CONTROL OF PHYCOCYANIN PRODUCTION FROM *Spirulina platensis* IN FED-BATCH REACTOR USING MID COURSE CORRECTION POLICY

NOR FATIHAH BINTI AB GHANI

A thesis submitted in fulfillment of the requirements

for the award of the degree of

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UNIVERSITI MALAYSIA PAHANG

JUNE 2012

I hereby declare that I have checked this thesis and in my opinion, this thesis is adequate in

terms of scope and quality for the award of the degree of Bachelor of Chemical Engineering (Biotechnology).

Signature :……

Name of Supervisor: MS ROHANA BINTI ABU

Position: LECTURER

Date :……

STUDENT'S DECLARATION

I declare that this thesis entitled "Control of Phycocyanin Production from Spirulina Platensis in Fed-Batch Reactor Using Mid Course Correction Policy" is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidate of any other degree.

Signature :
Name : NOR FATIHAH BINTI AB GHANI
ID. Number : KE08023
Date : 19th JUNE 2012

DEDICATION

Special dedication to,

My parents

Ab Ghani bin Ismail and Azizah binti Mohamed

My beloved brothers and sisters
and
all of my friends

for all of the supports and faith in me.

ACKNOWLEGDEMENT

Praises to God Almighty, with his blessing that I managed to complete this thesis successfully. This thesis will not complete without guidance and helps from many people. I would like to express my appreciation especially to the following.

Foremost, I would like to express my sincere gratitude to my supervisor, Mr Shahril bin Mohamad for the knowledge shared and his guidance, support and motivation during completing this thesis. His guidance and comments helped me in my research and thesis.

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Last but not least, million thanks to my fellow friends for the helps, supports and guidance giving to me throughout my research.

ABSTRACT

Phycocyanin is a product of cultivation of Spirulina platensis, a blue-green microalga. Phycocyanin is widely used in medical treatment as a cancer inhibitor and as a natural dye for cosmetics and foods. Spirulina platensis has been cultivated recently in fed batch reactor to optimize the cell growth and product formation. Large scale production of Spirulina platensis gives more advantage especially in production cost but it is hard to control the final product to the desired value due to the disturbance occurred during the cultivation process. Therefore, Mid-Course Correction (MCC) policy is introduced to the process. Throughout the MCC, a model predictor is constructed to predict the final product concentration based on the previous experimental data. A control model is build for phycocyanin production based on the light intensity adjustment. The adjustment is made on day 5 of the cultivation process and simulation is done to recheck whether the final phycocyanin production is fall in the desired range.

ABSTRAK

Phycocyanin adalah produk yang dihasilkan dari penanaman Spirulina platensis iaitu mikroalga biru-hijua. Phycocyanin digunkan secara meluas di dalam rawatan perubatan untunk mengurangkan penyebaran kanser serta pewarna semulajadi untuk kosmetik dan makanan. Kebelakangan ini Spirulina platensis ditanam di dalam reaktor fed-batch Untuk mengoptimiskan pembesaran sel dan pembentukan produk. Penghasilan Spirulina platensis dalam skala yang besar member kelebihan terutamanya di dalam kos penghasilan. Walaubagaimanapun, ia adalah sukar untuk mengawal produk akhir kepada nilai yang diingini akibat gangguan yang berlaku semasa proses penanaman. Oleh itu, dasar Mid-Course Correction (MCC) yang diperkenalkan dalam proses. Melaluui MCC, peramal model dibina untuk meramal kepekatan produk akhir berdasarkan data eksperimen sebelumnya. Satu model kawalan membina untuk pengeluaran phycocyanin berdasarkan pelarasan keamatan cahaya. Pelarasan itu dibuat pada 5 hari proses penanaman dan simulasi dilakukan periksa sama ada pengeluaran phycocyanin akhir adalah termasuk dalam julat yang dikehendaki.

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LIST OF ABBREVIATIONS

MCC	Mid course correction
MLR	Multiple linear regression
PLS	Partial least square
NASA	*National Aeronautics and Space Administration*
U-VIS	UV-Visible
SBR	Sequencing batch reactor
MPLS	Multivariate partial least square
RIP	Rolling identification prediction
X_0	Sample taken at initial time process
X	Online measurement taken at time t_s
X_m	Offline measurement taken at time t_s
t_s	Time at control action can be made for the process
X_P	Value of phycocyanin concentration
%	Percentage
UPL	Upper and lower limit
$\ddot{X}_n$	$\ddot{X}_n$ is the sample mean
A	t-distribution value
Sn	Variance
n	Sample number
C_x,	Cell concentration
C_p	Phycocyanin concentration
C_s	Substrate concentration

CHAPTER 1

INTRODUCTION

1.1 Background of Study

The cultivation of *Spirulina platensis* is done in open pond before changing the environment of microalgae cultivation in photobioreactor, fed-batch bioreactor and tubular bioreactor. These systems are easy for controlling and monitoring the cultivation process than in open pond. Open pond also need high maintenance in keeping the water clean and of course the cost production will increase (Singh and Gu, 2010).

Phycocyanin is a protein storage that is widely used in pharmaceutical and blue pigment contain in it is used as natural dye for foods and cosmetics (Belay et al., 1993; Borowitzka, 1994). The strain used for the cultivation of Spirulina platensis is from India. There are two strains that is studied for their excellent of contain high amount of chlorophyll-a content, phycobiliprotein and carotenoids. The strain chosen is from Jal-mahal (Kumar et al., 2011).

Heterotrophic culture is culture that is added carbon substrate into it without light energy while photo autotrophic culture is a culture contains light energy for the microalgae to do the photosysthesis. In the other hand, mixotrophic culture or also known as photoheterotrophic culture is a combination of photoautotrophic and heterotrophic culture. The culture is used to improve the cell density as well as phycocyanin production (Garcia et al.. 2011) .

