulated in such a way that the kinetics hybrid model can be fitted to the experimental data. Further, in practical terms, a kinetics hybrid model is easier to evaluate than the complicated kinetics multi-scale model [79]. Thus, it would not be necessary to evaluate such complex model to study the effect of aeration rate and stirrer speed on bioreactor performance, as this would save computational burden. Therefore, the implementation of the kinetics hybrid model by using CCD approach is sufficient to

obtain the optimum conditions of both aeration rate and stirrer speed to achieve maximum bioreactor performance.

5.4 CONCLUSIONS

As a conclusion, the optimization problem formulated is to achieve maximum bioreactor performance with the account of both aeration rate and stirrer speed in batch bioreactor. Optimization results present satisfactory optimal solutions for both yield and productivity. The incorporation of a more detailed kinetics model alleviates the need for constant yield and productivity coefficients and parameterization of cellular growth into distinct pathways [80]. Based on the optimal results:

- CCD approach is effective in determining the optimum conditions of both aeration rate and stirrer speed to achieve maximum bioreactor performance. ANOVA results for all three modeling approaches show significance and adequacy in each model proposed.
- The kinetics hybrid model is suggested to be utilized for maximum yield and productivity predictions by using the optimum conditions predicted. This model is suggested due to its simplicity in model construction and better accuracy. On the other hand, there are significant effects of aeration rate and stirrer speed on bioreactor performance based on statistical analysis. Thus, both aeration rate and stirrer speed are important to be taken into account to study its effect on bioreactor performance and future predictions.

Based on the optimization analysis, it is observed that both aeration rate and stirrer speed have significant effects on yield and productivity, i.e. bioreactor performance. Due to the accuracy of the kinetics hybrid model in bioreactor performance prediction, this model shows potential in the application of control strategy in batch bioreactor. The optimum conditions of aeration rate and stirrer speed will be considered as input variables for the control of the bioreactor performance since aeration rate and stirrer speed show significant effects on the bioreactor performance. Investigations in control strategy will be further discussed in the next chapter.

Chapter 6 Bioreactor Control Strategy

6.1 INTRODUCTION

The objective of this chapter is to explore the potential and practicability of the proposed models for control strategy of bioreactor. The optimum conditions of both aeration rate and stirrer speed are considered as input variables to achieve maximum bioreactor performance. Considering these input variables, a nonlinear model-based controller is designed, with the implementation of the proposed models. Therefore, both aeration rate and stirrer speed will be the manipulated variables to control the bioreactor system in order to achieve maximum bioreactor performance and to maintain the desired bioreactor performance.

In this chapter, the control strategy designed is implemented into a continuous bioreactor system instead of a batch bioreactor system. It is implemented in such a way since continuous bioreactor is widely used in chemical and biological processes in the pharmaceutical, food and chemical industries. Therefore, it is suggested to investigate the viability of the proposed control strategy in a continuous bioreactor system first in order to ensure that this strategy is useful and practical to be implemented into the industry.

This chapter is divided into five sections. Section 6.2 outlines the control strategy scope and assumptions to study the effect of aeration rate and stirrer speed on bioreactor performance. Section 6.3 explains the control strategy approach to investigate the potential of aeration rate and stirrer speed in the control of bioreactor performance. The controller design specifications are proposed in this section. Section 6.4 outlines the case studies involved in the control strategy investigation. In

Section 6.5, the control strategy results for the case studies are analyzed. Finally, concluding remarks are presented at the end of this chapter.

6.2 SCOPE AND ASSUMPTIONS

In our study, the control design objective is to investigate the potential of the proposed models in the control of continuous bioreactor. Aeration rate and stirrer speed are considered as manipulated variables. Both feed concentration, *So* and dilution rate, *D* are considered as disturbance variables, since both interact to affect the microbial growth in the fermentation process [81]. Biomass, substrate and product concentrations as well as yield and productivity are considered as output variables.

Figure 6-1 outlines the feedback control of a continuous fermentation process with the implementation of aeration rate and stirrer speed as manipulated variables, whereby *sp* is the set-point of the output variables. The set-point measurements are the optimum conditions of both aeration rate (*AR*) and stirrer speed (*SS*) obtained.

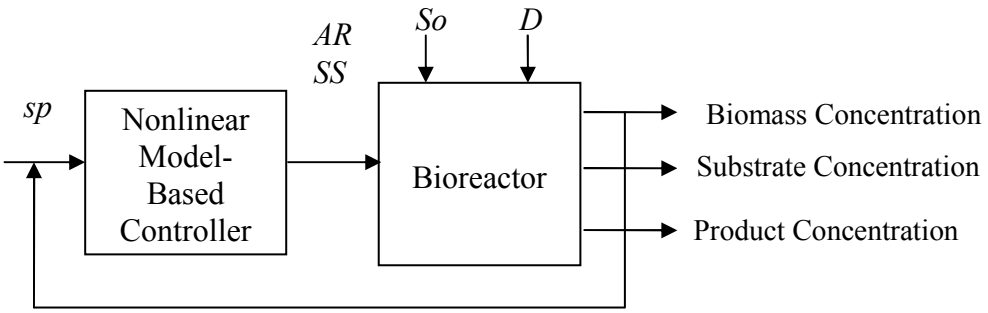


Figure 6-1 System Layout of Continuous Fermentation with the Implementation of Aeration Rate and Stirrer Speed as Input Variables

In our study, the data-based and kinetics hybrid models are employed in the nonlinear model-based controller. The control performances for both models are compared to investigate the potential of each model in the control of continuous bioreactor. It is of interest to investigate the potential of the kinetics model due to the versatility of the Monod's model. Thus, it is possible to control the system at any

steady state by manipulating the disturbance variables, i.e. feed concentration, *So* and the dilution rate, *D* [82].

The kinetics multi-scale model is considered as the bioreactor plant due to its best prediction on yield and productivity as explained in Chapter 5. The goal of these implementations is to investigate the potential of aeration rate and stirrer speed in controlling the bioreactor system to achieve maximum yield and productivity, i.e. bioreactor performance.

6.3 CONTROL STRATEGY APPROACH

In our study, Generic Model Control (GMC) is suggested for feedback control to describe the nonlinear bioreactor performance. This control law emphasizes integral action, which can be motivated by the presence of unmeasured disturbances. The additional of integral action in nonlinear control designs can improve the robustness and disturbance rejection properties of the controller. On the other hand, error trajectory is also considered, as the choice of an estimation error trajectory also arises in the design of nonlinear control. Through this kind of control system, it is suggested that the nonlinear properties of the bioreactor system could be investigated by controlling the input variables, i.e. aeration rate and stirrer speed, with respect to manipulated variables. Therefore, it is important to design the controller based on the specifications required to be taken into account in this study. The controller design specifications for the bioreactor are discussed earlier in Chapter 2 (Section 2.3.3).

6.4 CASE STUDIES

With the proposed control strategy, two case studies are considered. Case study 1 involves a $\pm$10% step perturbation of disturbance variables to be employed to the bioreactor system. On the other hand, in case study 2, a $\pm$30% step perturbation of disturbance variables is considered. The steady state conditions for all the input, output and manipulated variables, used for both case studies are summarized in Table 6-1. These conditions are the optimum conditions obtained from the optimization of the kinetics hybrid model. These conditions are considered at the open-loop and closed loop dynamics.

Table 6-1 Summary of Steady State Conditions for All Variables

Description	**Steady State Conditions**
Yield	21.150%
Productivity	0.150g/L.hr
Biomass Concentration	30.0g/L solution
Substrate Concentration	48.0g/L solution
Product Concentration	5.2g/L solution
Aeration Rate (*AR*)	1.43LPM
Stirrer Speed (*SS*)	250rpm

6.5 RESULTS AND DISCUSSION

6.5.1 Open-Loop Dynamics

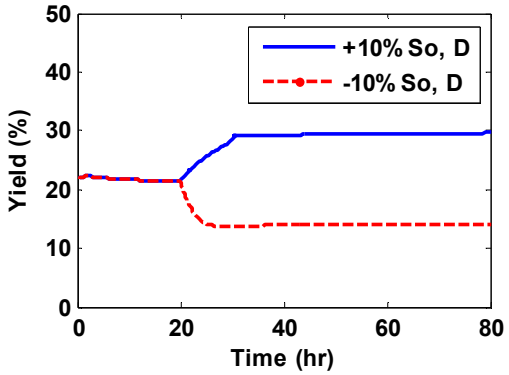
The open-loop dynamics of both case studies are investigated to analyze the robustness, stability and rough estimation of the input and output derivatives before proceeding to the closed-loop analysis, whereby GMC will be employed in the control structure.

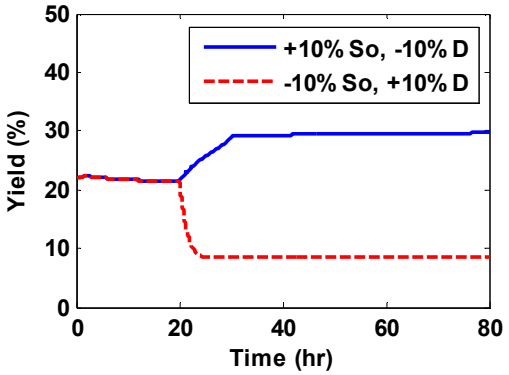
6.5.1.1 ±10% Step Perturbation Disturbance Variables (Case Study 1)

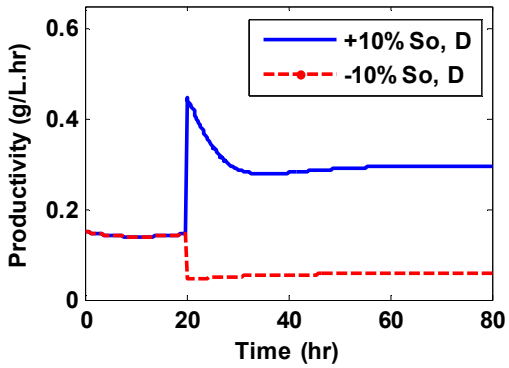
Table 6-2 shows the disturbance variables values after step perturbation of ±10%. The open-loop dynamics of the bioreactor performance are simulated based on the conditions presented in Table 6-2. Results of the open-loop dynamics are shown in Figure 6-2.

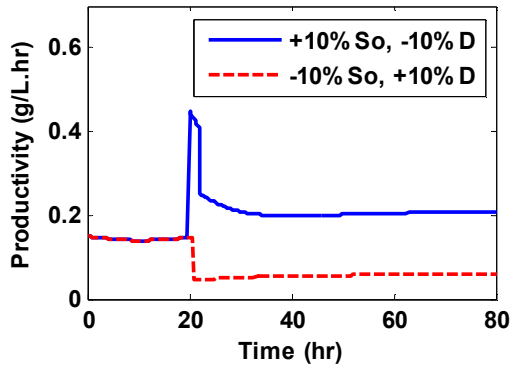
Table 6-2 ±10% Step Perturbation Values of Disturbance Variables (Case Study 1)

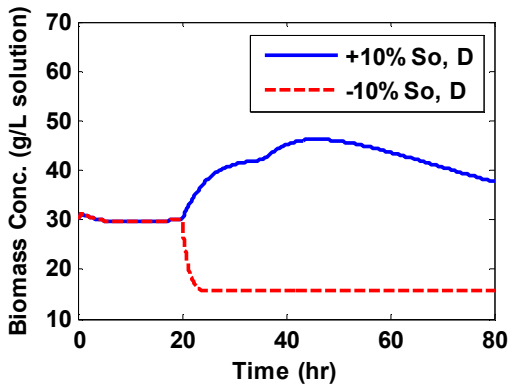
Description	**Up**	**Down**
Feed Concentration (S_0)	55	45
Dilution Rate (*D*)	1.1	0.9

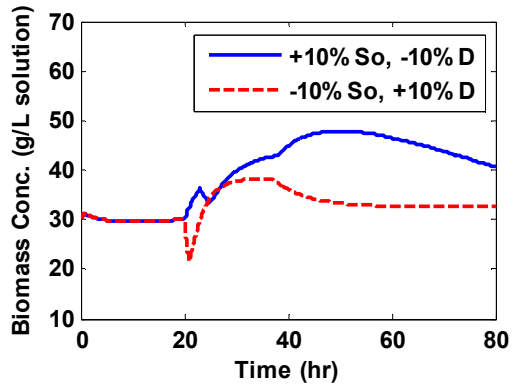

+10% So, D
-10% So, D
Yield (%)
Time (hr)

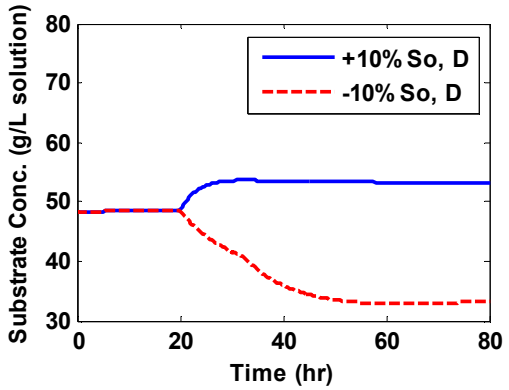

+10% So, -10% D
-10% So, +10% D
Yield (%)
Time (hr)

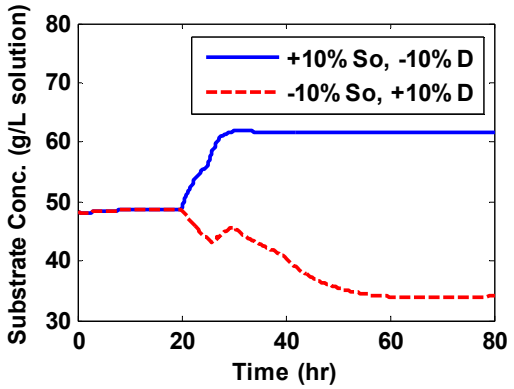

+10% So, D
-10% So, D
Productivity (g/L.hr)
Time (hr)


+10% So, -10% D
-10% So, +10% D
Productivity (g/L.hr)
Time (hr)


+10% So, D
-10% So, D
Biomass Conc. (g/L solution)
Time (hr)


+10% So, -10% D
-10% So, +10% D
Biomass Conc. (g/L solution)
Time (hr)


+10% So, D
-10% So, D
Substrate Conc. (g/L solution)
Time (hr)


+10% So, -10% D
-10% So, +10% D
Substrate Conc. (g/L solution)
Time (hr)

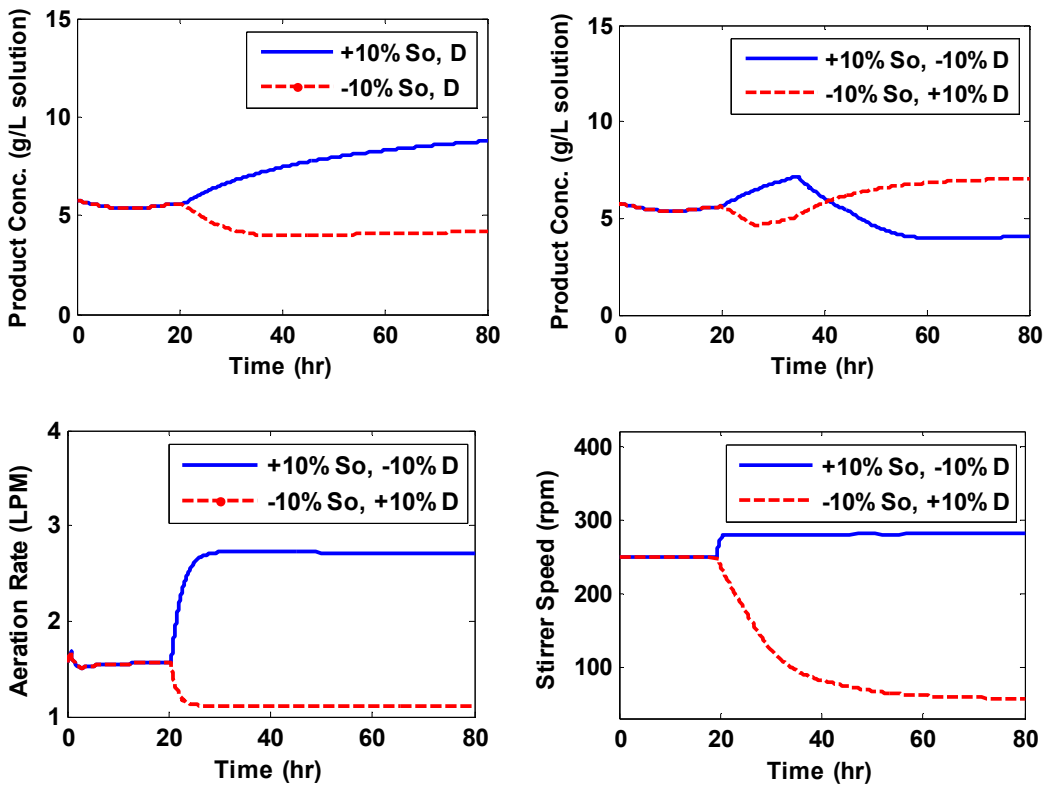


Figure 6-2 Open-Loop Dynamics of Yield, Productivity, Biomass Concentration, Substrate Concentration, Product Concentration, Aeration Rate and Stirrer Speed for ±10% Step Perturbation (Case Study 1)

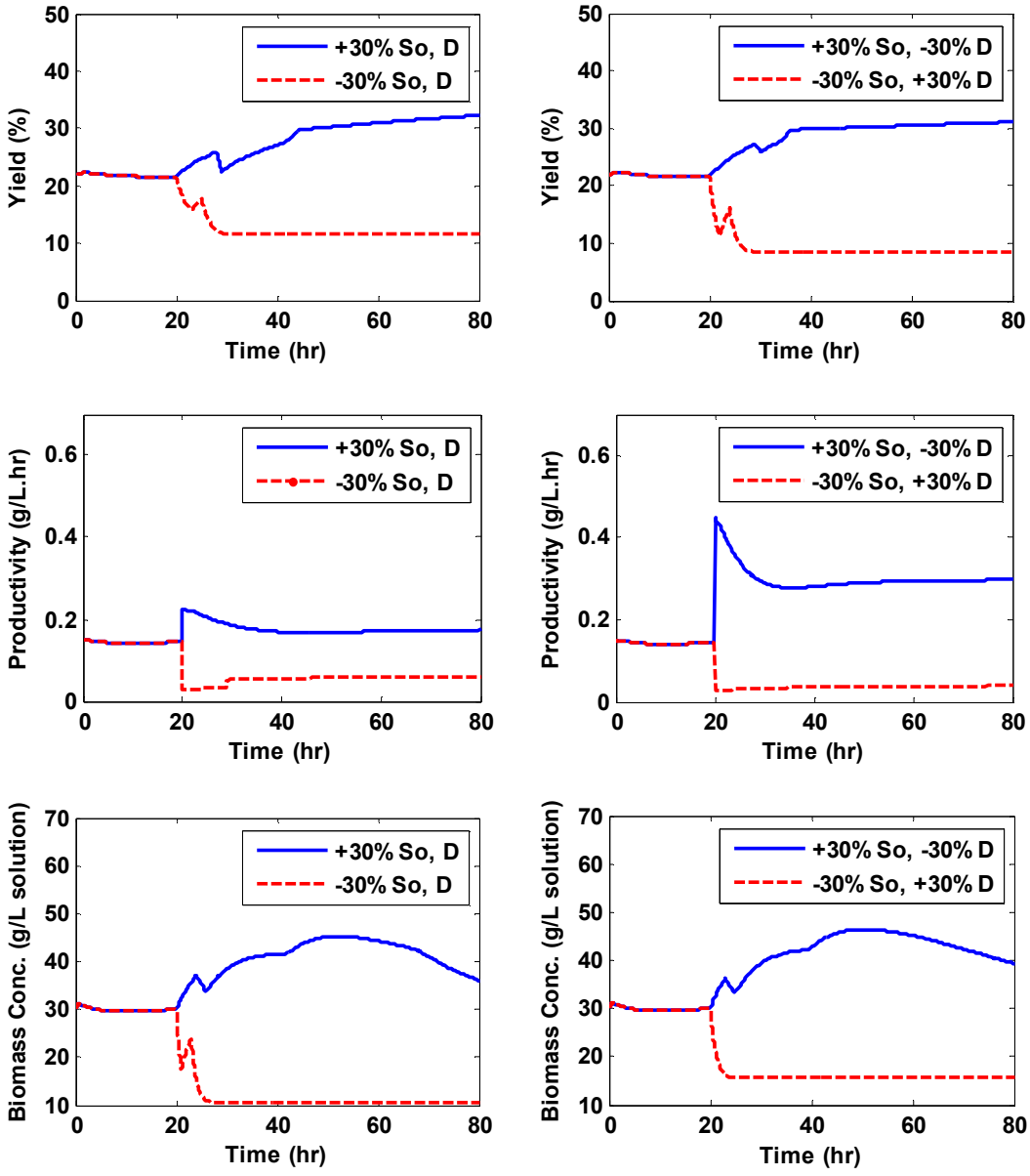
As observed in Figure 6-2, the dynamics of productivity is faster than yield. Based on the responses of the magnitude, yield and productivity can be controlled by manipulating the feed concentration (S_0) and dilution rate (D). Furthermore, the dynamics of substrate concentration is the fastest as compared to that of biomass and product concentrations. Only slight changes in substrate concentrations are observed after the initial period. Gradual dynamical observations can be seen during the 20th to 40th hour of the open-loop system for biomass concentration. These results show that the manipulation of dilution rate (D) have a big impact on the open-loop performances, especially for biomass, substrate and product concentrations during the 20th to 40th hour of the process. Thus, it is of interest to observe the closed-loop dynamics of the bioreactor system to investigate the potential of the nonlinear model-based controller to control the bioreactor system in order to achieve and maintain the desired conditions of the bioreactor.