In order to meet the customer satisfaction, a consistent and uniform product is needed (Dorsey and Lee, 2002). In a large scale production, controlling the final product quality is normal. However, it is difficult to control (Yabuki and MacGregor, 1997). This is due to the disturbance and it is complicated to alter the disturbance because of dynamic model for non linear is always not available for industrial process (Flores-Cerrillo and MacGregor, 2002).

Yabuki and MacGregor (1997) proposed mid-course correction policy to control the final product quality. In the MCC, a predictor model is developed by using historical data experiment. The model predictor is used to predict the final quality of a product, in this study is final phycocyanin concentration. Then the predictor model is checked its validity and feasiblility for the phycocyanin. After that a control model is build based on the historical data from previous experiment.

The control model is used when the final prediction value fall outside of the control region. In this study, light intensity adjustment is determined by using control model. After that the batch is simulated by using the light intensity adjustment at day 5 to determine its final value.

1.2 Problem Statement

Many researchers have done for the synthesis of phycocyanin in fed batch reactor. However, process designing is always becomes problem in the large scale of cultivation of Spirulina platensis (Zhang et al, 1998). In the meantime, the phycocyanin production needs to follow the requirement and specification. However, during early batch process, many quality variables are deviated from expected result due to the disturbance occurred in the middle of the process. The product that can not satisfy customer requirements will increase the production cost. Therefore, it is important keep the final product quality to the optimum and at most yield.

However, it is difficult to control the final product quality in fed batch reactor (Yabuki and MacGregor, 1997). This is due to the existence of disturbance during the process (Flores-Cerrillo and MacGregor, 2002). Disturbance is difficult to detect by operator especially without existence of online measurements. The online instrument however is almost never available. A system is needed to control and monitor the operation. The system also should be able to detect any variations of normal operation and correct it when needed. Therefore, MCC policy is proposed to the phycocyanin production.

1.3 Objectives

Three objectives are used to fulfil this study. There are:

a) To establish MCC final product predictor for phycocyanin production from *Spirulina platensis* in fed-batch reactor
b) To establish MCC control algorithm phycocyanin production from *Spirulina platensis* in fed-batch reactor
c) To validate the developed MCC approach of phycocyanin production from *Spirulina platensis* in fed-batch reactor.

1.4 Scope of Study

In this study, the concentration of *Spirulina platensis* growth, phycocyanin formation and glucose utilization are determined by simulating process. The identification of kinetic modelling, prediction model and control model will be determined during this study. In order to complete this study, few scopes of study are highlighted and should be followed. The scopes of this study are:

a) To develop established bioprocess model in simulation environment
b) To generate adequate number of normal batches under initial variations
c) To develop initial final product concentration prediction model based on single/dual sampling using different techniques (linear/nonlinear)
d) Single predictor
e) MLR
f) PLS
g) To test final product concentration prediction under abnormal conditions and re-optimize if' necessary
h) To develop initial control algorithm using normal data
i) To test control algorithm under abnormal conditions and re-optimize
j) Final validation of MCC under abnormal conditions

CHAPTER 2

LITERATURE REVIEW

2.1 Cultivation of *Spirulina platensis*

Spirulina platensis are a photosynthetic microalga that are widely plant for its nutritional value for humans and animals and is the most popular microalgae used for foodstuffs, food supplements and animal feed (Anupama, 2000; Belay et al., 1997). Though photosynthesis is the main carbon-fixation route in the cultivation of S*pirulina platensis*, but autotrophic photosynthesis and heterotrophic assimilation can be combined in a process called mixotrophy during light phase of cultivation (Marquez et al., 1993; Villarejo et al., 1995; Chen et al., 1996).

Zarouk medium is the first synthetic medium used in cultivation of most *Spirulina* as well as *Spirulina platensis*. These Zarouk medium is used as standard medium. Components in Zarouk medium like suggested by Costa et al. (2004) are 16.8 g/L $NaHCO_3$, 2.5 g/L $NaNO_3$, 0.5 g/L K_2HPO_4, 1.0 g/L NaCl, 0.2 g/L $MgSO_4.7H_2O$, 0.04 g/L $CaCl_2$, 0.01 g/L 0.08 g/L EDTA and micronutrients. The medium is also added with 2 g/L glucose substrate as suggested by Zhang et al. (1998).

Zhang et al. (1998) did a study on growth of *Spirulina platensis* in batch and fed-batch reactor using glucose as their substrate. The medium was sterilised at 121^0C for 15 minutes to prevent any contamination during the process. The temperature used

in this culture is 30^0C and agitated at 300 rpm. The pH was adjusted at 9.5. The cultivation of *Spirulina platensis* takes 13 days to complete.

Several factors are been considered in selecting the culture media for *Spirulina platensis* such as pH, temperature, nutrients concentration, salinity and light but most importantly are light. Light is used as an energy source for the cell growth in heterotrophic and mixotrophic culture and usually used as limiting factor in *Spirulina* cultivation to increase the biomass production (Costa et al., 2004). Other factors are can influenced the cultivation of *Spirulina* too like pH and salinity of a medium. Medium that contains high pH and salinity can prevent contamination of the reactor by bacteria, algae and protozoa (Walach et al., 1987).

2.1.1 Photoautotrophic

Spirulina platensis is cultured photoautotrophically because microalga needs light to undergo photosynthesis and makes its own food. However, different organism possesses different light intensity to growth. *Spirulina* also have their own range of light intensity (Samuel et al., 2010). If the light intensity is too low or too high, the growth of cell will decrease. Chen et al (1996) has study on effect of light intensity on phycocyanin production. Figure 2.2 shows the effect of light intensity on phycocyanin production when different light intensity is applied on the *Spirulina platensis* cultivation.

From the figure, the highest phycocyanin content is at 4 Klux followed by 2 and 0.4 Klux. They also suggested on the stepwise increased in light intensity to increase high cell density and productivity (Chen et al, 1996). The increasing of light intensity step-wisely is also proposed by Zhang et al. (1998). They fitted the cultivation time which is 13 days with light intensity is between 80 to 160$\mu Em^{-2}s^{-1}$. At day one to day 4, the light intensity is maintained at 80 but increased about 20 for each 2 days interval. Lastly at day 11 to 13, the light intensity is increased to 160$\mu Em^{-2}s^{-1}$. The result of effect of light intensity to phycocyanin concentration is shown in Figure 2.3.

From the figure, the highest phycocyanin concentration is when the light intensity increased step-wisely at 640+10t and at 320 when light intensity constant is applied to the fed-batch reactor. It is therefore, it can be concluded that light intensity can increased phycocyanin concentration especially when light intensity is gradually added day by day.

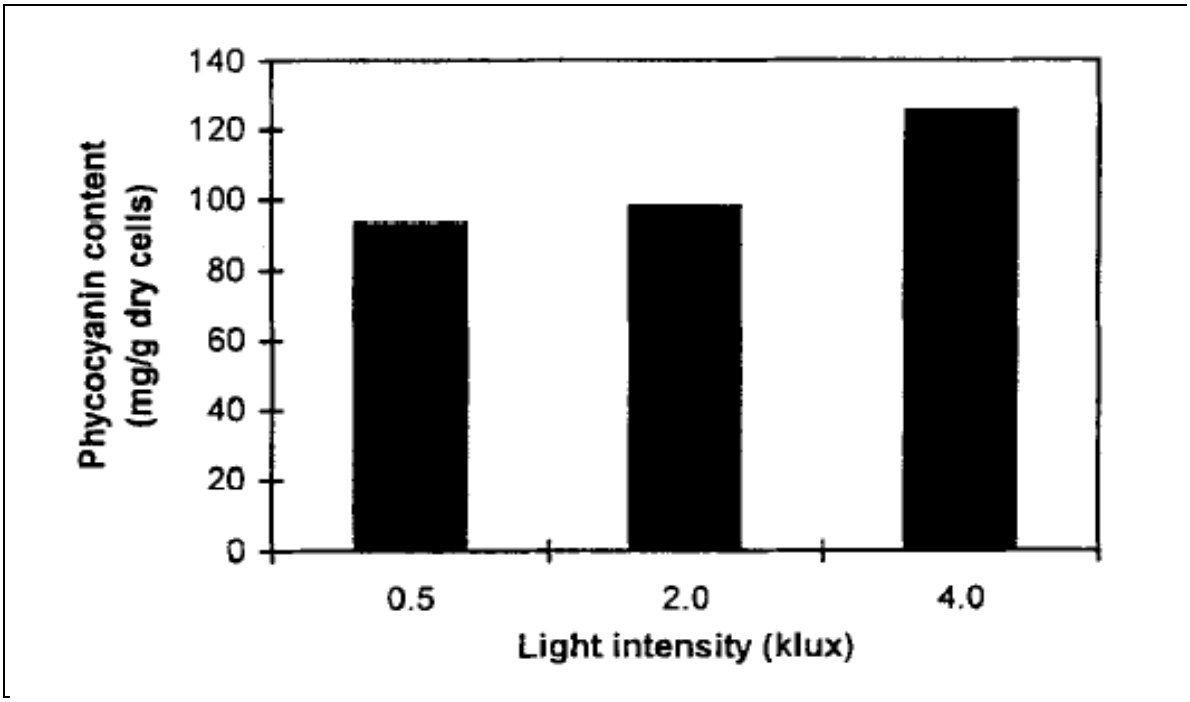

Figure 2.1: Phycoyanin content versus light intensity (Chen et al., 1996)