6.5.1.2 ±30% Step Perturbation Disturbance Variables (Case Study 2)

Table 6-3 summarizes the step perturbation values of the disturbance variables for case study 2. Results of the open-loop dynamics are shown in Figure 6-3.

Table 6-3 ±30% Step Perturbation Values of Disturbance Variables (Case Study 2)

Description	**Up**	**Down**
Feed Concentration (S_0)	65	35
Dilution Rate (D)	1.3	0.7



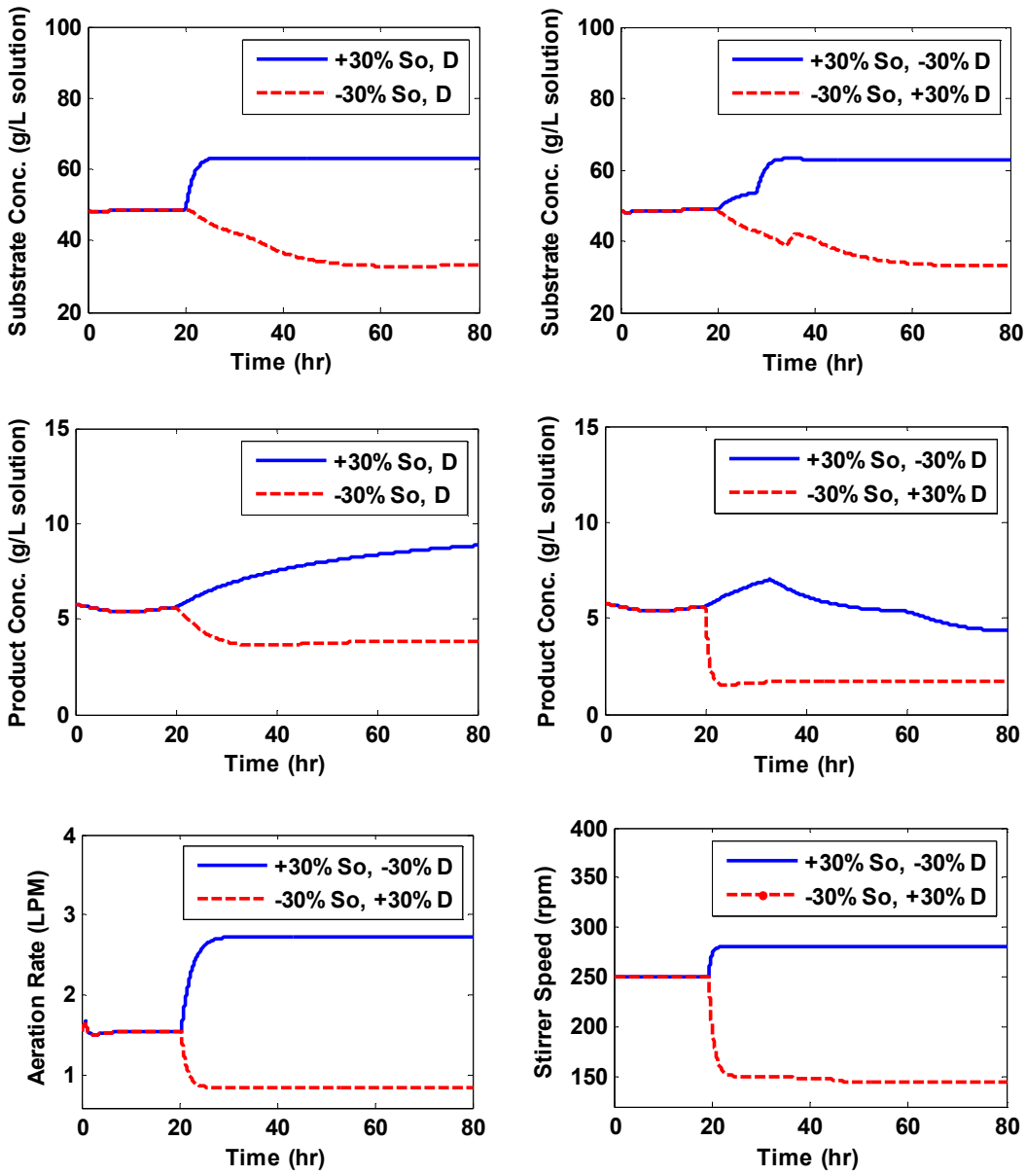


Figure 6-3 Open-Loop Dynamics of Yield, Productivity, Biomass Concentration, Substrate Concentration, Product Concentration, Aeration Rate and Stirrer Speed for ±30 Step Perturbation (Case Study 2)

As observed from Figure 6-3, the dynamics of productivity is faster than other variables, which is similar as case study 1. The productivity increases much more rapidly when dilution rate (D) is set at -30% compared to +30% while maintaining feed concentration (S_0) at +30%. On the other hand, not much dynamical changes is observed for productivity when feed concentration (S_0) was maintained at -30%. Thus, these results demonstrate that the decrease in dilution rate (D) gives large impact to the dynamics of productivity in the bioreactor system. Different

observations are seen for yield instead, whereby during the 20th to 40th hour, yield increases gradually and reaches steady state. The dynamics for dilution rate (D) at +30% is faster than -30% when feed concentration (S_0) is maintained at +30%. More dynamics is observed especially during the 20th to 40th hour of the bioreactor system as compared to the dynamics of case study 1. These results suggest that the percentage of step perturbation and the manipulation of dilution rate (D) have a big impact on the yield performance. For productivity, the dynamics is experiencing similar trend in regardless of the changes in step perturbation. Thus, both yield and productivity performances are highly dependent on the manipulation of dilution rate (D).

Apart from yield and productivity, the control of dilution rate (D) towards biomass, substrate and product concentrations affect the bioreactor performance as well. More dynamics is observed for biomass concentration during the 20th hour when dilution rate (D) is manipulated at -30%. When dilution rate (D) is manipulated at +30%, not much dynamical performances are observed. The biomass concentration decreases rapidly until reaches steady state. These show the need of controlling the bioreactor performance in order to achieve steady state conditions of the bioreactor. Therefore, it is crucial to investigate the effect of both aeration rate (AR) and stirrer speed (SS) in the control of bioreactor performance and the potential of the proposed nonlinear model-based controller in achieving the steady state bioreactor performance.

6.5.2 Closed-Loop Dynamics

The performances of the closed-loop dynamics are investigated and comparisons are made with the open-looped dynamics for both case studies.

6.5.2.1 ±10% Step Perturbation Disturbance Variables (Case Study 1)

The respective closed-loop dynamics of both data-based and kinetics hybrid models are shown in Figure 6-4.

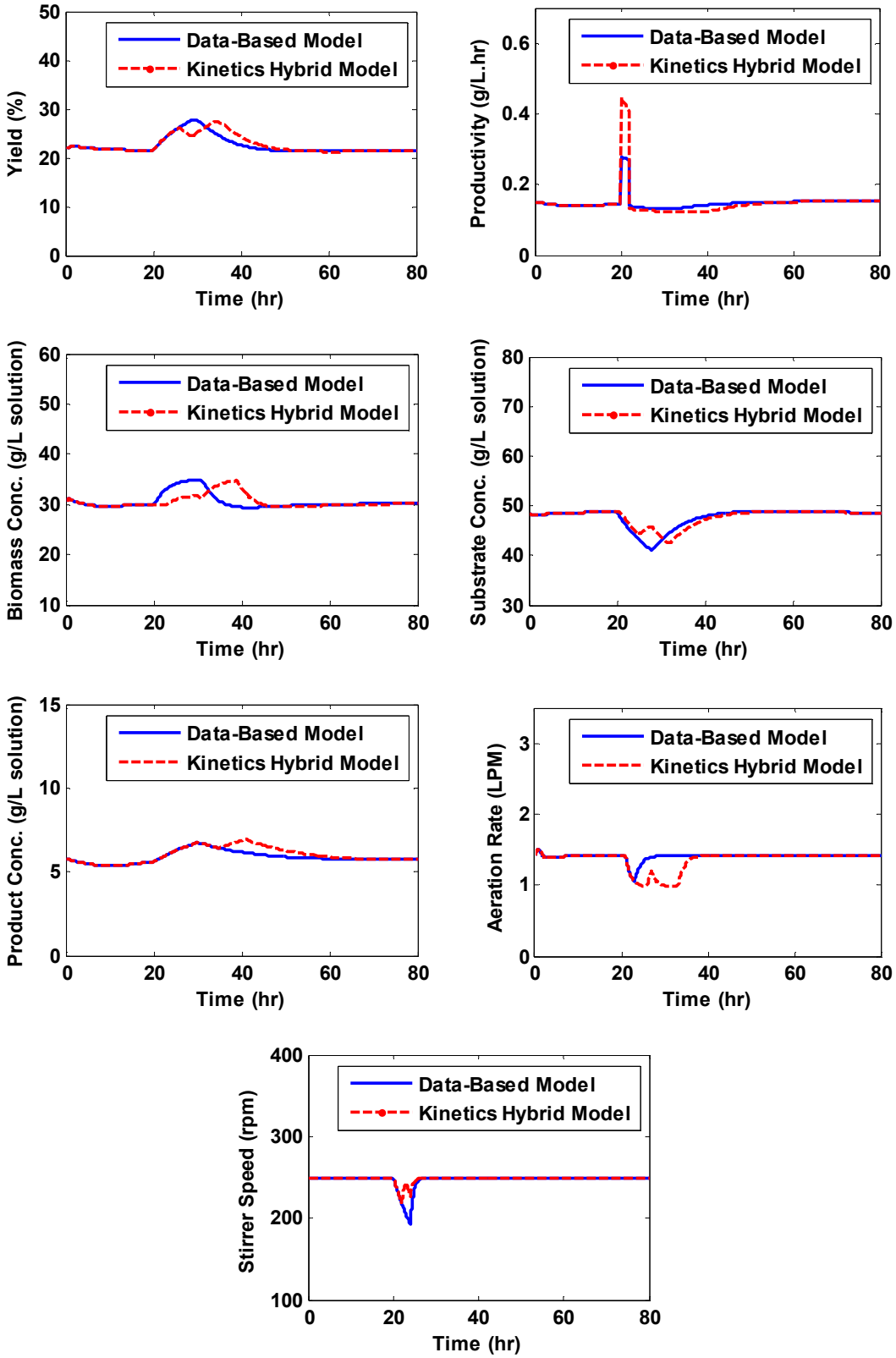


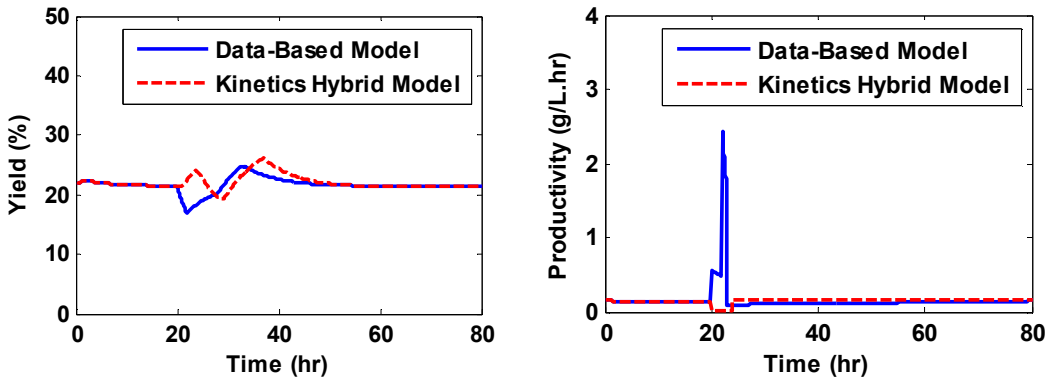
Figure 6-4 Closed-Loop Responses for Case Study 1 (+10% S_0, +10% D)

Results show that both controllers are able to achieve and maintain the output variables to their set-point values, by manipulating both aeration rate (AR) and stirrer

speed (*SS*). A step change of +10% is made to the feed concentration (S_0) and dilution rate (*D*) at the 20th hour. It is observed that both controllers perform well, whereby there are not much oscillation observed in the closed-loop dynamics of yield but more dynamics is observed for productivity. The kinetics hybrid model controller shows lesser oscillations with higher overshoot and requires longer time to return to the set-point. On the other hand, the performance of the simple data-based controller is slightly better.

Overall, both controllers are able to achieve and maintain the output variables to their set-point values for 10% changes. These results show that both aeration rate (*AR*) and stirrer speed (*SS*) can be operated at the suggested optimum conditions to achieve desired yield and productivity. Thus, both aeration rate (*AR*) and stirrer speed (*SS*) have significant effects on the control of bioreactor performance to achieve desired control performance.

Further analysis is done for other responses in order to investigate the potential of both data-based and kinetics hybrid models in the control performance of bioreactor. The closed-loop analysis is done for the responses under (+10% S_0, -10% *D*) which are shown in Figure 6-5.



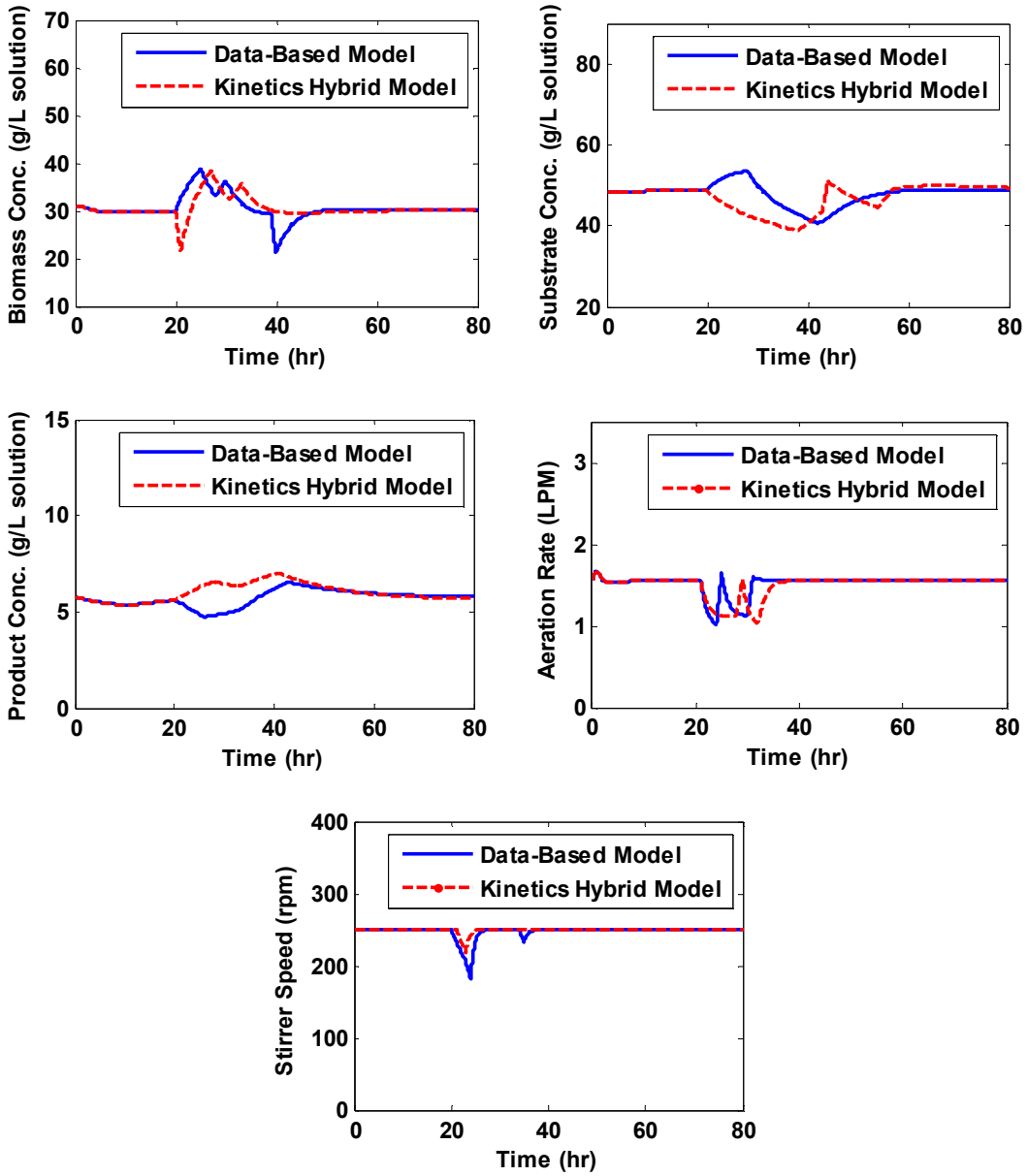
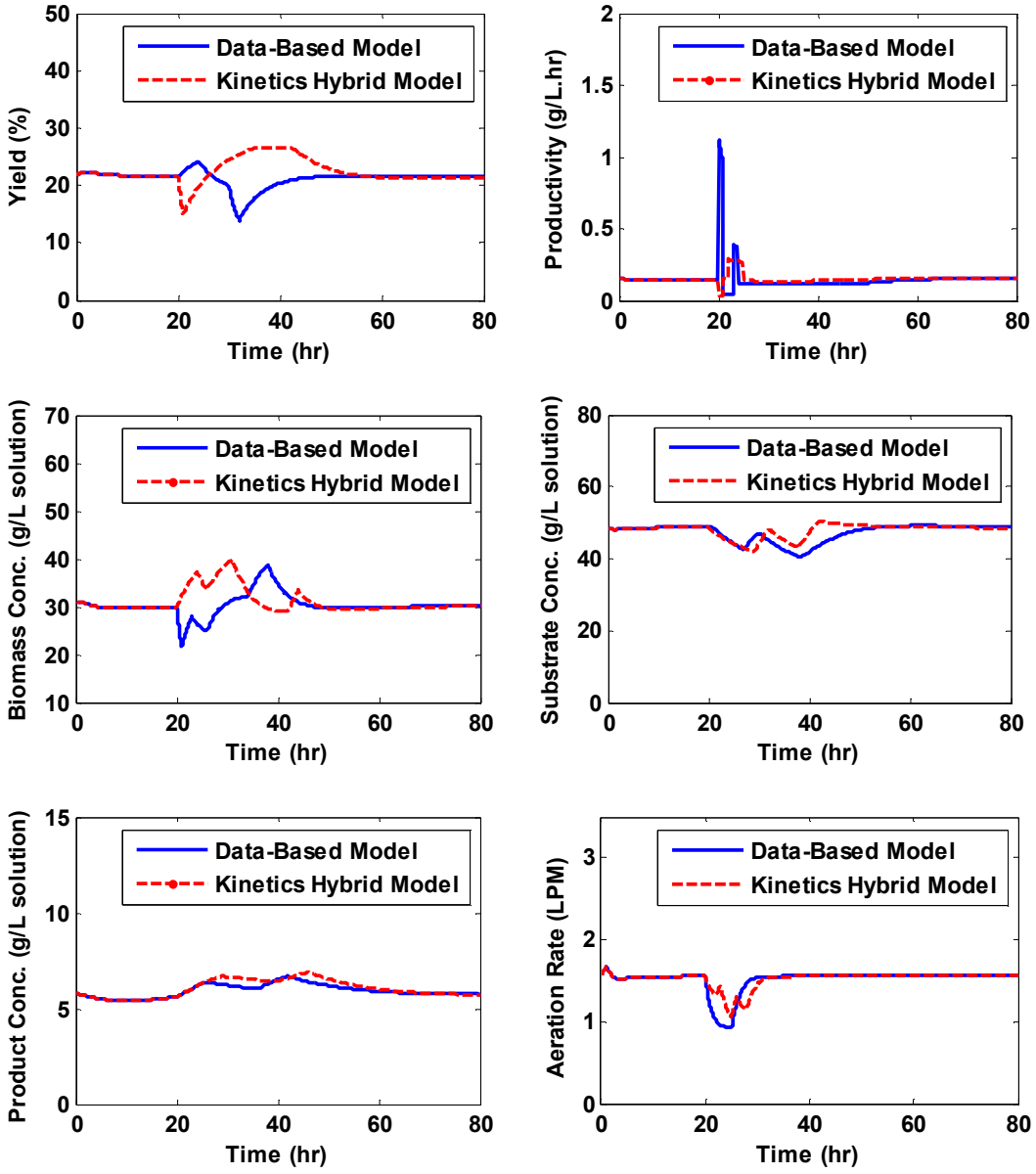


Figure 6-5 Closed-Loop Responses for Case Study 1 (+10% S_0, -10% D)

Results indicate that both controllers are able to maintain the output variables to their set-point values by the manipulation of aeration rate (AR) and stirrer speed (SS). But more dynamics is observed for each variable compared to that of the previous case (+10% S_0, +10% D). Both the data-based and kinetics hybrid model controller perform more aggressively before reaching steady state during the 20th hour. On the other hand, the data-based model controller demonstrates and shows better performance in achieving higher productivity. The kinetics hybrid model controller does not perform well as compared to that of the data-based model controller. More oscillations are observed for the kinetics hybrid model controller. More time is

required to return to the set-point, especially for substrate concentration, whereby it reaches the set-point at the 80th hour. Therefore, the data-based model controller performs better than the kinetics hybrid model controller. Figure 6-6 shows the closed-loop responses of (-10% S_0, +10% D).