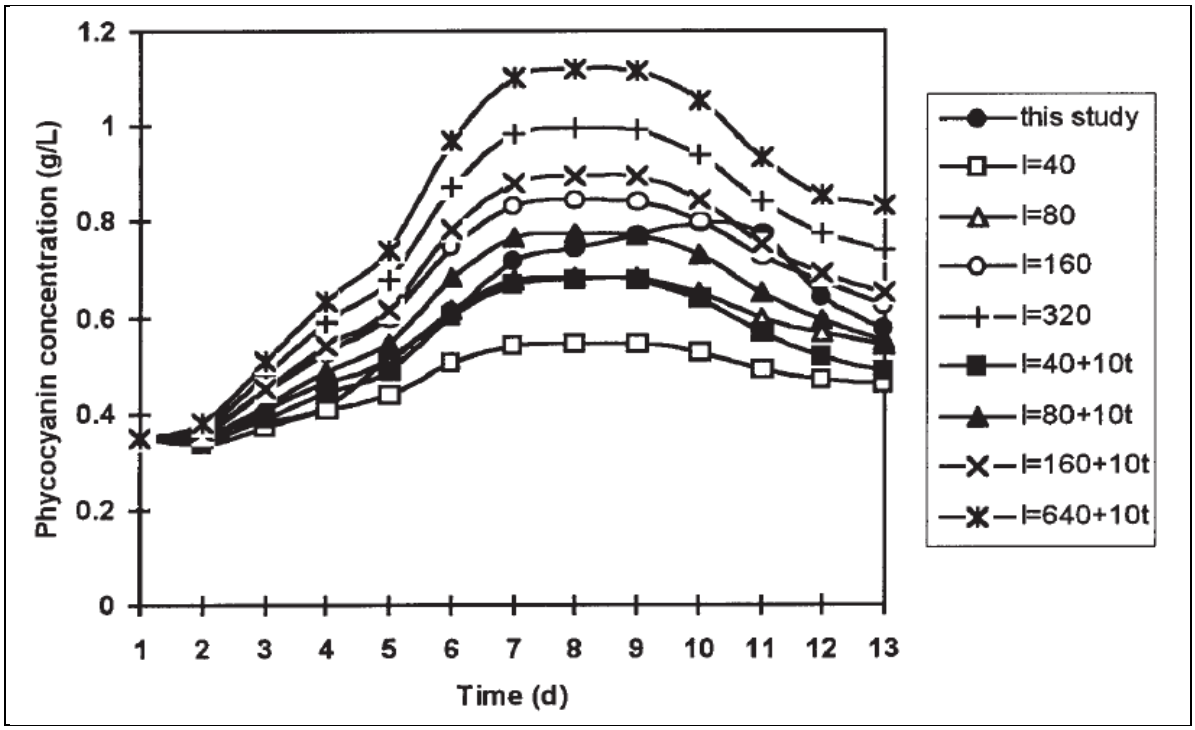

Figure 2.2: Effect of light intensity on phycocyanin concentration (Zheng et al., 1998)

2.1.2 Heterotrophic

Heterotrophy is the use of organic compound for growth and the heterotrophy organism that derives substrate and energy from organic compounds synthesized by other organisms. Therefore, they do not depend on light energy like photoautotrophic algae. In the heterotrophic culture, an organic carbon is added into the reactor and is utilized by microorganism inside it. Chen and Chen (2006) said that heterotrophic system provides a high degree o growth control as well as lower harvesting cost because of the higher cell densities achieved. It is also support by Miao and Wu (2006) study under similar conditions on *C. Protothecoides* where the lipid content in heterotrophic cells is 55%, 4 times higher than in a photoautotrophic cell which is 15%.

Heterotrophic culture is unsuitable for many microalgae since most of microalgae undergo photosynthesis. Hence, heterotrophic is only appropriate to those microalgae that grown in darkness such as Chlorella which gives 5.5 higher than

cultured under photoautotrophic growth. (Yang et al, 2000). However, heterotrophic culture need lower cost of production because of lower cost of harvesting and also minimal cost of set-up. This is due to the higher cell density acquired in the cultivation process. Phycocyanin is not suitable in the heterotrophic culture because phycocyanin is the major photosynthetic accessory pigment (Chen et al., 1996). Moreover, Marquez et al. (1993) has shown that cultivation of *Spirulina platensis* is suitable in the medium under photoautotrophic conditions than heterotrophic.

2.1.3 Mixotrophic

Spirulina platensis is one of the microalgae that can use mixotrophic culture to growth. Moreover, *Spirulina platensis* can produce more phycocyanin when cultivated in mixotrophic condition. In mixotrophs, the cell growth is not dependent on light only but also on the organic substrate (Andrade and Costa, 2007). Brennan and Owende (2009) said that light energy is not a limiting factor for cell growth in the mixotrophic cultivation because during dark and light phase, *Spirulina platensis* will undergo aerobic respiration and photosynthesis process.

A study of mixotrophic *Spirulina platensis* growth has been conducted by Andrade and Costa (2007) in a photobioreactor with varies of molasses concentration. The 25 days of cultivation shows the relationship between growth rate, light intensity and substrate utilization. They show that molasses is the main factor that effects the biomass concentration even with the existence of light. This is because light intensity only increases the cell growth after 11 days of cultivation.

2.2 Carbon Substrate

Carbon substrate is essential in the cultivation of *Spirulina platensis* which can influence cell growth of the microalgae. *Spirulina platensis* can utilize organic carbon to growth (Chen et al., 1996). Chosen the right carbon sources for *Spirulina* cultivation

can reduce the production cost for phycocyanin. Chen and Zhang (1997) have done a study on phycocyanin production from *Spirulina platensis* using glucose as their carbon substrate. It turns out that the increasing of biomass and product formation.

A research from Borsari et al. (2007) which is about growth of *Nostoc* sp in mixotrophic culture using glucose, sucrose and molasses shows that molasses is the best carbon sources among these three. Using molasses as the substrate had increased the biomass and phycobilin proteins formation. This is because of high nutrients in the molasses. Therefore, it not only has a great carbohydrate concentration but also nitrogenous substances, vitamins and trace elements.

2.3 Phycocyanin

Spirulina has high demand in nutritional food due to its high value of phytonurients and pigments. The content in *Spirulina* is used in the health foods, animal feeds, therapeutic and diagnostic (Becker, 1994; Richmond, 1992). It also been considered as food of the future and NASA has suggested *Spirulina* as an ideal food for astronauts. Additionally, about 74% proteins dry weight, along with high concentrations of minerals, pigments, unsaturated fatty acids and vitamins are contained in cyanobacterium *Spirulina* (Cohen, 1997).

One of the products from *Spirulina* is phycocyanin and is the most chosen among these protein pigments (Raoof *et al*., 2005). The single visible absorption for this pigment is between 615 and 620 nm while the maximum emission of fluorescence is at 650 nm. Other than have high absorption and visible wavelengths, phycocyanin has large Stokes shift and high quantum efficiency as well. All these properties make phycocyanin able to be used in a variety of immunological assays and as fluorescent labels for cell-sorting (Kronick, 1986).

2.3.1 Downstream Process of Phycocyanin Production

Figure2.4 shows the schematic diagram of cultivation microalgae until it becomes product. Downstream process in the production of phycocyanin is harvesting, solid recovery and extraction of algae oil.

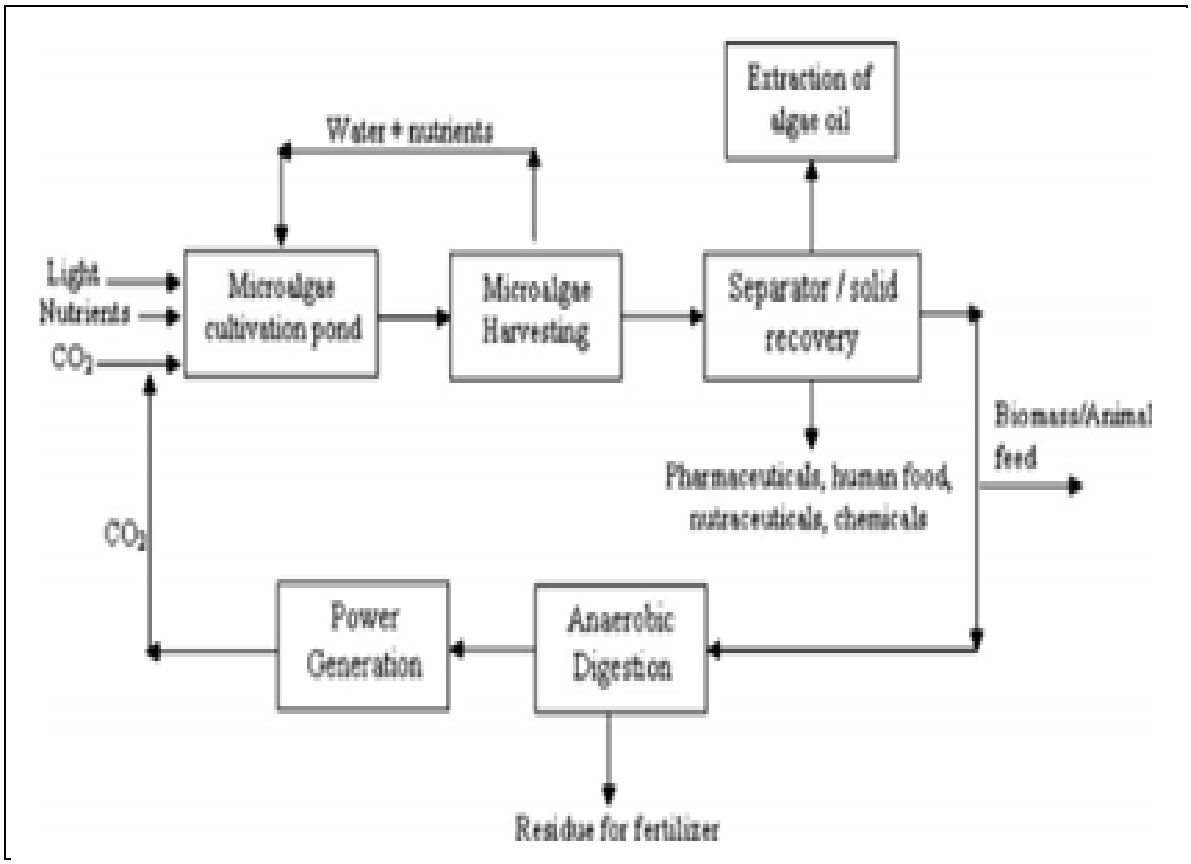

Figure 2.3: Schematic Diagram of Cultivation of Microalgae

2.3.1.1 Harvesting of Phycocyanin

Harvesting a micro alga needs a consideration on the size, density and the value of the target product since any technique chosen for harvesting must consider all these characteristics. There are two stages of harvesting process which bulk harvesting and thickening. Bulk harvesting is separation process while thickening is slurry concentrating. Bulk harvesting technique includes flocculation, flotation or gravity

sedimentation and it depends on the concentration of initial biomass (Brennan and Owende, 2009).

Technique use in thickening is centrifugation, filtration and ultrasonic aggregation and its objective is to get the concentrated slurry. In the phycocyanin production, *Spirulina* is harvested by using biomass filtration. This is because *Spirulina* is large microalgae and filtration is the conventional method used in harvesting *Spirulina.* Under pressure operations the efficiency of filtration is improved by filtration aids such as cellulose and diatomaceous earth (Brennan and Owende, 2009).

2.3.1.2 Extraction

Breanna and Owende (2009) said that there are five methods in extracting phycocyanin from wet biomass; water extraction, homogenisation of cells in mortar and pestle, freezing and thawing, homogenizatio in virtimixer and acid extraction. In the first method, the suspended *Spirulina* in distilled water undergo extraction process. The leachate is phycocyanin and is estimated by using spectrophotometrically. In the second method, with the presence of acid washed neutral sand, biomass is homogenized in a mortar and pestle. Then the extract was centrifuged and phycocyanin contain in supernatant. In the third method, phycocyanin is extracted by freezing and thawing the cell repeatedly in 50 mM phosphate buffer at pH 6.8. Then the fourth method is the homogenization process at 5, 10, and 20×1000 rpm for 10 minutes in Virtimixer at different speeds. The biomass is suspended in 50mm phosphate buffer at pH 6.8. in the last method, the wet biomass is treated with different HCl concentration at room temperature. Samples were centrifuged and supernatant is phycocyanin and will be estimated. All the estimation of phycocyanin is calculated by using equation

$$OD_{at\ 615nm-0.474} - (OD_{at\ 625nm})/5.34 \qquad (2.1)$$

2.4 Fed-batch Reactor

Microalgae like *Spirulina platensis* are cultivated conventionally in an open pond which has low cell concentration. This is because open pond can provide a sufficient light intensity for the cell growth (Chojnacka and Noworyta, 2003). The energy from light will be absorbed by the microalgae and fix inorganic carbon (Lodi et al., 2005). Fed-batch process is inconstant process variable where a substrate is added in steps along the process and therefore has lack of stationary phase (Gregersen and Jorgensen, 1999).