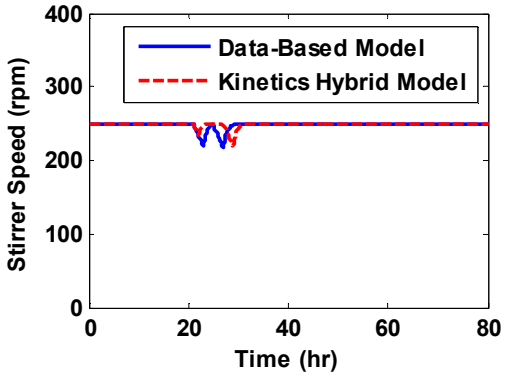


Figure 6-6 Closed-Loop Responses for Case Study 1 (-10% S_0, +10% D)

Overall, both data-based and kinetics hybrid model controllers are able to control the bioreactor performance with lesser oscillations. The simple data-based model controller demonstrates better performance as compared to that of the complex kinetics hybrid model controller. The dynamics of productivity is observed to be faster than other variables. This shows that the performance of productivity is highly influenced by the manipulation of feed concentration (S_0) and dilution rate (D).

As a whole, the data-based model controller performs better than the kinetics hybrid model controller. The manipulations of both feed concentration (S_0) and dilution rate (D), strongly influence the dynamics of the bioreactor system. Thus, aeration rate (AR) and stirrer speed (SS) play an important role in counterbalance the effects of both manipulated variables. The data-based model controls the effects and manages to reach set-point. Thus, with the use of nonlinear model-based controller, it is possible to assess the dynamic behaviour of the fermentation process and control the performance of bioreactor despite of the complicated system of the bioreactor [83].

6.5.2.2 ±30% Step Perturbation Disturbance Variables (Case Study 2)

Figure 6-7 shows the closed-loop responses for both data-based and kinetics hybrid model-based controllers for +30% step perturbation of disturbance variables.

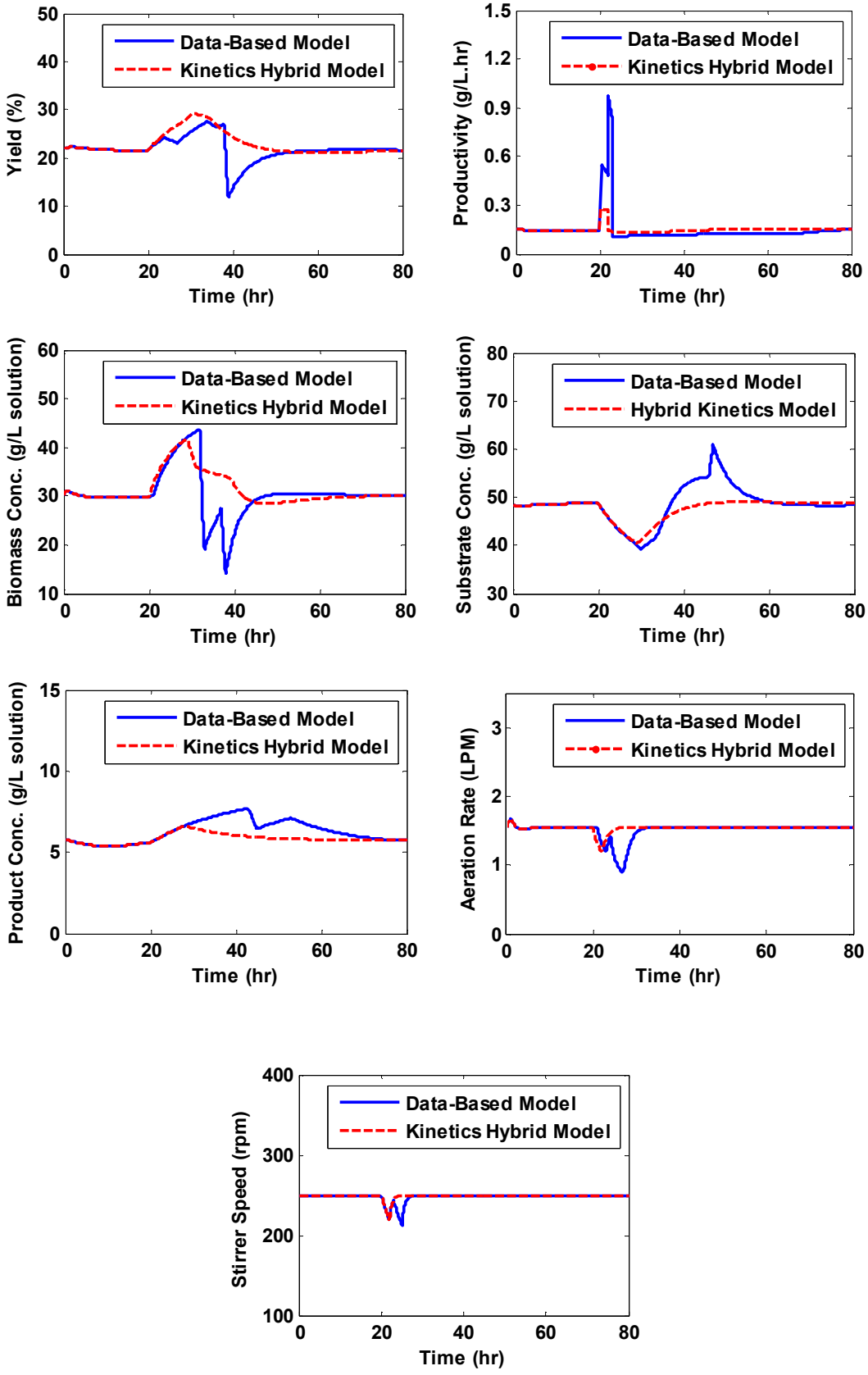
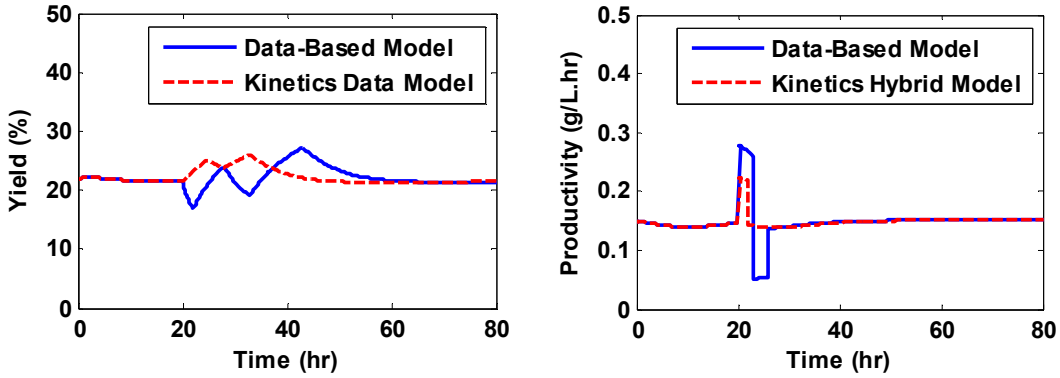


Figure 6-7 Closed-Loop Responses for Disturbance for Case Study 2 (+30% S_0, +30% D)

Overall, both controllers are able to maintain the output variables to their set-point values. However, the kinetics hybrid model controller performs much better than the data-based model controller. It is observed that the data-based model controller shows more oscillations and demands longer period of settling time to bring the process back to the set-point. Besides, higher overshoot is observed especially for yield, productivity, biomass concentration and substrate concentration.

Note that both aeration rate (*AR*) and stirrer speed (*SS*) hit their upper limits, indicating nonlinear dynamics of the bioreactor system. Due to the highly nonlinear dynamics of the bioreactor system, the kinetics hybrid model controller produces better closed-loop performance as compared to the data-based controller. The kinetics hybrid model controller has potential in controlling the bioreactor performance, especially when the fermentation process is experiencing more dynamics and nonlinearity as demonstrated by a higher step perturbation. This shows that the kinetics hybrid model could capture the nonlinear dynamics of the bioreactor system. However, if the manipulation effect is not "big", the simple data-based controller should be sufficient. To further investigate the dynamics of case study 2, further analysis is done under the settings of (+30% S_0, -30% D) which are shown in Figure 6-8.



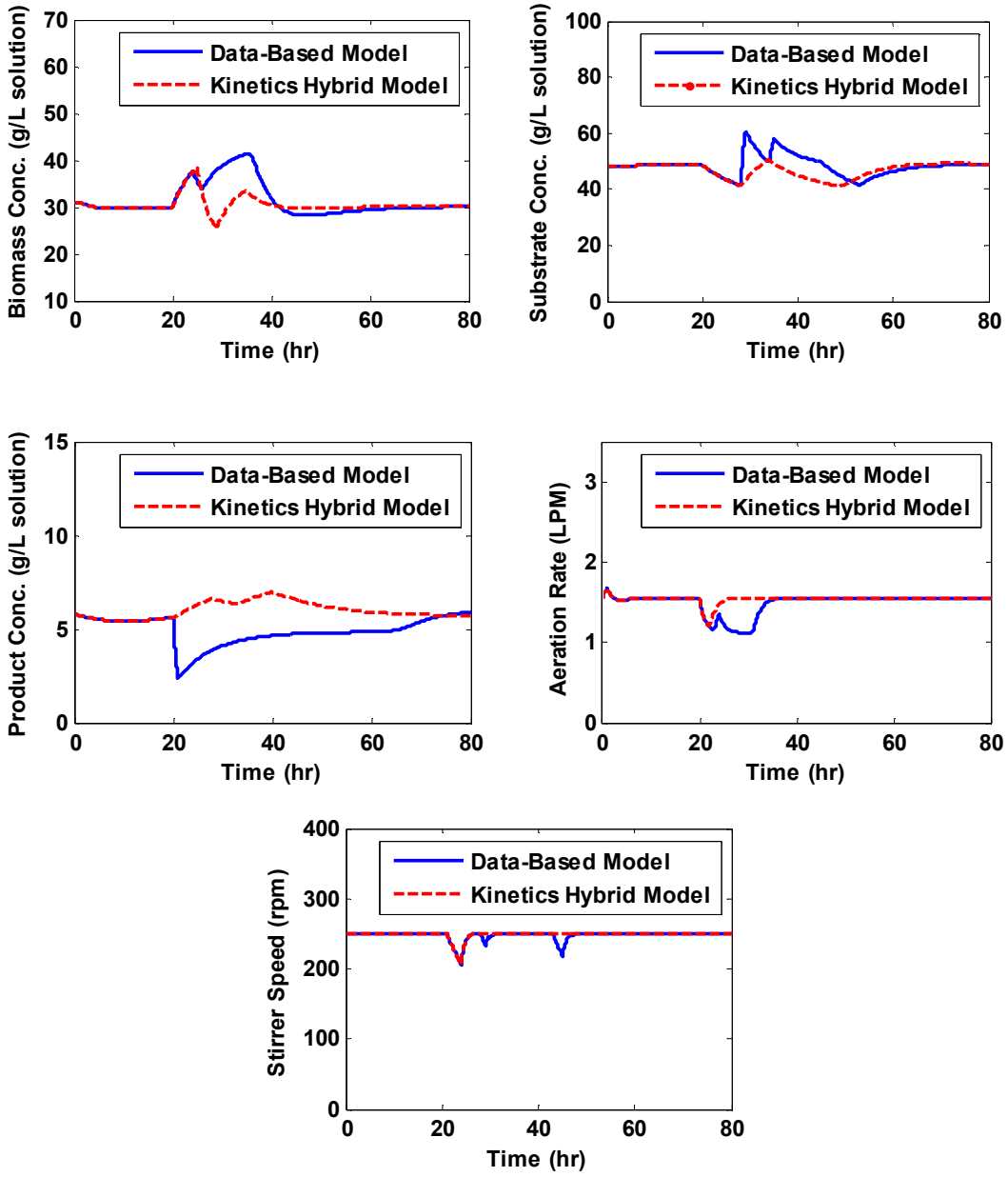
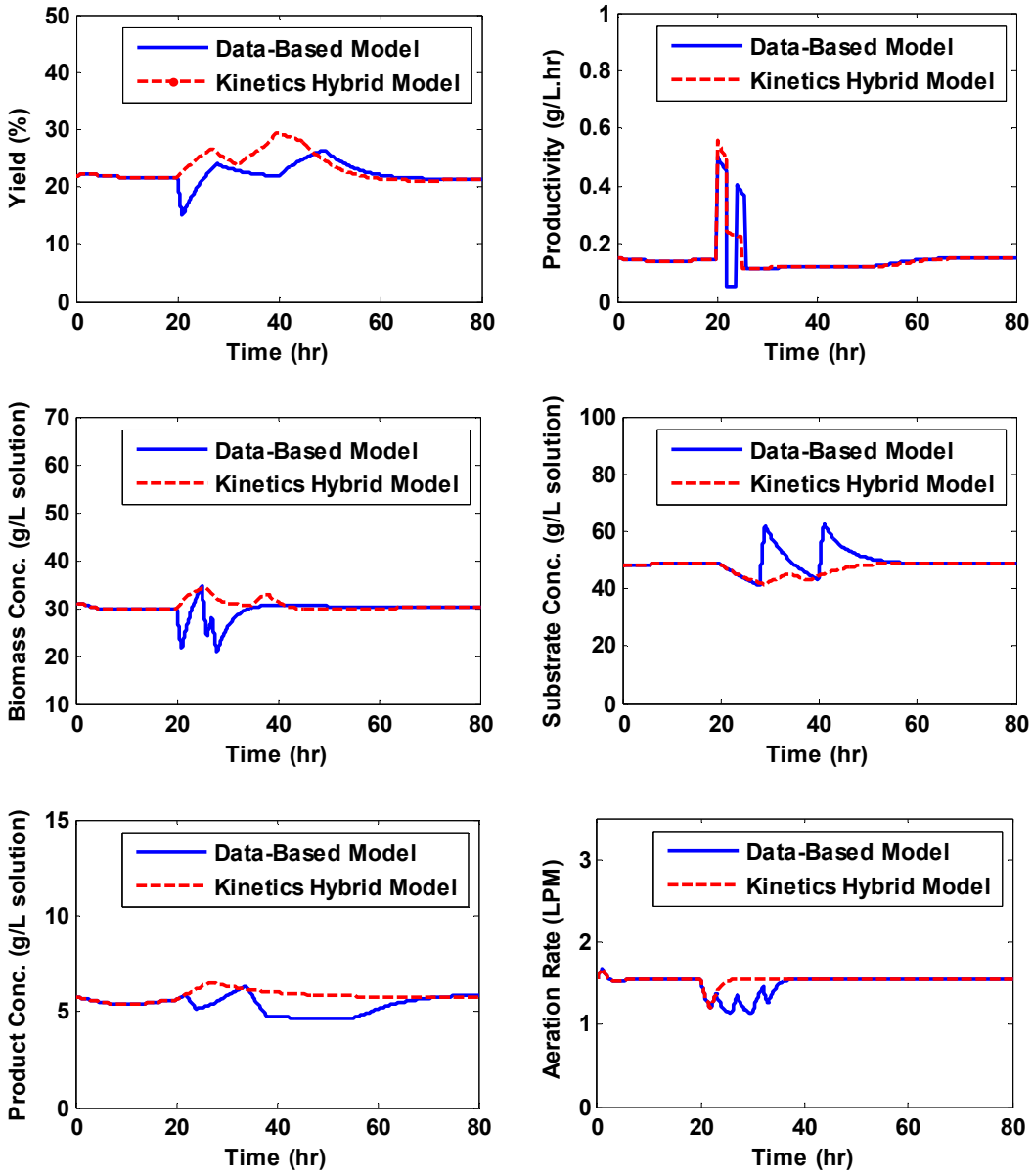


Figure 6-8 Closed-Loop Responses for Disturbance for Case Study 2 (+30% S_0, -30% D)

In this case, results indicate that both controllers are also able to maintain the output variables to their set-point values. The kinetics hybrid model controller shows lesser oscillations and reaches to the set-point faster than the data-based model controller. These results show the ability of the kinetics hybrid model controller in responding to the manipulation of feed concentration (S_0) and dilution rate (D), thus affecting the behaviour of the controller. The tuning procedure is able to adjust the behaviour of the output variables, avoiding oscillations.

Finally, Figure 6-9 shows the dynamical performances of the data-based and kinetics hybrid model controllers under the settings of (-30% S_0, +30% D).



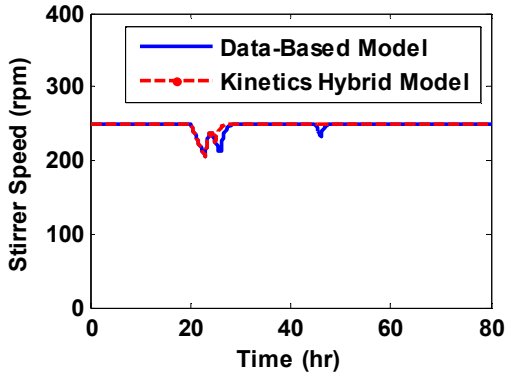


Figure 6-9 Closed-Loop Responses for Disturbance for Case Study 2 (-30% S_0, +30% D)

All variables show more dynamics under the control of the data-based model controller. It is suggested that the kinetics hybrid model controller offers better performance for higher step perturbation. More oscillations are observed at higher step perturbation, but the set-points are able to be achieved within the process time. It is recommended that the data-based model performs better in lower step perturbation and the kinetics hybrid model provides better control performance in higher step perturbation. These show that it is necessary to intricate suitable control strategies to deal with different conditions of manipulated and output variables in order to predict the dynamic behaviour of the complex bioreactor system [83]. Different perturbations provide different kinetics and dynamics into the bioreactor system. Thus, it is suggested to engage nonlinear control to achieve the best control performance. Especially for fermentation process, the process is highly nonlinear due to the complexity of the microbial activities. Therefore, the implementation of both integral action and error trajectory offer improved nonlinear model-based controller designs.

In addition, the control strategy proposed demonstrates that both feed concentration (S_0) and dilution rate (D) have significant effects on the bioreactor performance. Both data-based and kinetics hybrid models could be used to control the bioreactor performance. Both controllers are able to regulate the operating conditions in order to accommodate the perturbations with the lowest possible alterations in the process outputs. Thus, the proposed control strategy is useful in treating the highly nonlinear bioreactor system.

6.6 CONCLUSIONS

A new model-based control design for non-ideally mixed fermentation process has been presented, whereby models with different complexity are employed for the controller design. Both feed concentration (S_0) and dilution rate (D) are considered as disturbance variables. Studies have revealed that:

- Model-based nonlinear control strategy, i.e. Generic Model Control (GMC) has been incorporated directly into a controller structure, whereby it is suitable to be implemented into a fermentation process [84]. The controller structure is simple, disturbances can be compensated easily and the controller parameters can be easily tuned [85], in regardless of the complexity of the fermentation process.
- The choice of the nonlinear controller would depend on the expected disturbances on the process. For a relatively small manipulation scenario, the data-based controller is sufficient. However, for a significantly large manipulation, the kinetics hybrid model controller is able to enhance the closed-loop performance. These show that it is necessary to perform suitable control strategies to deal with different conditions of disturbances and output variables in order to predict the dynamic behaviour of the complex bioreactor system.
- Both data-based and kinetics hybrid models are able to achieve and maintain the output variables to their set-point values, by manipulating both feed concentration (S_0) and dilution rate (D). Therefore, both aeration rate (AR) and stirrer speed (SS) play important role in counterbalance the effects of both manipulated variables. Thus, with the use of nonlinear model-based controller, it is possible to predict and control the dynamic behaviour of the continuous bioreactor system.

The proposed data-based and kinetics hybrid model in the control strategy of bioreactor show the importance in engaging aeration rate (AR) and stirrer speed (SS) in the modeling and control of bioreactor. Both aeration rate (AR) and stirrer speed (SS) play important role in counterbalance the effects of both manipulated variables. Therefore, there is potential of engaging the data-based and kinetics hybrid models in the control of bioreactor.

Chapter 7 Conclusions and Recommendations

7.1 CONCLUSIONS

This thesis has addressed the modeling and control strategies of a non-ideally mixed bioreactor for fermentation process. The presence of such modeling and control system has considerably improved the evaluations and implications of the bioreactor, whereby ideally mixed assumption has been implemented over the decades. These interactions have therefore been a key issue in modeling and control discussed in this thesis.

The main contribution of this thesis has been a systematic approach in modeling and control of non-ideally mixed bioreactor for an ethanolic micro-aerobic fermentation process. Both modeling analysis and control strategy design are performed in a systematic manner by following procedures proposed in Chapters 4, 5 and 6.

By using the procedures, three modeling approaches are proposed based on evaluations done experimentally. Both aeration rate and stirrer speed are taken into account in each modeling approach in order to describe the non-ideally mixed mechanism of the bioreactor. The interactions resulting from each approach are then analyzed to observe their effect on the overall achievable bioreactor performance. As a result of this analysis, the control strategy is designed in such a way that the proposed models are able to control the whole bioreactor performance. Hence, the challenge has been to investigate which proposed model will result in achievable bioreactor performance.