Moreover, using fed-batch reactor for the Spirulina platensis cultivation gives much advantage. Fed-batch can avoid repressive effect caused by carbon sources because carbon sources which may be present are rapidly removed by the microorganism being cultivated. Repressive also cause by shortage nitrogen that limits the alga growth in the system with low alga growth (Fried et al, 2003). Other than that, the effect of toxic components in fed batch is restricted by dilution plus medium viscosity is also reduced in the fed-batch reactor (Ward, 1989).

2.5 Control quality of a product

Quality of a product is described as the characteristic of the product that satisfies the customer specifications and requirements. Therefore, to meet the satisfaction, an objective of production of uniform and consistent is made throughout the production process (Dorsey and Lee, 2002). In industrial plants, it is common to control the quality product (Flores-Cerrilo and Macgregor, 2002).

However, control of the final quality product is quite difficult as said by Yabuki and MacGregor (1997). The reason is the nonlinear dynamic behaviour from batch or semibatch reactor is very high and if there is dynamic model for the nonlinear, it is not often available for industrial process. Therefore, it is difficult to alter the disturbance.

Other than that, the changes in raw material properties such as viscosity, stability and opacity make it harder to achieve final product (Flores-Cerrillo and MacGregor, 2002).

Control of final product can be achieved via several processes. One of them is by using real time online monitoring such as UV-VIS. According to Langergraber et al, real time online monitoring is applied in-situ where the results displayed without doing sampling or sample treatment. It was applied in the control a pilot-scale sequencing batch reactor (SBR). In this process, UV-VIS is installed to the reactor and measures several parameters simultaneously in real time by range of wavelength is between 200 and 750nm in 15s time interval for every measurement (Langergraber et al).

Other than that, control of final quality product can also be attained by using multivariate partial least square (MPLS) where history or previous batch process data were used to obtain the empirical models. These models then will be used in monitoring the upcoming batch runs. In the study of styrene-butadiene batch reactor, Nomikos and MacGregor (1995) had applied MPLS to predict the final product by online predictor for used in control the quality product. However, MPLS are prone to measurement error and difficult to control if the process is having enough variables to the product quality.

2.5.1 Predictor model

The quality of a final product is usually can be control by using various methods available nowadays. These methods usually derived from the requirements of a plant production to generate a control system for an easier used in controlling the quality of a product. Predictor model is created to predict final quality of a product based on their requirements and satisfactory.

Prediction model is used to ensure the final product can be identified and between in the range of upper and lower limit (Kourti et al, 1995). In the process of developing a predictor model, a historical database is needed and must be readily

available in any computer-monitored industrial batch process (Ferreira et al, 2006). Moreover, multivariate methods and Multiway Partial Least Square are used to get the information from trajectories or from measured batch variables and will be used in defining low dimensional space (Kourti et al, 1995).

Study of a model-based predictor for penicillin concentration is introduced by Yuan et al. (2001); there are 5 stages process where at initial fermentation, the biomass concentration increased rapidly and after the stage, the biomass increases almost linearly. Then the decreases of product formation are observed and at the next stage is the stationary stage for biomass concentration. The online and offline data measurement is taken at the same time. Yuan et al also use technique proposed by Guo et al. (1995) called Rolling identification Prediction (RIP) for online identify the parameter that they choose. The outline of RIP is shown in figure 2.5.