Results from this study has indicated that the kinetics hybrid model is the best model to control the overall bioreactor performance, satisfying the achievable yield and productivity of the fermentation process. Other contributions of this work are summarized as follows:

1. New approaches to non-ideally mixed bioreactor analysis in which the interactions of both aeration rate and stirrer speed are taken into account. This is because previous studies were only done on temperature and pH as manipulated variables.
2. The use of cassava as main substrate to measure the achievable yield and productivity as compared to that of glucose as main substrate. Cassava is able to achieve higher yield and productivity compared to glucose.
3. The use of Central Composite Design (CCD) in process optimization as indicators for optimum conditions determination for the proposed non-ideally mixed bioreactor models. CCD is simple to be utilized and effective in determining the optimum conditions for the fermentation process due to its flexibility.
4. Alternative control strategies to improve the overall bioreactor performance are investigated. Both aeration rate and stirrer speed are considered as input variables and controlled to achieve desirable yield and productivity. Non-ideally mixed bioreactor model is implemented into the control system to describe the nonlinear behaviour of the bioreactor. On the other hand, it is also used as controllers to achieve desirable bioreactor performance.

Furthermore, some insights into modeling and control strategy have been gained from the application of the proposed methodology to the two case studies: glucose and cassava substrates. These are summarized as follows:

7.1.1 Strategies Developed for Modeling and Control of Non-Ideally Mixed Bioreactor

- This study describes three modeling approaches potentially utilized in portraying the non-ideality of a mixing bioreactor, i.e. the data-based, kinetics hybrid as well as kinetics multi-scale model. Both aeration rate and stirrer speed are taken into account in each modeling approach in order to describe the non-ideally mixed mechanism of the bioreactor.

- Statistical results indicate that among the three modeling approaches, the data-based model is significant for both glucose and cassava substrates. The kinetics hybrid model and kinetics multi-scale model are significant for glucose substrate but not significant for cassava substrate. On the other hand, statistical results also show that by engaging both aeration rate and stirrer speed into the kinetics model, the existing Herbert's kinetics model is improved. Glucose and cassava substrates show different kinetic behaviours by fitting the respective experimental data into the kinetics model. Glucose substrate show good model fitting but for cassava substrate, it does not fit well into the kinetics model. Thus, only glucose is considered for the extension studies of kinetics multi-scale model buildup.
- The kinetics hybrid model shows good competence with the experimental data of glucose substrate. CFD simulations demonstrate the mixing behaviour within the bioreactor, whereby different conditions of aeration rate and stirrer speed influence the mixing mechanism. Thus, different conditions of aeration rate and stirrer speed contribute to the differences in dynamics and kinetics within the bioreactor. These differences show that both aeration rate and stirrer speed play important role in the non-ideally mixed mechanism of the bioreactor.
- Model-based nonlinear control strategy, i.e. Generic Model Control (GMC) has been incorporated directly into a controller structure, whereby it is suitable to be implemented into an alcoholic fermentation process [84]. The controller structure is simple, disturbances can be compensated easily and the controller parameters can be easily tuned [85], in regardless of the complexity of the fermentation process.

7.1.2 Experimental and Modeling Analysis of Glucose and Cassava Substrates

- Experimental results show that with the utilization of glucose as the main substrate, ethanol production is highest at the maximum settings of experimental range, i.e. at aeration rate of 1.5LPM and stirrer speed of 250rpm. It is observed that under these settings, approximately 24% of ethanol yield and 0.16g/L.hr productivity. On the other hand, for cassava as the main substrate, ethanol production is highest at the baseline settings, i.e.

at aeration rate of 1.25LPM and stirrer speed of 200rpm, with approximately 49% of ethanol yield and 0.99g/L.hr productivity. These results show that different substrates produced different amount of yield and productivity under different conditions of aeration rate and stirrer speed. Different conditions of aeration rate and stirrer speed demonstrated different mixing mechanism in order to achieve desired yield and productivity.

- Experimental and modeling analyses show that aeration can greatly improve the ethanol yield and productivity [66]; [86] as well as reduce the formation of by-product, i.e. glycerol [87]. On the other hand, low stirrer speed can lead to poor mixing in the bioreactor causing poor yield. In addition to that, excessively high stirrer speed can cause physiological stress to the microbial cells which in turn leads to poor yield [88];[89]. Thus, both aeration rate and stirrer speed affect the mixing mechanism in a bioreactor and directly affect the yield and productivity of the desired end products. It is important to incorporate both aeration rate and stirrer speed into process models in order to describe the non-ideally mixed mechanism of the bioreactor, as the traditional assumption of ideally mixed mechanism is no longer valid.

7.1.3 Optimization of Ethanolic Fermentation Process

- Central Composite Design (CCD) is effective in determining the optimum conditions for the fermentation process due to its flexibility and simplicity. Three modeling approaches which had been proposed, i.e. data-based, kinetics hybrid and kinetics multi-scale models are statistically analyzed and the optimum of each models are obtained.
- Statistical results show significance and adequacy in all the models proposed. This ensured that the optimization procedure could proceed and optimum results proposed are statistically accurate.
- Predicted and experimental/verified results are comparable for all three modeling approaches, thus this demonstrated the usefulness and efficiency of this method in optimization. It is suggested that the kinetics hybrid model give the most comparable results of maximum yield and productivity among the three proposed models. Thus, this model is suggested for the determination of both maximum yield and productivity due to its simplicity in

model construction, as compared to the data-based and kinetics multi-scale models.

7.1.4 Bioreactor Control Strategy

- The choice of the nonlinear controller would depend on the expected manipulations done on the process. For a relatively small manipulation scenario, the data-based controller is basically sufficient. However, for a significantly large manipulation effect, the kinetics hybrid controller is able to enhance the closed-loop performance.
- The manipulation of dilution rate has a big impact on the open-loop performances, especially for biomass, substrate and product concentrations for relatively small disturbance scenario. For large disturbance scenario, both yield and productivity are influenced by the manipulation of dilution rate instead.
- Both data-based and kinetic hybrid models are able to maintain the controlled variable in their set-point values, by manipulating both aeration rate and stirrer speed. On the other hand, the kinetics multi-scale model is used to describe the nonlinear behaviour of the bioreactor. Results show that both aeration rate and stirrer speed play an important role in counterbalance the effects of both manipulated variables, i.e. substrate concentration and dilution rate. The kinetics multi-scale model is able to demonstrate the nonlinearity of the bioreactor. Therefore, the mixing mechanism of the bioreactor is demonstrated despite of the nonlinear behaviour of the bioreactor. Thus, with the use of mathematical model, it is possible to assess the dynamic behaviour of the fermentation process. Both aeration rate and stirrer speed are important variables in describing the mixing mechanism of the bioreactor.

7.1.5 Evaluations and Implications of Present Study

- The combination investigations of aeration rate and stirrer speed could be employed to study the mixing behaviour of a bioreactor in order to explore the kinetics and dynamic behaviour of a mixing bioreactor.
- It is proven from experiments and statistical analysis that different aeration rate and stirrer speed provide significant differences in kinetics and mixing

behaviour of bioreactor. Oxygen supplied affects the metabolite formation since the reaction of product formation, i.e. ethanol, is dependent on oxygen [90];[91]. Agitation, on the other hand, improves the mass transfer characteristics with respect to substrates, products, byproducts and oxygen, which results in better mixing of the fermentation medium [91]. Thus, both aeration rate and stirrer speed are factors which affect a bioreactor's efficiency in supplying microbial cells with oxygen and mass transfer within the bioreactor by agitation. There is a need to describe the mixing mechanism of a fermentative bioreactor with respect to aeration rate and stirrer speed.

- Strategies developed in modeling and control of bioreactor is suitable in evaluating and investigating the non-ideality behaviour of the mixing mechanism within a fermentative bioreactor. Statistical results imply that the non-ideally mixed bioreactor models proposed are significantly appropriate and adequate in describing the mixing behaviour of a bioreactor. On the other hand, the control strategy developed show that the mixing mechanism of a fermentative bioreactor is able to be controlled. This could be done with the adjustments of both aeration rate and stirrer speed, in order to meet the desired set-points. The tolerable range of both aeration rate and stirrer speed is set in order to evaluate its effects on the kinetic and dynamic behaviours of the system.

7.2 RECOMMENDATIONS

In addition to some promising results, this study also indicates a number of challenging problems that needed further investigations. These issues are recommended for future work and summarized as follows:

1. Extend Kinetics Modeling and Control Analysis to Cassava Substrate

Although the strategies developed have been successful in evaluating the mixing mechanism of a bioreactor, investigation is not performed further for cassava substrate in kinetics modeling. This is due to the differences in chemical structure between glucose and cassava, as well as the more complicated composition of cassava. The metabolism and kinetics of cassava by *Saccharomyces cerevisiae* has yet to be investigated in depth to date. Thus, further work needs to be performed to

have a comprehensive understanding of cassava in the mixing mechanism and non-ideality of a bioreactor of an ethanolic fermentation process.

2. **Expand the Current Proposed Kinetics Models to a Number of Further Case Studies**

The optimization of the performance of the existing bioreactor requires screening of various mixing configurations. This would allow researchers to spend more time on evolving creative and innovative mixer configurations rather than validating and screening the established configurations [51]. It is suggested to conduct more studies in the future, especially in scaling up the bioreactor system so that the system could be utilized in practice in industry in the future.

In this research, three modeling approaches, i.e. data-based, kinetics hybrid and kinetics multi-scale models have been proposed. These three models have been implemented for control studies for continuous bioreactor. In this research, as the control strategy has only been tested by using simulations, it is suggested to conduct experimental verification of the proposed controller for further validation. On the other hand, further work could be considered to simulate more mathematical models in order to further improve the comprehensiveness and limitations of the models proposed in this research. The control studies can be extended for application in batch bioreactor. This would not only expand the tolerable range but also to widen the range of applications, for example, models proposed could be utilized in any operating conditions and substrates. This would not only save time in terms of computational burden, but also diversify the experimental applications to various substrates.

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Appendix A

Experimental Procedures and Analysis

A.1 Experimental Procedures (Glucose and Cassava Substrates)

In this study, two case studies are conducted by utilizing different substrate for each case study, i.e. glucose and cassava respectively. Basically, the experimental procedure for both glucose and cassava substrates are the same except that cassava is required to be hydrolyzed before experiment begins. Generally, for both glucose and cassava, 1.5 litre of the medium culture is prepared based on the medium formulation for each glucose and cassava, which is presented in Section 3.2.1.2 and Section 3.2.1.3. The medium formulation is based on Thatipamala, Rohani and Hill (1992) [92] whereby the culture medium is sterilized at 121^{o}C and subsequently to be cooled down under room temperature.

For glucose, the medium is sterilized for 20 minutes whereas for cassava, the medium is sterilized for 45 minutes to further breakdown the complex structure of cassava to simple glucose. 0.040 litre of inoculum is added to the fermentation medium (See Table 3-2 for glucose fermentation medium formulation). Temperature and pH conditions are maintained and controlled at 30°C and pH 5 respectively. The fermentation process is stopped after approximately 72 hours and the samples are taken in every 2-3 hours for biomass, substrate (glucose), product (ethanol) and byproduct (glycerol) concentrations analysis. Ethanol, glucose and glycerol are analyzed using R-Biopharm test kits and UV-spectrophotometer, right after samples

are taken from the medium culture. Analysis is done right after the samples are taken from the medium culture in order to avoid further contamination and to obtain better accuracy of measurements. All analytical methods are done according to the instructions stated in the R-Biopharm instruction guides, including the determination of wavelength to be set at the UV-spectrophotometer. The fermentation process is considered finished when the glucose concentration is less than 5g/L at the end of the process, whereby the microbial cells are subjected to death.

For cassava, acid hydrolysis is done to modify the cassava starch granule structure in order to produce soluble products with altered gelatinization behaviour [93]. 0.75 litre of 0.1M of sulphuric acid solution is mixed with cassava. Next, the medium is mixed with other nutrients (See Table 3-3 for cassava fermentation medium formulation) before the addition of 0.040 litre of inoculum. Both temperature and pH conditions are maintained and controlled at 30°C and pH 5 respectively. The fermentation process is stopped after approximately 72 hours and the samples are taken in every 2-3 hours for biomass, substrate (glucose), product (ethanol) and byproduct (glycerol) concentrations analysis. By using R-Biopharm test kits and UV-spectrophotometer, ethanol, glucose and glycerol are analyzed right after samples are taken from the medium culture in order to avoid contamination and to obtain better accuracy of concentration measurements. All analytical methods are done according to the instructions stated in the R-Biopharm instruction guides, including the determination of wavelength to be set at the UV-spectrophotometer. The fermentation process is considered finished when the glucose concentration is less than 5g/L at the end of the process.

A.2 Inoculum Preparation

Before experiments begin, it is important to prepare inoculum to be inoculated in the fermentation medium for microbial growth. It is vital to note that the inoculum is to be prepared in a contamination free environment since the physiological condition of the inoculum has a major effect of performance of the fermentation when it is transferred to the fermentation medium.

In this study, inoculum preparation is done based on the formulation by Thatipamala, Rohani and Hill (1992) [92] which are outlined in Table A-1, along with an addition of 1g of Baker's yeast.

Table A-1 Inoculum Preparation Formulation

Constituents	**Amount (g/L)**
Glucose	50
Yeast extract	5.0
NH_4Cl	2.5
Na_2HPO_4	2.91
KH_2PO_4	3.0
$MgSO_4$	0.25
$CaCl_2$	0.08
Citric acid	4.3
Sodium citrate	3.0

Before preparing the inoculum, it is important to ensure that the conical flask is sterilized before use in order to ensure that the flask is not contaminated. Steam is used for sterilization and is applied at 15psi for an hour. 0.25 litre of inoculum is prepared in the sterilized conical flask and is mixed thoroughly before allowing the inoculum to stand for around 8 hours for microbial growth. To prevent contamination, the conical flask is wrapped and covered with cotton wool and aluminium foil. Figure A-1 shows the inoculum appearance after 8 hours.

Figure A-1 Appearance of Inoculum after 8 hours

A.3 Medium Preparation (Glucose)

In this section, the preparation of glucose medium is outlined. Table A-2 summarizes the glucose medium formulation of the fermentation medium prepared in the laboratory.

Table A-2 Glucose Fermentation Medium Formulation

Constituents	**Amount (g/L)**
Glucose	50
Yeast extract	5.0
NH_4Cl	2.5
Na_2HPO_4	2.91
KH_2PO_4	3.0
$MgSO_4$	0.25
$CaCl_2$	0.08
Citric acid	4.3
Sodium citrate	3.0

Based on the fermentation medium formulation summarized in Table A-2, the medium culture is prepared and stirred thoroughly after sterilization of the bioreactor. Steam is used to sterilize the culture medium and is applied at 15psi for an hour. Figure A-2 shows the glucose medium culture after the medium is mixed thoroughly. It can be observed that the medium culture is yellowish in colour.

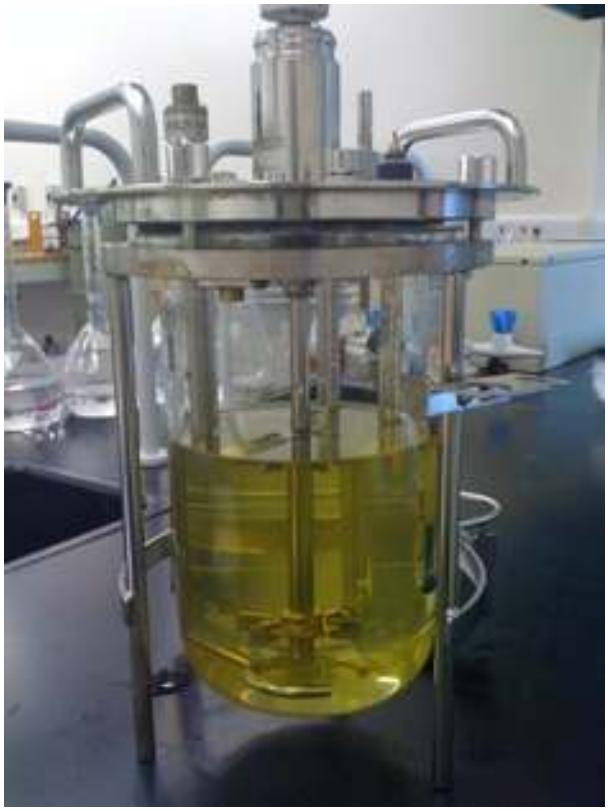

Figure A-2 Glucose Culture Medium

After the medium culture is prepared in the sterilized bioreactor, the medium is sterilized and allowed to cool down under room temperature for approximately 4 hours for the bioreactor and the medium culture to be completely cooled down. Experiment begins right after the bioreactor and medium culture are cooled down to avoid any contaminations of the bioreactor and medium culture.

A.4 Medium Preparation (Cassava)

In this section, the cassava medium preparation is outlined, whereby Table A-3 summarizes the cassava medium formulation of the fermentation medium. The formulation is similar to that of glucose medium, except that the amount of cassava is

twice as more as glucose in order to generate more ethanol due to the more complex structure of cassava which is tougher to breakdown. Yeast, on the other hand is reduced to 1g/L instead of 5g/L in order to investigate the ability of cassava in producing ethanol. All of these constituents are then be hydrolyzed with the addition of 0.75 litre of 0.1M (i.e. 0.2N) sulphuric acid solution.

Table A-3 Cassava Fermentation Medium Formulation

Constituents	**Amount (g/L)**
Cassava	100
Yeast extract	1.0
NH_4Cl	2.5
Na_2HPO_4	2.91
KH_2PO_4	3.0
$MgSO_4$	0.25
$CaCl_2$	0.08
Citric acid	4.3
Sodium citrate	3.0

Based on the fermentation medium formulation summarized in Table A-3, the medium culture is prepared and stirred thoroughly after sterilization of the bioreactor. Steam is used to sterilize the medium culture and is applied at 15psi for an hour. Figure A-3 shows the cassava medium culture, whereby the medium culture is orange yellowish in colour.

Figure A-3 Cassava Medium Culture

The bioreactor along with the hydrolyzed cassava starch is sterilized and cooled down under room temperature for approximately 4 hours for complete cool down of the bioreactor and medium culture. Experiment begins right after the bioreactor and medium culture are cooled down to avoid any contaminations of the bioreactor and medium culture.

A.5 Analytical Methods

In order to measure the biomass, glucose, ethanol and glycerol concentrations, a UV-spectrophotometer is utilized, aided with R-Biopharm test kits whereby different test kits are required for each concentration measurements. Figure A-4 shows the UV-spectrophotometer (Lambda 25) used for analysis in the laboratory.

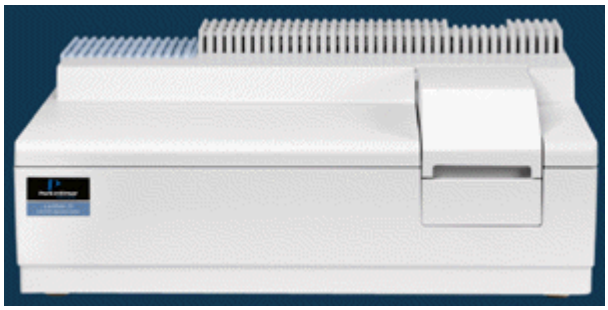

Figure A-4 UV Spectrophotometer (Lambda 25)

On the other hand, Figure A-5 shows the test kits for glucose, ethanol and glycerol concentrations. Preparations are done according to the test kits manuals allocated for different concentration measurements. Glass cuvettes are utilized during the analytical process.

Figure A-5 R-Biopharm Test Kits for Glucose, Ethanol and Glycerol Concentrations

A.5.1 Biomass Concentration Measurements

For the biomass concentration measurements, cells are diluted in 0.8% (w/v) sodium chloride (NaCl) solution before the biomass concentrations are measured by using UV- spectrophotometer at optical density of OD_{600nm}.

A.5.2 Substrate (Glucose and Cassava) Concentration Measurements

Glucose concentration measurements are done according to the glucose test kit manual, whereby a wavelength of 340nm is used under a temperature of 20°C. Absorbance values are recorded for the concentration calculation, which is based on the general equation below:

$$\Delta A = (A_1 - A_2)_{sample} - (A_1 - A_2)_{blank} \tag{61}$$

where A_1 is the absorbance value upon reaction after approximately 3 minutes

A_2 is the absorbance value upon reaction after approximately 10-15 minutes

$$c = \frac{V \times MW}{\varepsilon \times d \times v \times 1000} \times \Delta A \ \text{[g/L]} \tag{62}$$

where V = final volume [mL]

v = sample volume [mL]

MW = molecular weight of the substance to be assayed [g/mol]

d = light path = 1 [cm]

ε = extinction coefficient of NADPH at 340nm = 6.3 [L × $mmol^{-1}$ × cm^{-1}]

A.5.3 Ethanol Concentration Measurements

To measure the concentration of ethanol, similar analysis is done based on the analysis of glucose concentration, except for certain calculations.

$$\Delta A = (A_1 - A_2)_{sample} - (A_1 - A_2)_{blank} \tag{63}$$

where A_1 is the absorbance value upon reaction after approximately 3 minutes

A_2 is the absorbance value upon reaction after approximately 5-10 minutes

$$c = \frac{V \times MW}{\varepsilon \times d \times v \times 2 \times 1000} \times \Delta A \ \text{[g/L]} \tag{64}$$

where V = final volume [mL]

v = sample volume [mL]

MW = molecular weight of the substance to be assayed [g/mol]

d = light path = 1 [cm]

ε = extinction coefficient of NADPH at 340nm = 6.3 [L × mmol^{-1} × cm^{-1}]

A.5.4 Glycerol Concentration Measurements

For glycerol concentration measurements, similar analysis is utilized as compared to that of ethanol and glucose concentrations measurements. Calculations are similar to that of glucose concentration.

$$\Delta A = (A_1 - A_2)_{sample} - (A_1 - A_2)_{blank} \tag{65}$$

where A_1 is the absorbance value upon reaction after approximately 5-7 minutes

A_2 is the absorbance value upon reaction after approximately 5-10 minutes

$$c = \frac{V \times MW}{\varepsilon \times d \times v \times 1000} \times \Delta A \ \text{[g/L]} \tag{66}$$

where V = final volume [mL]

v = sample volume [mL]

MW = molecular weight of the substance to be assayed [g/mol]

d = light path = 1 [cm]

ε = extinction coefficient of NADPH at 340nm = 6.3 [L × mmol^{-1} × cm^{-1}]

Appendix B

Statistical Analysis (Experimental Data)

B.1 GLUCOSE SUBSTRATE

Table B-1 to Table B-8 show the ANOVA results for glucose, ethanol, glycerol and biomass concentrations, as well as yield, productivity, DO and OUR for glucose substrate.

Table B-1 ANOVA Results for Glucose Concentration (Glucose Substrate)

Source	**Sum of Squares**	**Degree of Freedom**	**Mean Square**	**F Value**	**p-value Prob > F**
AB	14.44	1	14.44	179.28	0.0070

Table B-2 ANOVA Results for Ethanol Concentration (Glucose Substrate)

Source	**Sum of Squares**	**Degree of Freedom**	**Mean Square**	**F Value**	**p-value Prob > F**
AB	16.51	1	16.51	219.18	0.0330

Table B-3 ANOVA Results for Glycerol Concentration (Glucose Substrate)

Source	**Sum of Squares**	**Degree of Freedom**	**Mean Square**	**F Value**	**p-value Prob > F**
AB	16.51	1	16.51	219.18	0.0460

Table B-4 ANOVA Results for Biomass Concentration (Glucose Substrate)

Source	Sum of Squares	Degree of Freedom	Mean Square	F Value	p-value Prob > F
AB	213.44	1	213.44	77.18	0.0330

Table B-5 ANOVA Results for Yield (Glucose Substrate)

Source	Sum of Squares	Degree of Freedom	Mean Square	F Value	p-value Prob > F
AB	63.61	1	63.61	179.48	0.0049

Table B-6 ANOVA Results for Productivity (Glucose Substrate)

Source	Sum of Squares	Degree of Freedom	Mean Square	F Value	p-value Prob > F
AB	27.96	1	27.96	277.18	0.0096

Table B-7 ANOVA Results for DO (Glucose Substrate)

Source	Sum of Squares	Degree of Freedom	Mean Square	F Value	p-value Prob > F
AB	25.19	1	25.19	139.57	0.0268

Table B-8 ANOVA Results for OUR (Glucose Substrate)

Source	Sum of Squares	Degree of Freedom	Mean Square	F Value	p-value Prob > F
AB	10.56	1	10.56	167.85	0.0421

B.2 CASSAVA SUBSTRATE

Table B-9 to Table B-16 show the ANOVA results for glucose, ethanol, glycerol and biomass concentrations, as well as yield, productivity, DO and OUR for cassava substrate.

Table B-9 ANOVA Results for Glucose Concentration (Cassava Substrate)

Source	Sum of Squares	Degree of Freedom	Mean Square	F Value	p-value Prob > F
AB	64.96	1	64.96	279.28	0.0239

Table B-10 ANOVA Results for Ethanol Concentration (Cassava Substrate)

Source	Sum of Squares	Degree of Freedom	Mean Square	F Value	p-value Prob > F
AB	116.51	1	116.51	248.18	0.0063

Table B-11 ANOVA Results for Glycerol Concentration (Cassava Substrate)

Source	Sum of Squares	Degree of Freedom	Mean Square	F Value	p-value Prob > F
AB	95.12	1	95.12	119.18	0.0329

Table B-12 ANOVA Results for Biomass Concentration (Cassava Substrate)

Source	Sum of Squares	Degree of Freedom	Mean Square	F Value	p-value Prob > F
AB	33.44	1	33.44	57.18	0.0074

Table B-13 ANOVA Results for Yield (Cassava Substrate)

Source	Sum of Squares	Degree of Freedom	Mean Square	F Value	p-value Prob > F
AB	53.45	1	53.45	199.12	0.0189

Table B-14 ANOVA Results for Productivity (Cassava Substrate)

Source	Sum of Squares	Degree of Freedom	Mean Square	F Value	p-value Prob > F
AB	67.45	1	67.45	296.18	0.0303

Table B-15 ANOVA Results for DO (Cassava Substrate)

Source	Sum of Squares	Degree of Freedom	Mean Square	F Value	p-value Prob > F
AB	135.19	1	135.19	239.74	0.0268

Table B-16 ANOVA Results for OUR (Cassava Substrate)

Source	Sum of Squares	Degree of Freedom	Mean Square	F Value	p-value Prob > F
AB	20.12	1	20.12	159.34	0.0452

Appendix C

Statistical Analysis (Kinetics Modeling)

C.1 DATA-BASED MODEL

C.1.1 Glucose Substrate

Table C-1 below shows the ANOVA results for yield for glucose substrate. Results show that the data-based model for yield is significant.

Table C-1 ANOVA Results for Yield (Glucose Substrate)

Source	**Sum of Squares**	**Degree of Freedom**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	57.06	3	19.02	250.83	0.0040	Significant
A – AR	27.77	1	27.77	366.17	0.0027	
B – SS	15.86	1	15.86	209.15	0.0047	
AB	13.44	1	13.44	177.18	0.0049	
Curvature	22.86	1	22.86	301.51	0.0033	Significant
Pure Error	0.15	2	0.076			
Cor Total	80.08	6				

Figure C-1 shows the half-normal plot for yield, obtained from glucose substrate. Figure C-1 reveals that the residuals, i.e. aeration rate (*AR*) and stirrer speed (*SS*) generally fall further away from the straight line, implying that both have interactions and important in terms of modeling. The interaction of *AB*, i.e. aeration rate (*AR*) and stirrer speed (*SS*) show response and this indicates that both show importance in interaction. Thus, both aeration rate (*AR*) and stirrer speed (*SS*) need to be engaged together in order to show better performance.

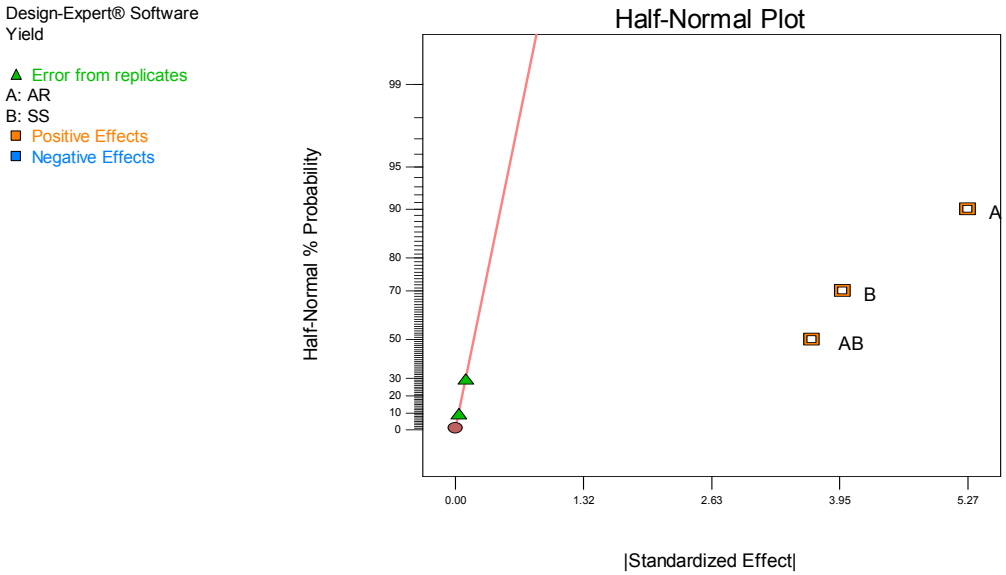

Figure C-1 Half-Normal Plot of Effects for Yield Data (Glucose Substrate)

Also, Figure C-2 shows the interaction between aeration rate (*AR*) and stirrer speed (*SS*), whereby within experimental range, there is contribution of interaction effect which is one of the significant model term. Results predict that yield is expected to be within 21% to 22%, which is the desired amount of yield. The curvature value as indicated in ANOVA, also supports this statement whereby the curvature is significant. Thus, both interaction of aeration rate (*AR*) and stirrer speed (*SS*) is predicted to be able to contribute to the desired amount of yield within experimental range.

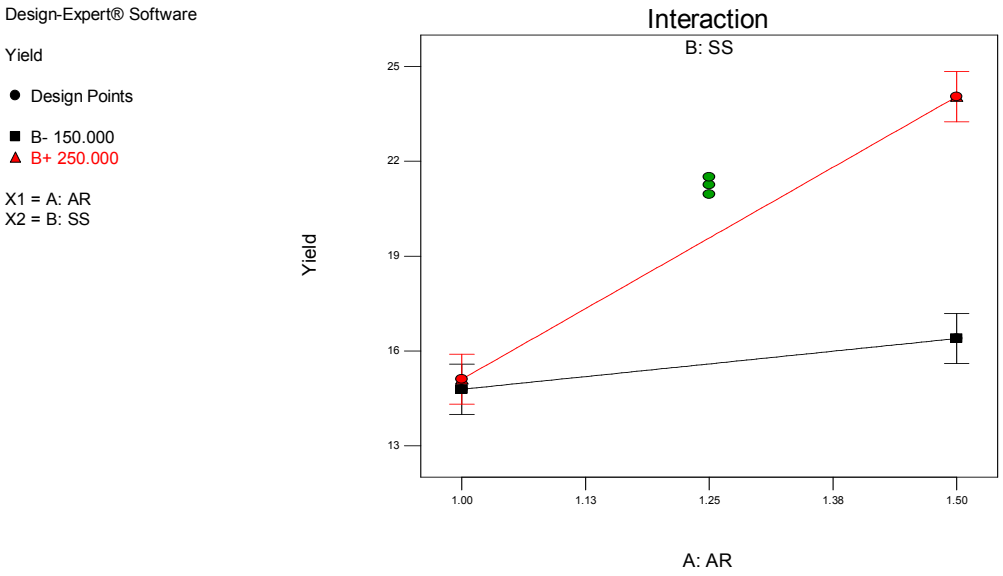

Figure C-2 Interaction Plot between Aeration Rate (*AR*) and Stirrer Speed (*SS*) for Yield Data (Glucose Substrate)

On the other hand, Figure C-3 shows the plot of residuals vs. predicted response for yield for glucose substrate. This figure reveals that they have no obvious pattern and unusual structure. The three design points are within both red lines which imply that there is no reason to suspect any violation of the independence or constant variance assumption.

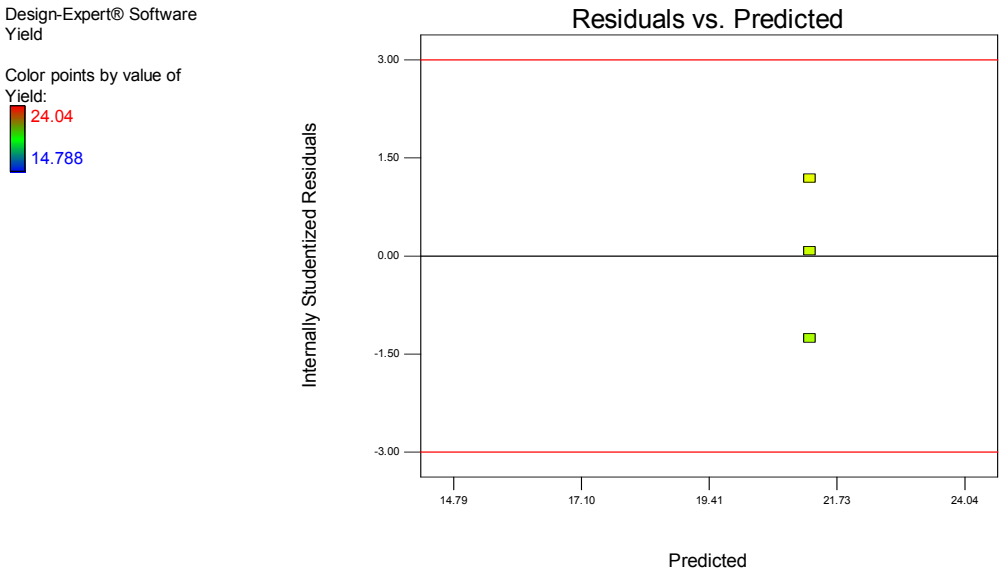

Figure C-3 Plot of Residuals vs. Predicted Response for Yield Data (Glucose Substrate)

Results from ANOVA and plots indicate that yield gave significant curvature results. The probability values for yield fall below the acceptable threshold of 0.05. The curvature F-value of 301.51 for yield implies that there is significant curvature, as measured by the difference between the average of the centre points and the average of the factorial points in the design space. There is only a 0.33% chance that a curvature F-value for yield this large could occur due to noise. Overall, curvature is significant and therefore, augmentation of the design could proceed for optimization.

Table C-2 shows the ANOVA results for productivity, obtained from glucose substrate. Results indicate that the data-based model for productivity is significant.

Table C-2 ANOVA Results for Productivity (Glucose Substrate)

Source	**Sum of Squares**	**Degree of Freedom**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	2.519E-3	3	8.396E-4	132.57	0.0075	Significant
A – AR	1.056E-3	1	1.056E-3	166.78	0.0059	
B – SS	8.122E-4	1	8.122E-4	128.25	0.0077	
AB	6.503E-4	1	6.503E-4	102.67	0.0096	
Curvature	6.361E-3	1	6.361E-3	1004.44	0.0010	Significant
Pure Error	1.267E-5	2	6.333E-6			
Cor Total	8.893E-3	6				

To further investigate the adequacy of the data-based model for productivity, a check of the plots in Figures C-4 to C-6 reveals the predicted responses for productivity in terms of aeration rate (*AR*) and stirrer speed (*SS*) for glucose substrate. Figure C-4 reveals that the residuals, i.e. aeration rate (*AR*) and stirrer speed (*SS*) generally fall further away from the straight line, implying that both have interactions and important in terms of modeling. The interaction of *AB*, i.e. aeration rate (*AR*) and stirrer speed (*SS*) shows response and this indicates that both shows importance in interaction. Thus, both aeration rate (*AR*) and stirrer speed (*SS*) need to be engaged together in order to show better performance.

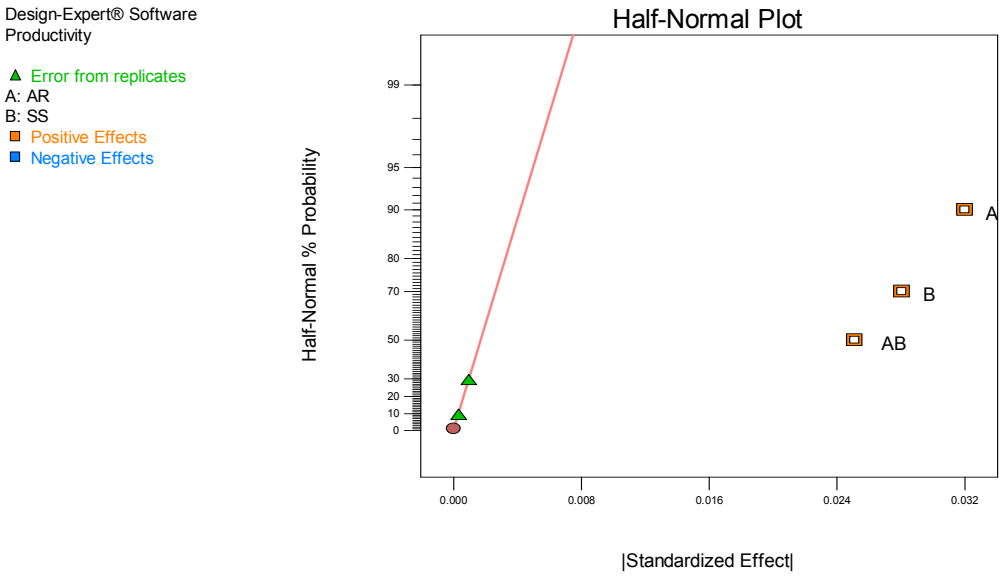

Figure C-4 Half-Normal Plot of Effects for Productivity Data (Glucose Substrate)

Also, Figure C-5 shows the interaction between aeration rate (*AR*) and stirrer speed (*SS*), whereby within experimental range, there is contribution of interaction effect which is one of the significant model term.

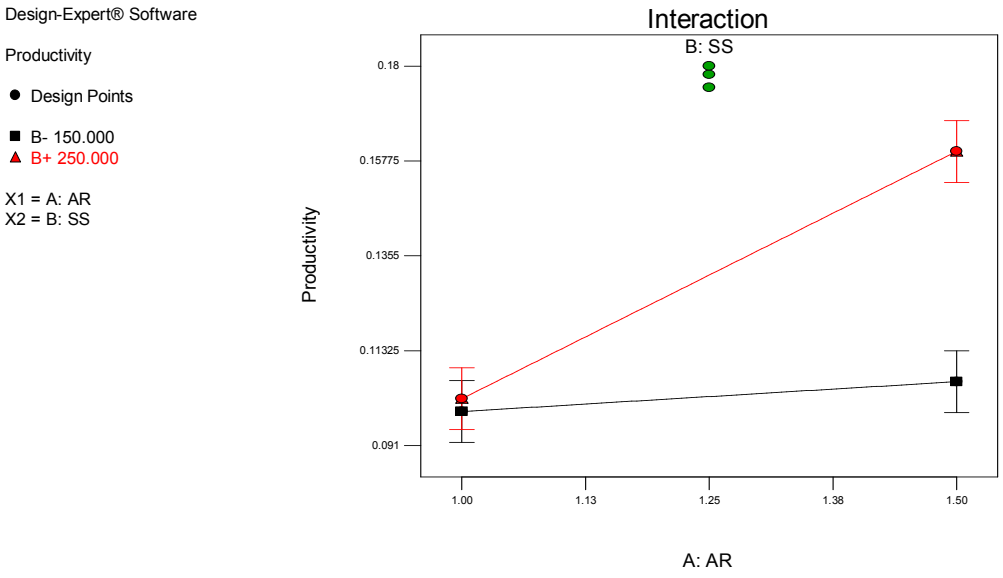

Figure C-5 Interaction Plot between Aeration Rate (*AR*) and Stirrer Speed (*SS*) for Productivity Data (Glucose Substrate)

Results predict that productivity is expected to be within 0.17% to 0.18%, which is the desired amount of productivity. The curvature value as indicated in ANOVA, also supports this statement whereby the curvature is significant. Thus, both interaction of aeration rate (*AR*) and stirrer speed (*SS*) is predicted to be able to contribute to the desired amount of productivity within experimental range.

On the other hand, Figure C-6 shows that they have no obvious pattern and unusual structure. The three design points are within both red lines which imply that there is no reason to suspect any violation of the independence or constant variance assumption.

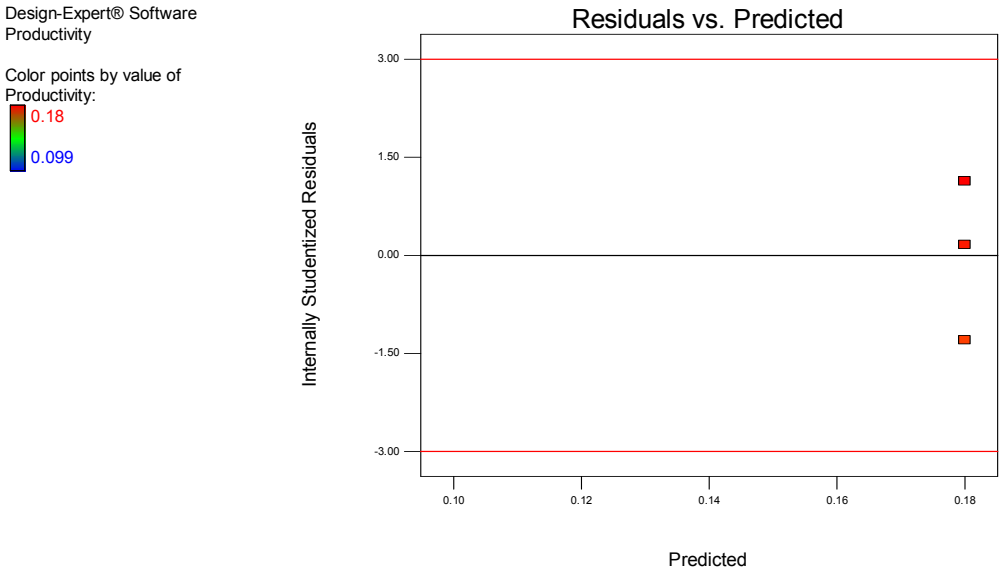

Figure C-6 Plot of Residuals vs. Predicted Response for Productivity Data (Glucose Substrate)

Results from ANOVA and plots indicate that productivity gives significant curvature results. The probability values for productivity fall below the acceptable threshold of 0.05. The curvature F-value of 1004.44 for productivity implies that there is significant curvature, as measured by the difference between the average of the centre points and the average of the factorial points in the design space. There is only a 0.75% chance that a curvature F-value for productivity this large could occur due to noise. Overall, curvature is significant and therefore, augmentation of the design could proceed for optimization.