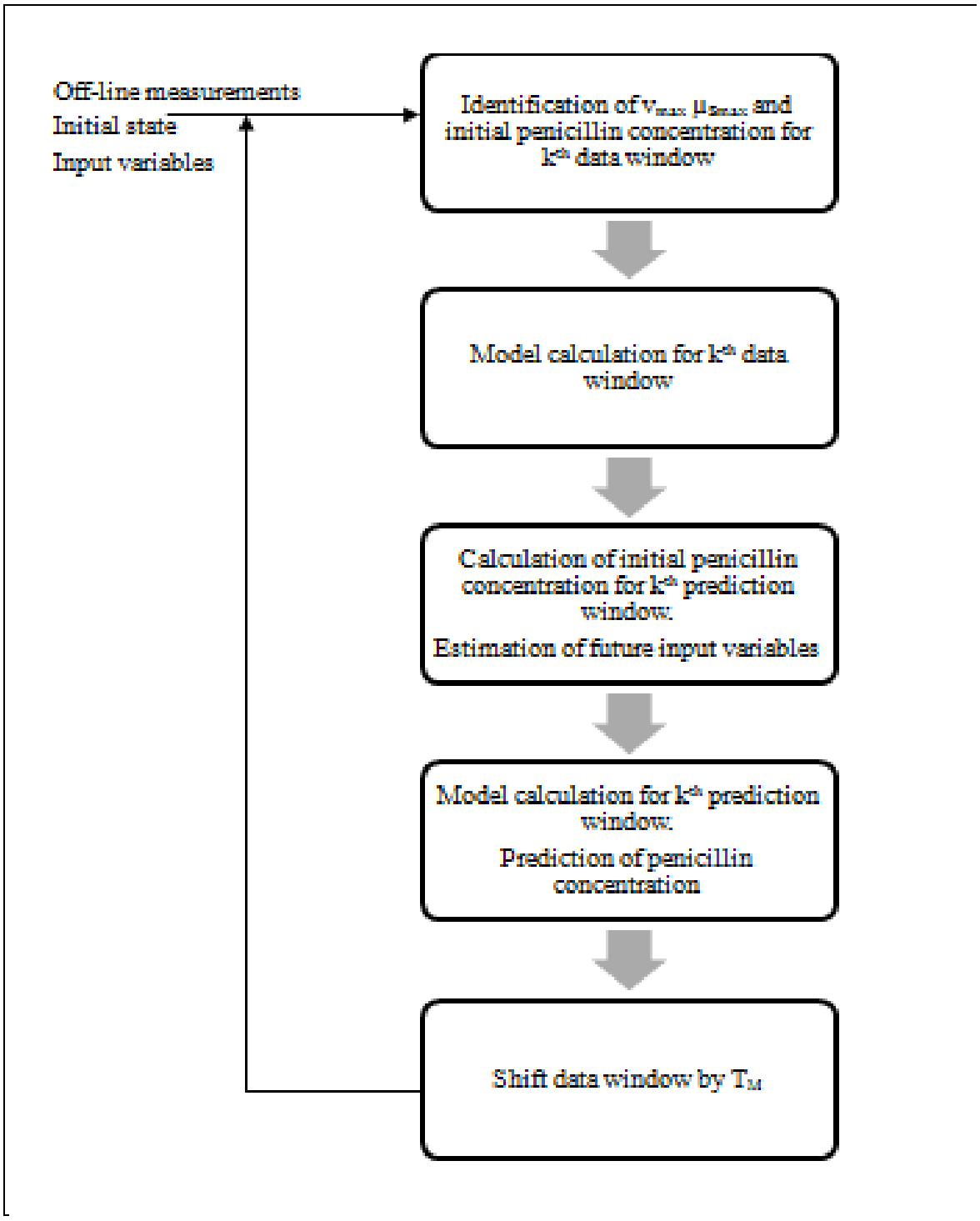

Figure 1.4: Rolling Identification Prediction

The identifying process was done to minimize the sum of square errors between model simulated, measured penicillin and sugar concentration in the related data window. Then the prediction is evaluated and the result shown in figure 2.6.

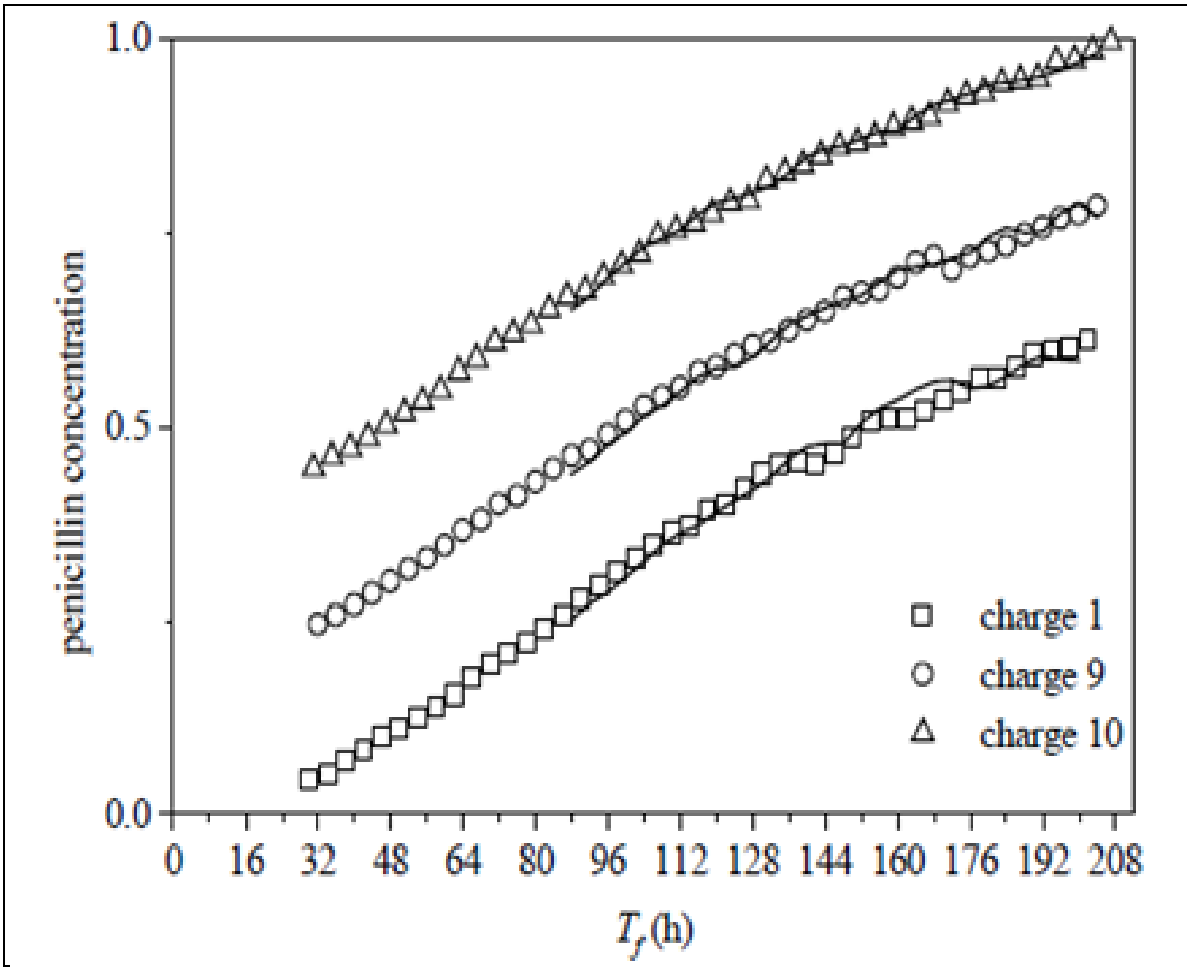

Figure 2.5: Comparison between rolling identification prediction (32 h ahead) and measurements of penicillin concentration for charges 1, 9 and 10. Symbols are measured data, lines are predictions

2.5.2 MCC policy

Among all the control of the final quality product, one of them is control by combination of online measurement and offline laboratories analysis called mid course correction (MCC) policies. An example of MCC applied is in the quality control of molecular weight and cross-link density in the semibatch emulsion polymerization of styrene-butadiene rubber by Yabuki and MacGregor (1997). Figure 2.1 shows application of MCC policy in a production. X_0 is the sample taken at initial time process, X and X_m is an online and offline measurement taken at time t_s. t_c is time at control action can be made for the process.