C.1.2 Cassava Substrate

Table C-3 shows the ANOVA results for yield, obtained from cassava substrate. Results show that the data-based model for yield is significant for cassava substrate.

Table C-3 ANOVA Results for Yield (Cassava Substrate)

Source	**Sum of Squares**	**Degree of Freedom**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	785.15	3	261.72	2481.15	0.0004	Significant
A – AR	272.38	1	272.38	2582.28	0.0004	
B – SS	507.33	1	507.33	4809.67	0.0002	
AB	5.43	1	5.43	51.51	0.0189	
Curvature	1057.66	1	1057.66	10026.98	<0.0001	Significant
Pure Error	0.21	2	0.11			
Cor Total	1843.02	6				

To further investigate the adequacy of the model, a check of the plots in Figures C-7 to C-9 reveals the predicted responses for yield in terms of aeration rate (*AR*) and stirrer speed (*SS*) for glucose substrate. Figure C-7 reveals that the residuals, i.e. aeration rate (*AR*) and stirrer speed (*SS*) generally fall further away from the straight line. For the two-level interaction of *AB*, i.e. the interaction of both aeration rate (*AR*) and stirrer speed (*SS*), is located close to the straight lime, implying that both have interactions but the interaction is not as strong as compared to glucose substrate. Regardless of this interaction, the interaction of both aeration rate (*AR*) and stirrer speed (*SS*) is still important in terms of modeling since there is a sign of interaction. Thus, both aeration rate (*AR*) and stirrer speed (*SS*) need to be engaged together in order to show better performance.

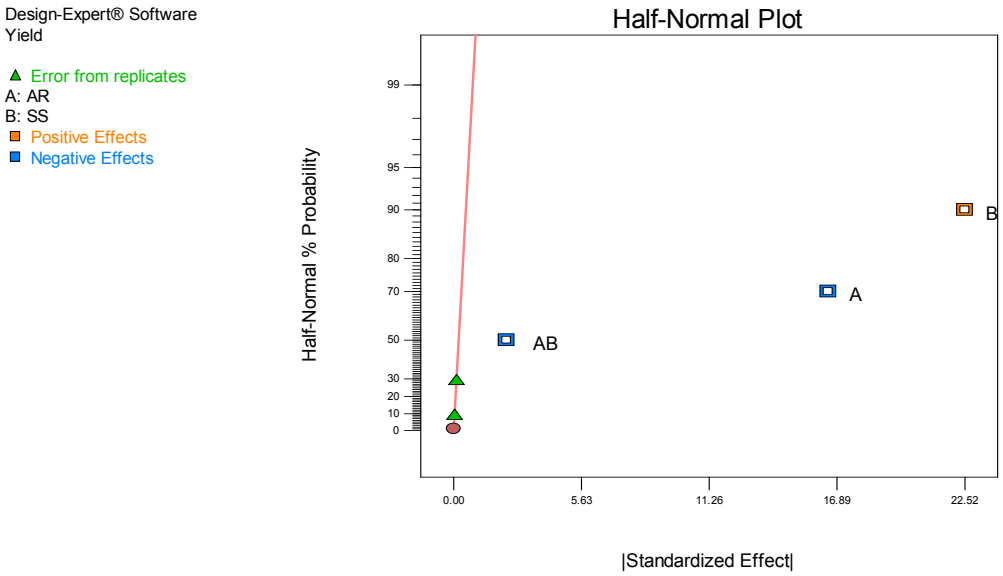

Figure C-7 Half-Normal Plot of Effects for Yield Data (Cassava Substrate)

Also, Figure C-8 shows the interaction between aeration rate (*AR*) and stirrer speed (*SS*), whereby within experimental range, there is contribution of interaction effect which is one of the significant model term. Results predict that yield is expected to be around 49%, which is the desired amount of yield. The curvature value as indicated in ANOVA, also supports this statement whereby the curvature is significant. Thus, both interaction of aeration rate (*AR*) and stirrer speed (*SS*) is predicted to be able to contribute to the desired amount of yield within experimental range.

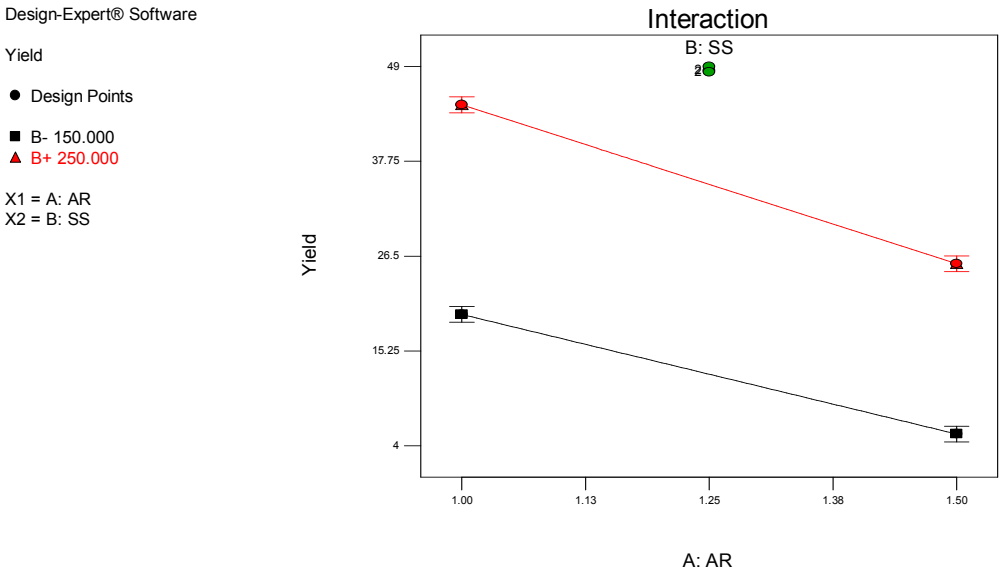

Figure C-8 Interaction Plot between Aeration Rate (*AR*) and Stirrer Speed (*SS*) for Yield Data (Cassava Substrate)

On the other hand, Figure C-9 reveals that they have no obvious pattern and unusual structure. The three design points are within both red lines which imply that there is no reason to suspect any violation of the independence or constant variance assumption.

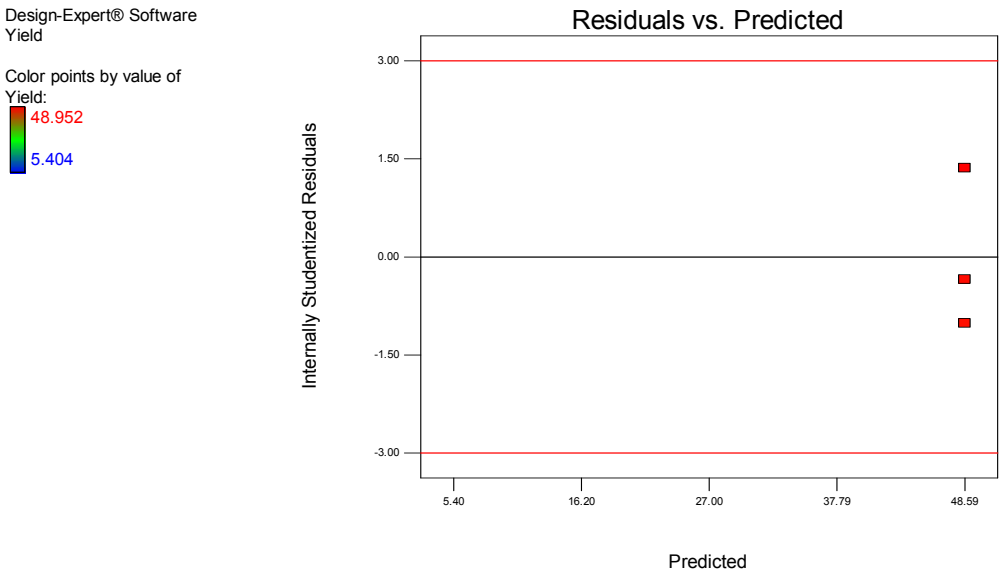

Figure C-9 Plot of Residuals vs. Predicted Response for Yield Data (Cassava Substrate)

Further, results from ANOVA and plots indicate that yield gives significant curvature results. The probability values for yield fall below the acceptable threshold of 0.05. The curvature F-value of 10026.98 for yield implies that there is significant curvature, as measured by the difference between the average of the centre points and the average of the factorial points in the design space. There is only a 0.04% chance that a curvature F-value for productivity this large could occur due to noise. Overall, curvature is significant and therefore, augmentation of the design could proceed.

The interpretation of ANOVA results for productivity for cassava substrate is presented in Table C-4. Results indicate that the model is significant.

Table C-4 ANOVA Results for Productivity (Cassava Substrate)

Source	**Sum of Squares**	**Degree of Freedom**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	0.18	3	0.060	44.52	0.0221	Significant
A – AR	0.040	1	0.040	29.82	0.0319	
B – SS	0.14	1	0.14	101.83	0.0097	
AB	2.550E-3	1	2.550E-3	1.89	0.0303	
Curvature	0.56	1	0.56	418.64	0.0024	Significant
Pure Error	2.696E-4	2	1.348E-3			
Cor Total	0.75	6				

To further investigate the adequacy of the model, a check of the plots in Figures C-10 to C-12 reveals the predicted responses for productivity in terms of aeration rate (*AR*) and stirrer speed (*SS*) for glucose substrate. Figure 4-10 reveals that the residuals, i.e. aeration rate (*AR*) and stirrer speed (*SS*) generally fall further away from the straight line. However, the two level interaction of *AB*, i.e. aeration rate (*AR*) and stirrer speed (*SS*) is nearer to the straight line, implying that both have interactions but not as strong as compared to that of glucose substrate. The interaction of *AB* shows response and this indicates that both show importance in interaction. Regardless of this interaction, the interaction of both aeration rate (*AR*) and stirrer speed (*SS*) is still important in terms of modeling since there is a sign of

interaction. Thus, both aeration rate (*AR*) and stirrer speed (*SS*) need to be engaged together in order to show better performance.

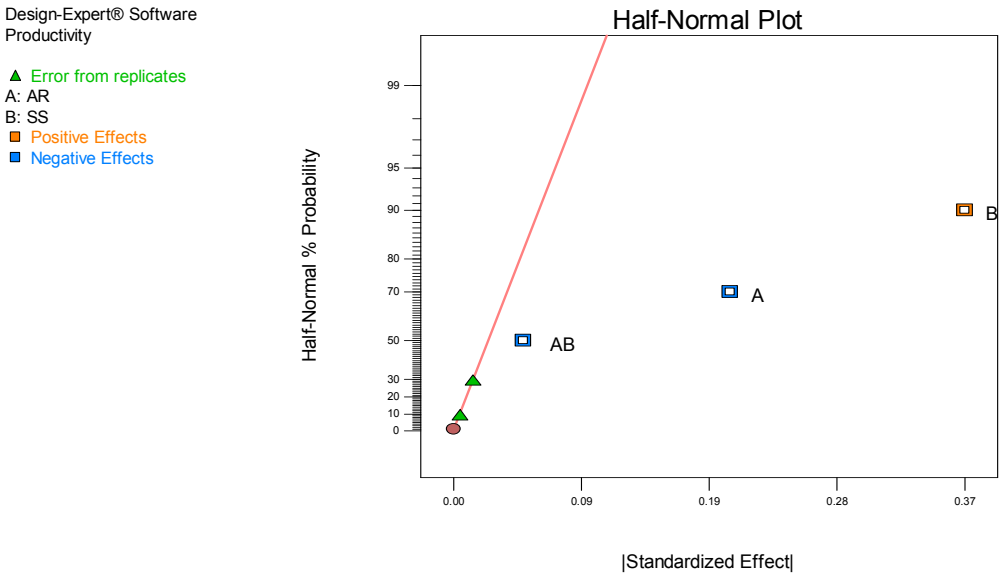

Figure C-10 Half-Normal Plot of Effects for Productivity Data (Cassava Substrate)

Also, Figure C-11 shows the interaction between aeration rate (*AR*) and stirrer speed (*SS*), whereby within experimental range, there is contribution of interaction effect which is one of the significant model term. Results predict that productivity is expected to be within 0.17% to 0.18%, which is the desired amount of productivity. The curvature value as indicated in ANOVA, also supports this statement whereby the curvature is significant. Thus, both interaction of aeration rate (*AR*) and stirrer speed (*SS*) is predicted to be able to contribute to the desired amount of productivity within experimental range.

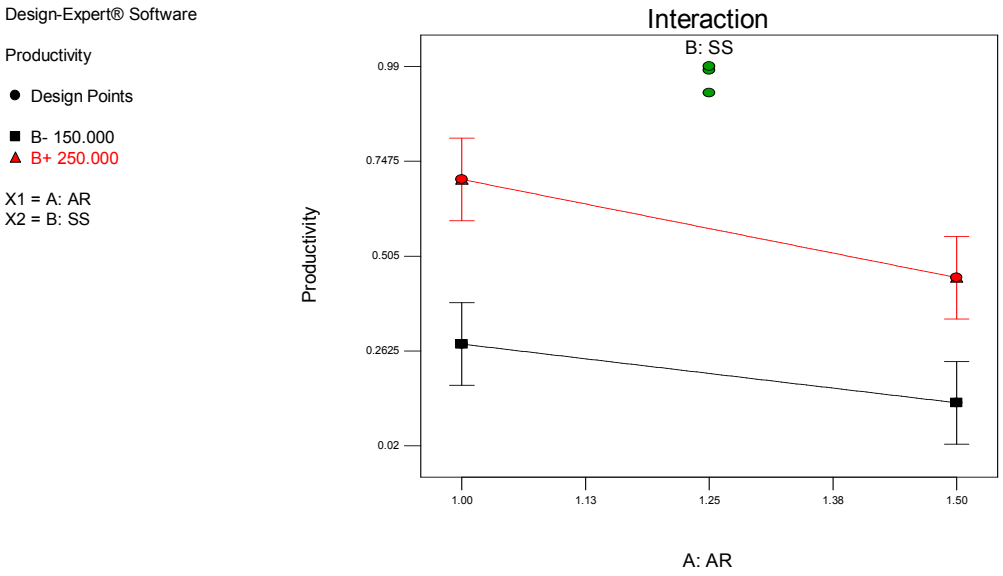

Figure C-11 Interaction Plot between Aeration Rate (*AR*) and Stirrer Speed (*SS*) for Productivity Data (Cassava Substrate)

On the other hand, Figure C-12 reveals that they have no obvious pattern and unusual structure. The three design points are within both red lines which imply that there is no reason to suspect any violation of the independence or constant variance assumption.

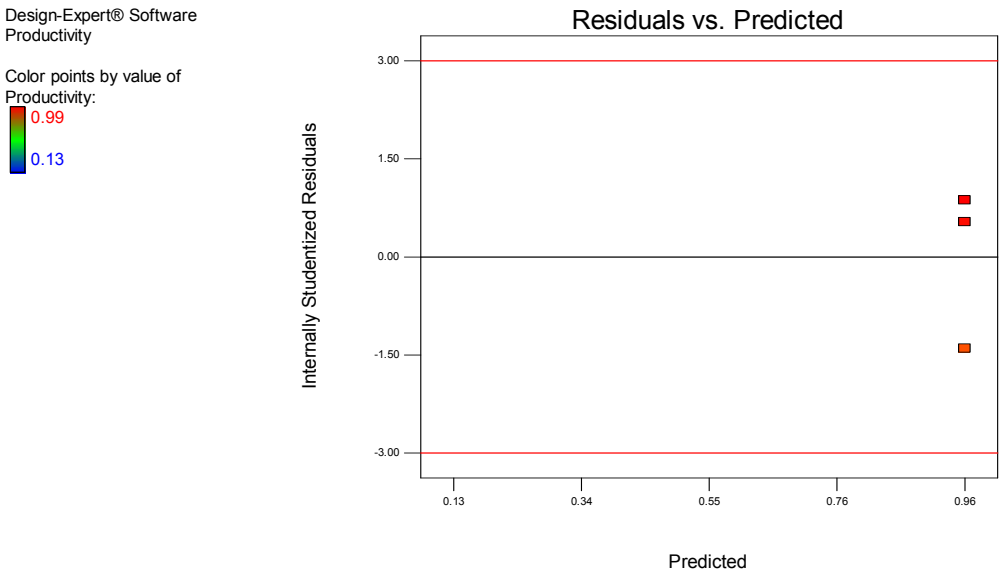

Figure C-12 Plot of Residuals vs. Predicted Response for Productivity Data (Cassava Substrate)

Further, results from ANOVA and plots indicate that productivity gives significant curvature results. The probability values for productivity fall below the acceptable threshold of 0.05. The curvature F-value of 418.64 for productivity implies that there is significant curvature, as measured by the difference between the average of the centre points and the average of the factorial points in the design space. There is only a 2.21% chance that a curvature F-value for productivity this large could occur due to noise. Overall, curvature is significant and therefore, augmentation of the design could proceed for optimization.

C.2 KINETICS HYBRID MODEL

Table C-5 shows the ANOVA results for kinetics hybrid model for glucose substrate, whereby the model is significant.

Table C-5 ANOVA Results for Kinetics Hybrid Model (Glucose Substrate)

Source	**Sum of Squares**	**Degree of Freedom**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	99.19	2	49.59	16.24	0.0007	Significant
Lack of Fit	30.54	2	15.27	5.29	0.0707	Not Significant
Pure Error	1.35	4	0.34			

Table C-6 shows the ANOVA results for kinetics hybrid model for cassava substrate. Results indicate that the model is not significant.

Table C-6 ANOVA Results for Kinetics Hybrid Model (Cassava Substrate)

Source	**Sum of Squares**	**Degree of Freedom**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	544.84	2	272.42	7.96	0.0632	Not Significant
Lack of Fit	12.28	1	12.28	0.27	0.6542	Not Significant
Pure Error	90.40	2	45.20			

C.3 KINETICS MULTI-SCALE MODEL

Table C-7 shows the ANOVA results for kinetics multi-scale model for glucose substrate. Results indicate that the model is significant.

Table C-7 ANOVA Results for Kinetics Multi-Scale Model (Glucose Substrate)

Source	**Sum of Squares**	**Degree of Freedom**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	138.40	5	27.68	29.49	0.0001	Significant
Lack of Fit	4.19	4	1.05	1.26	0.4136	Not Significant
Pure Error	3.32	4	0.83			

Appendix D

Statistical Analysis (Optimization)

D.1 DATA-BASED MODEL

Figures D-1 to D-2 reveal the predicted responses for yield in terms of aeration rate (*AR*) and stirrer speed (*SS*) for glucose substrate. Figure D-1 reveals that the residuals generally fall on the straight line implying that the errors are distributed normally. This implies that the model proposed is adequate and there is no reason to suspect any violation of the independence or constant variance assumption.

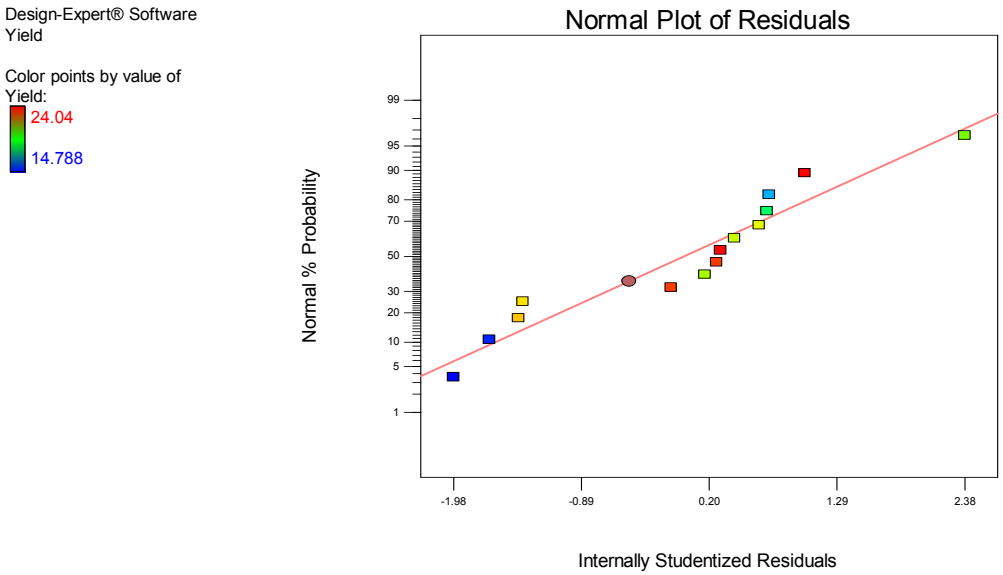

Figure D-1 Normal Probability Plot of Residuals for Yield Data

On the other hand, Figure D-2 shows the plot of residuals vs. predicted response for yield data. A check of the plot reveals that they have no obvious pattern and unusual structure. All the design points are within both red lines which imply that there are no outliers present, in accordance to the quadratic model fitted.

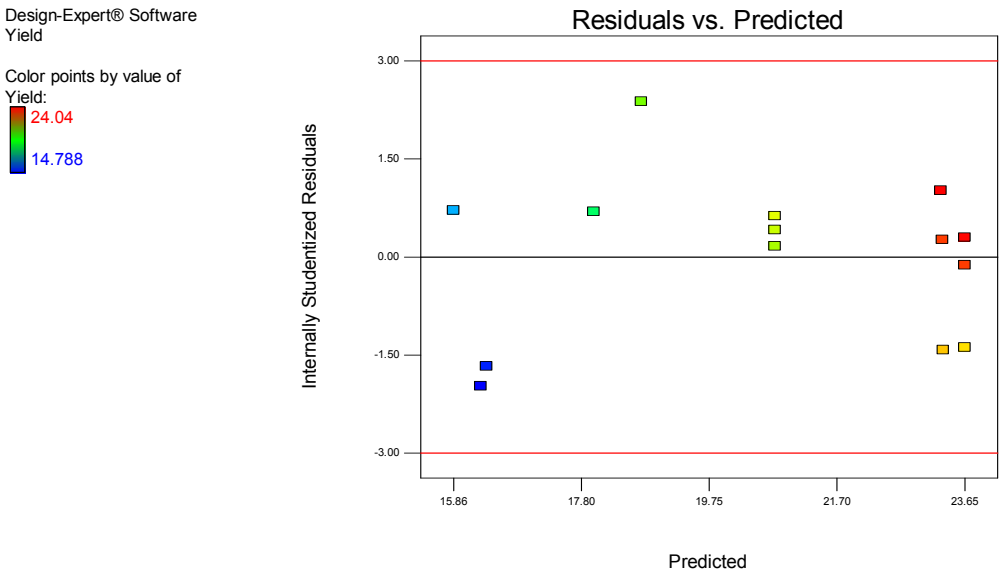

Figure D-2 Plot of Residuals vs. Predicted Response for Yield Data

Further, results from ANOVA and plots indicate that yield gives significant results. The probability values for yield fall below the acceptable threshold of 0.05. The model F-value of 9.73 for yield implies that there is significance, as measured by the difference between the average of the centre points and the average of the factorial points in the design space. As indicated by the analysis, there is only a 0.47% chance that a model F-value for yield this large could occur due to noise.

To further investigate the adequacy of the developed model, a check of the plots in Figures D-3 to D-4 reveals the predicted responses for yield in terms of aeration rate (*AR*) and stirrer speed (*SS*) for glucose substrate. Figure D-3 reveals that the residuals generally fall on the straight line implying that the errors are distributed normally. This implies that the model proposed is adequate and there is no reason to suspect any violation of the independence or constant variance assumption.

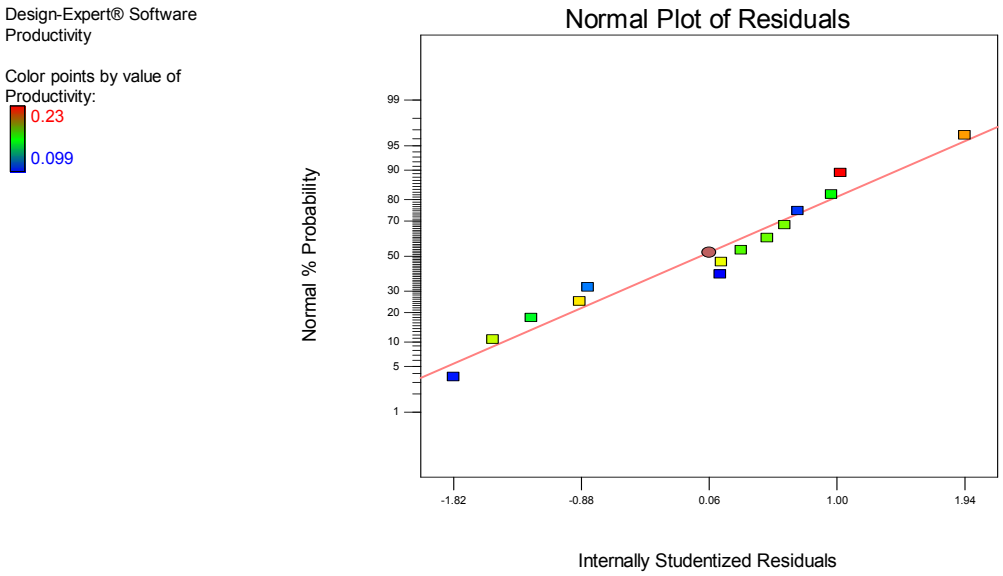

Figure D-3 Normal Probability Plot of Residuals for Productivity Data

On the other hand, Figure D-4 shows the plot of residuals vs. predicted response for productivity data. A check of the plot reveals that they have no obvious pattern and unusual structure. All the design points are within both red lines which imply that there are no outliers present, in accordance to the quadratic model fitted.

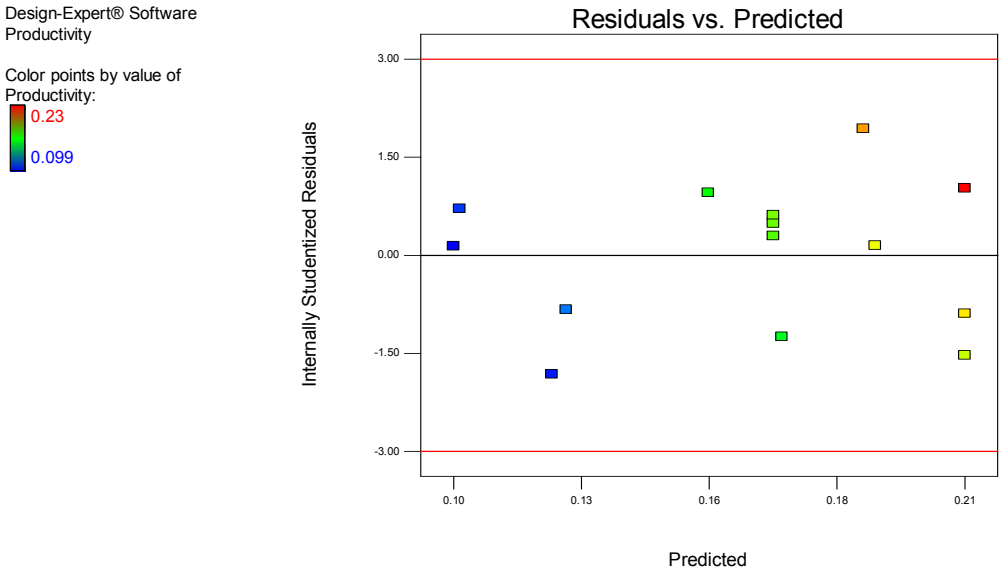

Figure D-4 Plot of Residuals vs. Predicted Response for Productivity Data

Further, results from ANOVA and plots indicate that productivity gives significant results. The probability values for yield fall below the acceptable threshold of 0.05. The model F-value of 9.30 for productivity implies that there is significance, as

measured by the difference between the average of the centre points and the average of the factorial points in the design space. There is only a 0.54% chance that a model F-value for productivity this large could occur due to noise. Overall, the developed model is significant and the optimum conditions of aeration rate (*AR*) and stirrer speed (*SS*) could be located, which is to be discussed in the next section of this chapter. The percentage differences between experimental and the developed model data are calculated in order to make comparisons on the credibility of the developed data-based model for productivity.

D.2 KINETICS HYBRID MODEL

Figures D-5 to D-6 reveal the predicted responses for yield in terms of aeration rate (*AR*) and stirrer speed (*SS*) for glucose substrate. Figure D-5 reveals that the residuals generally fall on the straight line implying that the errors are distributed normally, except for a point which is located slightly away from the straight line. This implies that the model proposed is quite adequate but there could be slight suspicion of any violation of the independence or constant variance assumption.

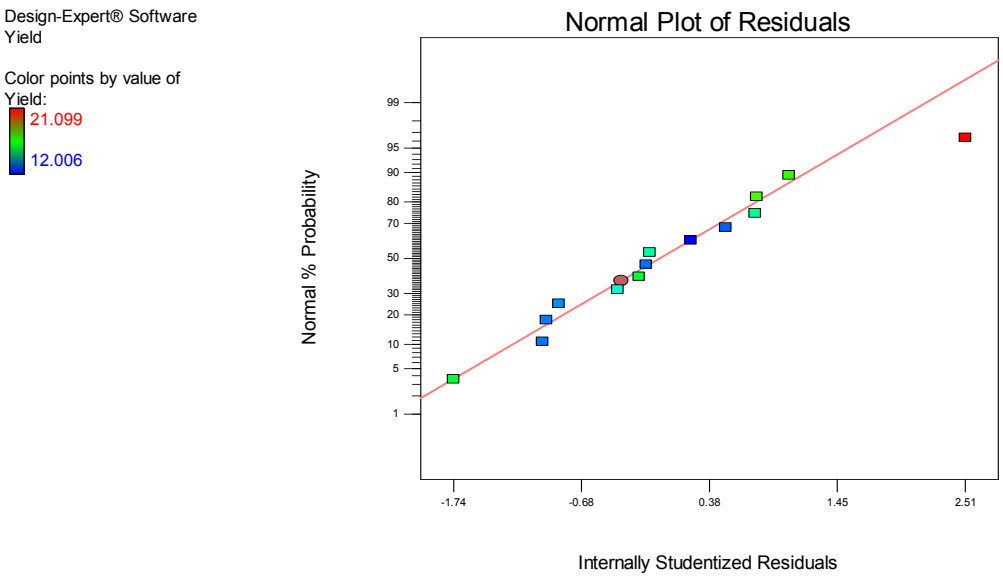

Figure D-5 Normal Probability Plot of Residuals for Yield Data

On the other hand, Figure D-6 shows the plot of residuals vs. predicted response for yield data. A check of the plot reveals that they have no obvious pattern and unusual structure. All the design points are within both red lines which imply that there are no outliers present, in accordance to the model fitted.

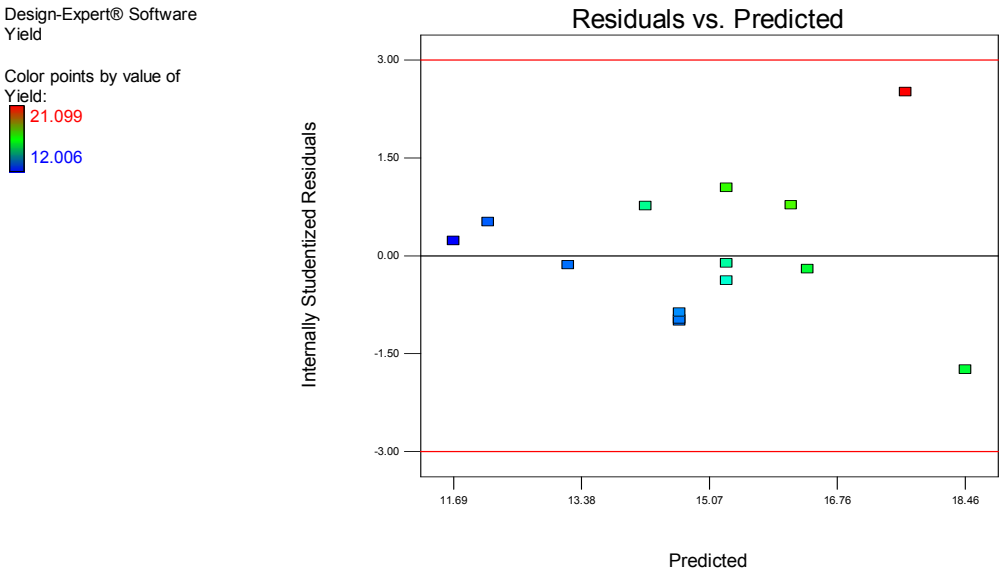

Figure D-6 Plot of Residuals vs. Predicted Response for Yield Data

Further, results from ANOVA and plots indicate that yield gives significant results. The probability values for yield fall below the acceptable threshold of 0.05. The model F-value of 7.20 for yield implies that there is significance, as measured by the difference between the average of the centre points and the average of the factorial points in the design space. From the analysis, it is indicated that there is only a 1.15% chance that a model F-value for yield this large could occur due to noise.

To further investigate the adequacy of the developed kinetics hybrid model, a check of the plots in Figures D-7 to D-8 reveals the predicted responses for productivity in terms of aeration rate (*AR*) and stirrer speed (*SS*) for glucose substrate. Figure D-7 reveals that the residuals generally fall on the straight line implying that the errors are distributed normally. This implies that the model proposed is adequate and there is no reason for suspicion of any violation of the independence or constant variance assumption.

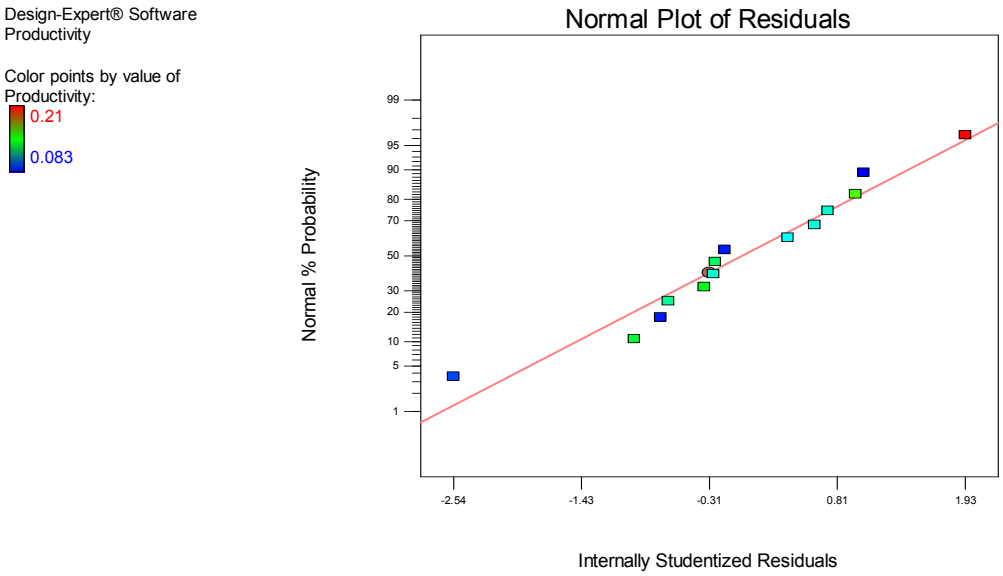

Figure D-7 Normal Probability Plot of Residuals for Productivity Data

On the other hand, Figure D-8 shows the plot of residuals vs. predicted response for yield data. A check of the plot reveals that they have no obvious pattern and unusual structure. All the design points are within both red lines which imply that there are no outliers present, in accordance to the model fitted.

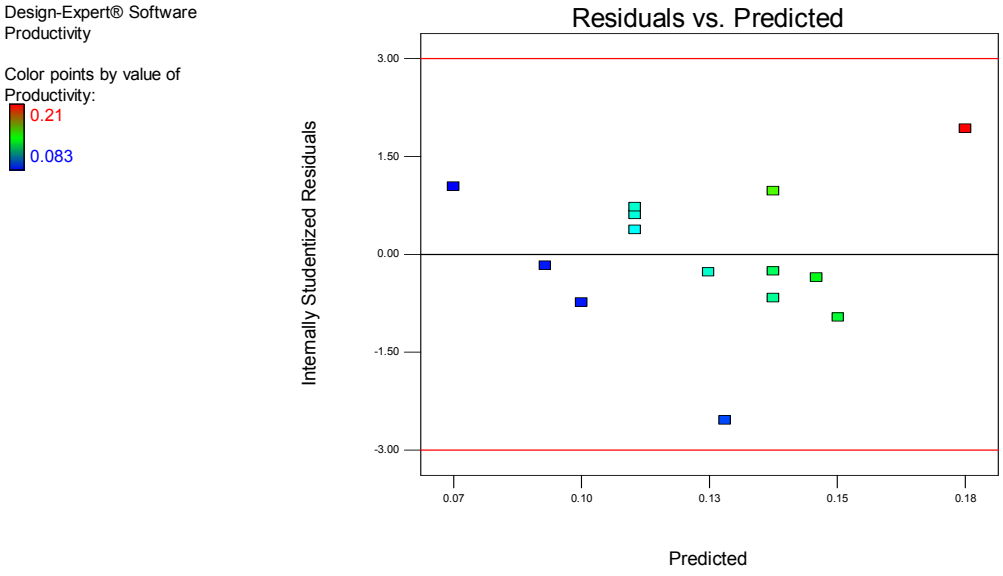

Figure D-8 Plot of Residuals vs. Predicted Response for Productivity Data

D.3 KINETICS MULTI-SCALE MODEL

To further investigate the adequacy of the developed kinetics multi-scale model, a check of the plots in Figures D-9 to D-10 reveals the predicted responses for yield in terms of aeration rate (*AR*) and stirrer speed (*SS*) for glucose substrate. Figure D-9 reveals that the residuals generally fall on the straight line implying that the errors are distributed normally. This implies that the model proposed is adequate and there is no reason that there is suspicion of any violation of the independence or constant variance assumption.

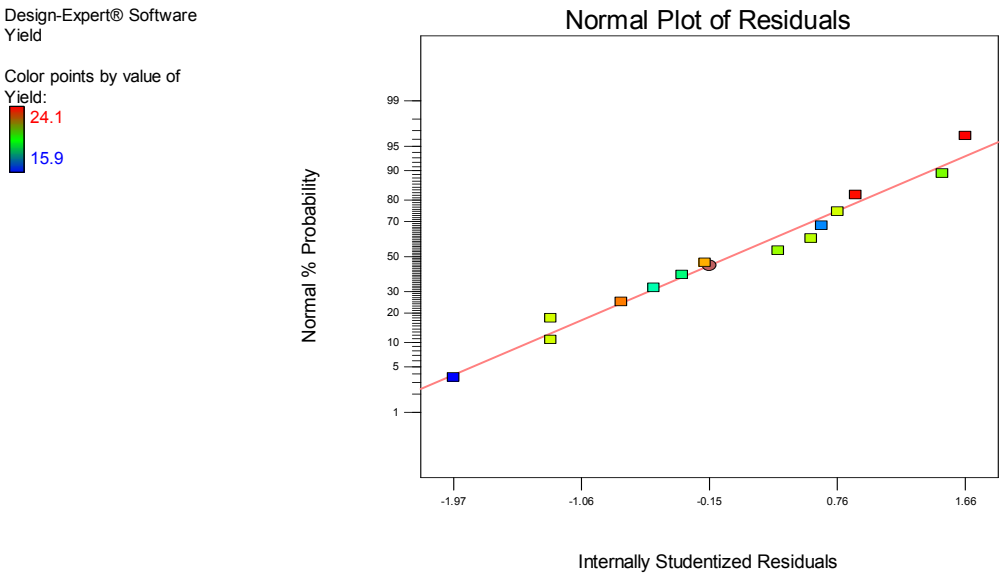

Figure D-9 Normal Probability Plot of Residuals for Yield Data

On the other hand, Figure D-10 shows the plot of residuals vs. predicted response for yield data. A check of the plot reveals that they have no obvious pattern and unusual structure. All the design points are within both red lines which imply that there are no outliers present, in accordance to the model fitted.

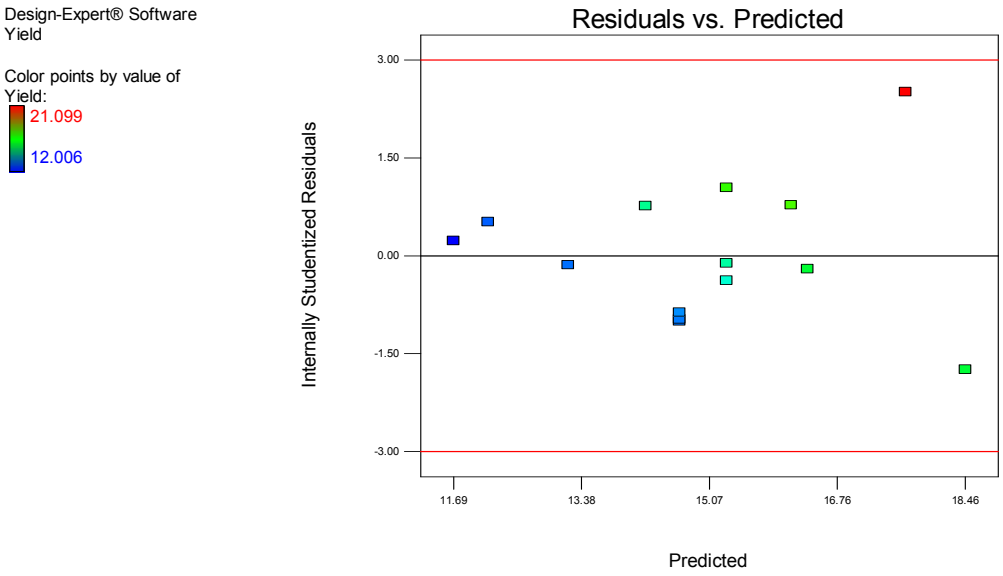

Figure D-10 Plot of Residuals vs. Predicted Response for Yield Data

Further, results from ANOVA and plots indicate that yield gives significant results. The probability values for yield fall below the acceptable threshold of 0.05. The model F-value of 7.10 for yield implies that there is significance, as measured by the difference between the average of the centre points and the average of the factorial points in the design space. It is indicated from the analysis that there is only a 1.15% chance that a model F-value for yield this large could occur due to noise.

To further investigate the adequacy of the developed kinetics hybrid model, a check of the plots in Figures D-11 to D-12 reveals the predicted responses for productivity in terms of aeration rate (*AR*) and stirrer speed (*SS*) for glucose substrate. Figure D-11 reveals that the residuals generally fall on the straight line implying that the errors are distributed normally. This implies that the model proposed is adequate and there is no reason for suspicion of any violation of the independence or constant variance assumption.

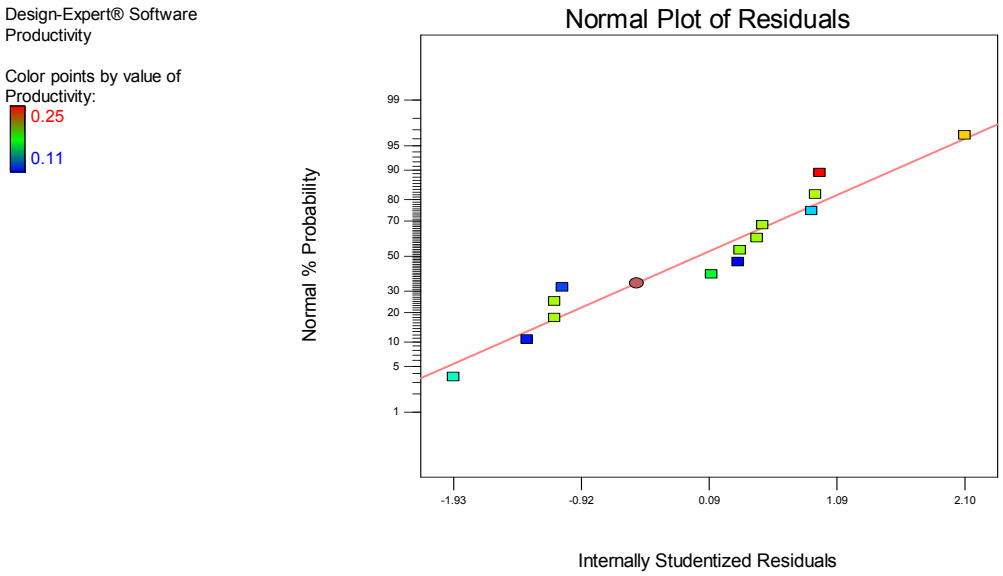

Figure D-11 Normal Probability Plot of Residuals for Productivity Data

On the other hand, Figure D-12 shows the plot of residuals vs. predicted response for yield data. A check of the plot reveals that they have no obvious pattern and unusual structure. All the design points are within both red lines which imply that there are no outliers present, in accordance to the model fitted.

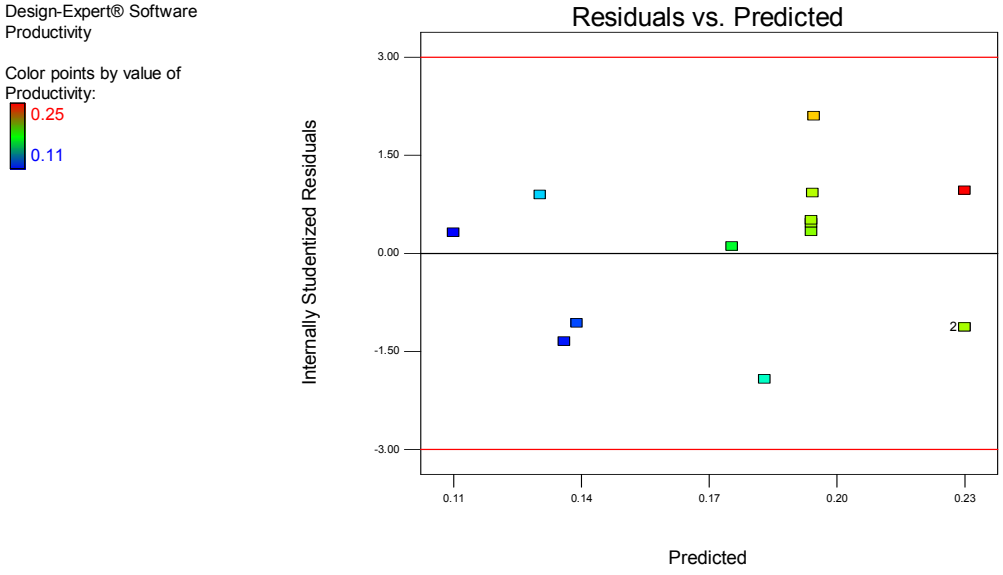

Figure D-12 Plot of Residuals vs. Predicted Response for Productivity Data

Further, results from ANOVA and plots indicate that productivity gives significant results. The probability values for yield fall below the acceptable threshold of 0.05.

The model F-value of 4.74 for productivity implies that there is significance. There is only a 3.28% chance that a model F-value for productivity this large could occur due to noise.

Appendix E

C Programming Language Codes for User-Defined Functions (UDFs)

A user-defined function (UDF) is a function that can be dynamically loaded with the FLUENT solver to enhance the standard features of the code. For example, a UDF can be used to define desired boundary conditions, material properties and source terms for desired flow regime. On the other hand, customized model parameters can be specified, for example multiphase flows, and to enhance post-processing. UDFs are written in the C programming language using any text editor. This appendix describes the C programming language codes utilized to describe the non-ideally mixed bioreactor model. The C programming language codes below is an example to describe the mixing mechanism under aeration rate of 1LPM and stirrer speed of 150rpm.

```
#include "udf.h"
#include "sg.h"

#define FLUID_ID 1
#define ua1 1.5158

#define va1 1.5158

#define ka1 2.2723e-2
#define ka2 6.7989
#define ka3 -424.18
```

```
#define ka4 9.4615e3
#define ka5 -7.7251e4
#define ka6 1.8410e5

#define da1 -6.5819e-2
#define da2 88.845
#define da3 -5.3731e3
#define da4 1.1643e5
#define da5 -9.1202e5
#define da6 1.9567e6

#define kb1 1.4085
#define kb2 1.4768
#define kb3 -5.704e-3
#define kc1 0.0001
#define kd1 0.6631
#define kd2 0.0878
#define kd3 -2.95e-4
#define ke1 0.10398
#define ke2 0.0511
#define ke3 2.835e-4
#define kf1 0.75582
#define kf2 -0.08432
#define kf3 -2.0376e-3
#define kg1 0.01425
#define kg2 -7.6e-3
#define kg3 -1e-6
#define AR 1
#define SS 150


DEFINE_PROFILE(fixed_u, thread, np)
{
 cell_t c;
 real x[ND_ND];
 real r;

 begin_c_loop (c,thread)
   {
/* centroid is defined to specify position dependent profiles*/
    C_CENTROID(x,c,thread);
    F_PROFILE(c,thread,np) =
          ua1;
}
 end_c_loop (c,thread)
}


DEFINE_PROFILE(fixed_v, thread, np)
{
```

```
  cell_t c;
  real x[ND_ND];

  begin_c_loop (c,thread)
    {
/* centroid is defined to specify position dependent profiles*/
     C_CENTROID(x,c,thread);
     F_PROFILE(c,thread,np) =
          va1;
}
  end_c_loop (c,thread)
}



DEFINE_PROFILE(fixed_ke, thread, np)
{
  cell_t c;
  real x[ND_ND];
  real r;

  begin_c_loop (c,thread)
    {
/* centroid is defined to specify position dependent profiles*/
     C_CENTROID(x,c,thread);
     r =x[1];
     F_PROFILE(c,thread,np) =
     ka1+(ka2*r)+(ka3*r*r)+(ka4*r*r*r)+(ka5*r*r*r*r)+(ka6*r*r*r*r*r);

    }
  end_c_loop (c,thread)
}



DEFINE_PROFILE(fixed_diss, thread, np)
{
  cell_t c;
  real x[ND_ND];
  real r;

  begin_c_loop (c,thread)
    {
/* centroid is defined to specify position dependent profiles*/
     C_CENTROID(x,c,thread);
     r =x[1];
     F_PROFILE(c,thread,np) =
     da1+(da2*r)+(da3*r*r)+(da4*r*r*r)+(da5*r*r*r*r)+(da6*r*r*r*r*r);

    }
```

```
  end_c_loop (c,thread)
}
DEFINE_PROFILE(fixed_kinetics, thread, np)
{
  cell_t c;
  real x[ND_ND];
  real K1;
  real K2;
  real K3;
  real K4;
  real K5;
  real K6;
  real X;
  real S;
  real P;
  real rgrowth;
  real rx;
  real rend;
  real rs;
  real rp;


  begin_c_loop (c,thread)
    {
/* centroid is defined to specify position dependent profiles*/
     C_CENTROID(x,c,thread);
     F_PROFILE(c,thread,np) =
     K1 = kb1 + kb2*(AR-1.25) + kb3*(SS-200);
     K2 = kc1;
     K3 = kd1 + kd2*(AR-1.25) + kd3*(SS-200);
     K4 = ke1 + ke2*(AR-1.25) + ke3*(SS-200);
     K5 = kf1 + kf2*(AR-1.25) + kf3*(SS-200);
     K6 = kg1 + kg2*(AR-1.25) + kg3*(SS-200);
     rgrowth = ((K1*X*S)/(K2+S))*exp(-K5*P);
     rend = (-K6)*X;
     rx = rgrowth + rend;
     rs = (-K3)*rgrowth;
     rp = (K4)*rgrowth;
    }
  end_c_loop (c,thread)
}
```

Appendix F

Matlab Software Codes

The function below is to analyse the effect of interactions of a bioreactor.

```
function [sys,x0,str,ts]= sfbioreactor(t,x,u,flag,X0,S0,P0)
%
switch flag
case 0 % Initialization

    str = []                          ;
    ts  = [0 0]                       ;

    s   = simsizes                    ;

        s.NumContStates      = 3      ;
        s.NumDiscStates      = 0      ;
        s.NumOutputs         = 3      ;
        s.NumInputs          = 4      ;
        s.DirFeedthrough     = 0      ;
        s.NumSampleTimes     = 1      ;

    sys = simsizes(s)                 ;

    x0  = [X0,S0,P0]        ;

case 1  % derivatives

    D   = u(1);      % dilution rate
    So  = u(2);      % substrate concentration
    AR  = u(3);      % aeration rate
    SS  = u(4);      % stirrer speed


   %
      sys =   biore(t,x,D,So,AR,SS) ;
```

```
case 3  % output

    sys = x                 ;

case {2 4 9}          % 2: discrete
                      % 4: calcTimeHit
                      % 9: termination
    sys = [];

otherwise

    error(['unhandled flag =',num2str(flag)]);

end
```

The function below is used to describe the bioreactor model and to calculate the bioreactor dynamics behaviour.

```
function dx = biore(t,x,D,So,AR,SS)
% ================================================================
% This function is to calculate the bioreactor dynamics behaviour
%
% ----------------------------------------------------------------
%                    Process Variables - Outputs
% ----------------------------------------------------------------
%
X       = x(1);      % Biomass optical concentration [-]
S       = x(2);      % Substrate concentration [g/L]
P       = x(3);      % Product concentration [g/L]


% ----------------------------------------------------------------
%                    Kinetic Parameters
% ----------------------------------------------------------------
% Model 6
k1 = 1.4085 - 0.2852*((AR-1.25)/0.25) + 0.3692*((SS-200)/50);
k2 = 0.0010;
k3 = 0.6631 - 0.0148*((AR-1.25)/0.25) + 0.0220*((SS-200)/50);
k4 = 0.1040 + 0.0142*((AR-1.25)/0.25) + 0.0128*((SS-200)/50);
k5 = 0.7558 - 0.1019*((AR-1.25)/0.25) - 0.0211*((SS-200)/50);
k6 = 0.0143 - 0.0001*((AR-1.25)/0.25) - 0.0019*((SS-200)/50);
%
% Function to calculate growth, substrate consumption and product
formation rates
% ----------------------------------------------------------------
-------
rxgrowth = ((k1*X*S/(k2+S))*exp(-k5*P));
rxend = -k6*X;
rx = rxgrowth + rxend;
rs = k3*rxgrowth;
rp = k4*rxgrowth;
%
% ------------------------------------------
%
% ----------------------------------------------------------------
% Initialization - Initial values (Assume initially at steady-state)
% ----------------------------------------------------------------
%dX = 0;
%dS = 0;
%dP = 0;
% ================================================================
%                        Solve ODE
% ----------------------------------------------------------------
% Substrate mass balance:
%
dX = -D*X + rx;
%
dS = D*(So-S) - rs;
%
dP = -D*P + rp;
%
%
dx  = [dX;dS;dP];
```

```
%
% END ------------------------------------------------------------
```

This function is used to analyse the effect of interactions of a data-based model controller.

```
function [sys,x0,str,ts]= sfcontroller1(t,x,u,flag)
%
switch flag
case 0 % Intialization

    x0  = []                          ;
    str = []                          ;
    ts  = [0 0]                       ;

    s   = simsizes                    ;

        s.NumContStates      = 0      ;
        s.NumDiscStates      = 0      ;
        s.NumOutputs         = 2      ;
        s.NumInputs          = 4      ;
        s.DirFeedthrough     = 4      ;
        s.NumSampleTimes     = 1      ;

    sys = simsizes(s)                 ;



case 3  % derivatives

    Y    = u(1);
    Prod  = u(2);
    Ey   = u(3);
    Ep = u(4);


   %
      sys =   controlcostError(Y,Prod,Ey,Ep);



case {1 2 4 9}         % 2: discrete
                     % 4: calcTimeHit
                     % 9: termination
    sys = [];

otherwise

    error(['unhandled flag =',num2str(flag)]);

end



function K = controlcostError(Y,Prod,Ey,Ep)
%
```

```
ARo=evalin('base','ARo');
SSo=evalin('base','SSo');
%
u0=[ARo SSo];
lb=[1 150];
ub=[1.5 250];
%
%
%If  Error >= 0.01;
options = optimset('Display','iter','LargeScale',
'off','MaxIter',1e4,...
  'MaxFunEvals',1e4,'TolCon',1e-8,'TolFun',1e-8,'TolX',1e-8);
%
[K,fval,exitflag,output]=fmincon(@Jcost,u0,[],[],[],[],lb,ub,[],opti
ons);
%
function C = Jcost(K)
%
AR=K(1);
SS=K(2);
% Data-based model
Yp = 33.09800 - 18.78500*AR - 0.14307*SS + 0.14670*AR*SS;
Prodp = 0.23400 - 0.13900*AR - 0.00099*SS + 0.00102*AR*SS;
%
deltaY2= (Yp-Y-Ey)/(24.044-13.357);
deltaP2= (Prodp-Prod-Ep)/(0.198 - 0.1);
deltaAR=(AR-ARo)/(1.5-1);
deltaSS=(SS-SSo)/(250-150);
%
C = ((deltaAR^2)+(deltaSS^2))+((deltaY2^2)+(deltaP2^2))
%
end
end
%
```

This function is used to analyse the effect of interactions of a kinetics hybrid model controller.

```
function K = controlcosthybridTest3(Y,Prod,Ey,Ep,X,S,P,D)
%
ARo=evalin('base','ARo');
SSo=evalin('base','SSo');
%
u0=[ARo SSo];
lb=[1 150];
ub=[1.5 250];
%
%
%If  Error >= 0.01;
options = optimset('Display','iter','LargeScale',
'on','MaxIter',1e4,...
  'MaxFunEvals',1e4,'TolCon',1e-10,'TolFun',1e-10,'TolX',1e-10);
%
[K,fval,exitflag,output]=fmincon(@Jcost,u0,[],[],[],[],lb,ub,[],opti
ons);
%
function C = Jcost(K)
%
AR=K(1);
SS=K(2);
% Hybrid model
k1 = 1.4085 - 0.2852*((AR-1.25)/0.25) + 0.3692*((SS-200)/50);
k2 = 0.0010;
k3 = 0.6631 - 0.0148*((AR-1.25)/0.25) + 0.0220*((SS-200)/50);
k4 = 0.1040 + 0.0142*((AR-1.25)/0.25) + 0.0128*((SS-200)/50);
k5 = 0.7558 - 0.1019*((AR-1.25)/0.25) - 0.0211*((SS-200)/50);
k6 = 0.0143 - 0.0001*((AR-1.25)/0.25) - 0.0019*((SS-200)/50);
%
rxgrowth = ((k1*X*S/(k2+S))*exp(-k5*P));
rxend = -k6*X;
rx = rxgrowth + rxend;
rs = k3*rxgrowth;
rp = k4*rxgrowth;
%
Yp=D*P/rs;
Prodp=rp/D;
%
deltaY2= (Yp-Y-Ey)/(24.044-13.357);
deltaP2= (Prodp-Prod-Ep)/(0.198 - 0.1);
deltaAR=(AR-ARo)/(1.5-1);
deltaSS=(SS-SSo)/(250-150);
%
C = ((deltaAR^2)+(deltaSS^2))+((deltaY2^2)+(deltaP2^2))
%
end
end
%
```

Figure F-1 below shows the bioreactor system used for Matlab simulations.

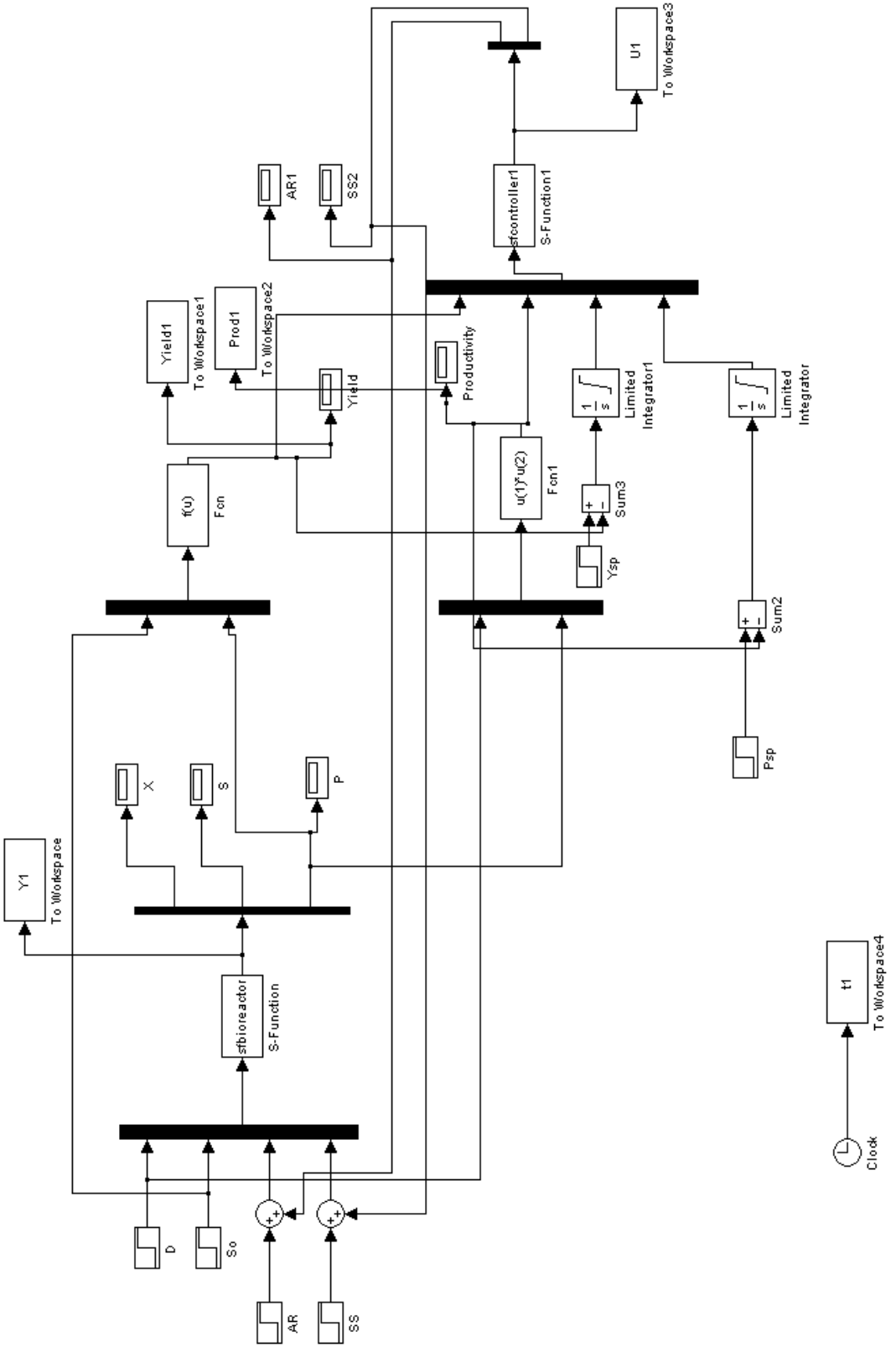

Figure F-1 Simulink Diagram for Bioreactor System