In the study From Yabuki and MacGregor (1997), after 200 min of semibatch is operated, a sample of latex is collected and undergoes lab analysis. Data collected form offline measurement is combined with online temperature measurement to build a predictor model to estimate the final properties of molecular weight and cross-link density. If the prediction falls inside the control region defined, so no mid-course adjustment is made. However if the prediction falls outside the control region, at time 230 min, a shot of initiator or either inhibitor is introduced to the semibatch reactor and the process is continue as usual. Figure 2.1 shows the whole process of polymerization process and mid-course control approach.

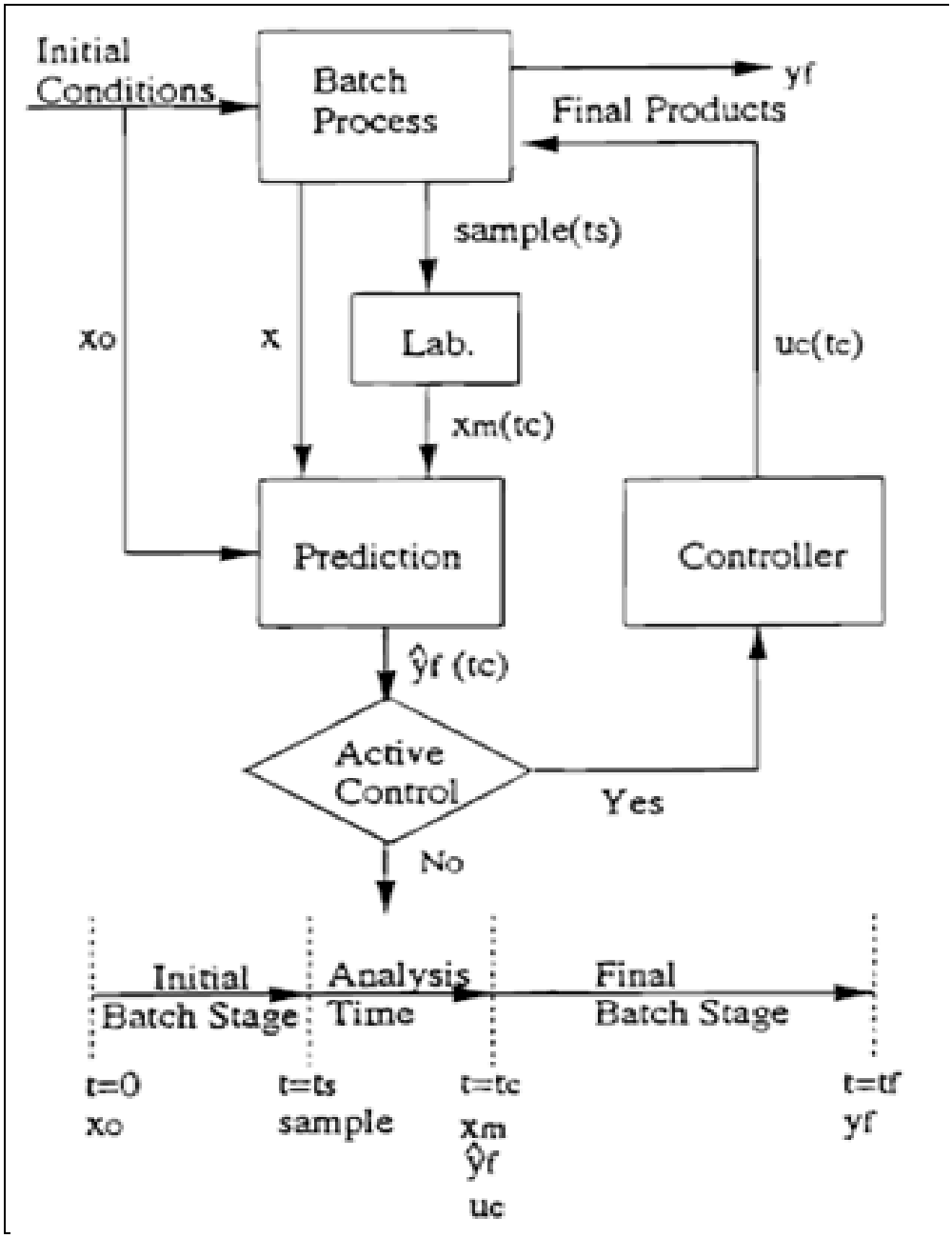

Figure 2.6: Mid-course strategy applied in the quality control of molecular weight and cross-link density in the semibatch emulsion polymerization of styrene-butadiene rubber (Yabuki and MacGregor, 1997)

CHAPTER 3

METHODOLOGY

This chapter of this study will focus mainly on the instrumentation of the study, and also the method used to in order conduct the study. This instrumentation and methodology will focus on prediction model for normal batch of production of phycocyanin by *Spirulina platensis* in abnormal condition and control of algorithm for abnormal condition of this batch process.

3.1 Methodology Flow Chart

This methodology flow chart represents the steps of process that needed to be done in order to complete this study.

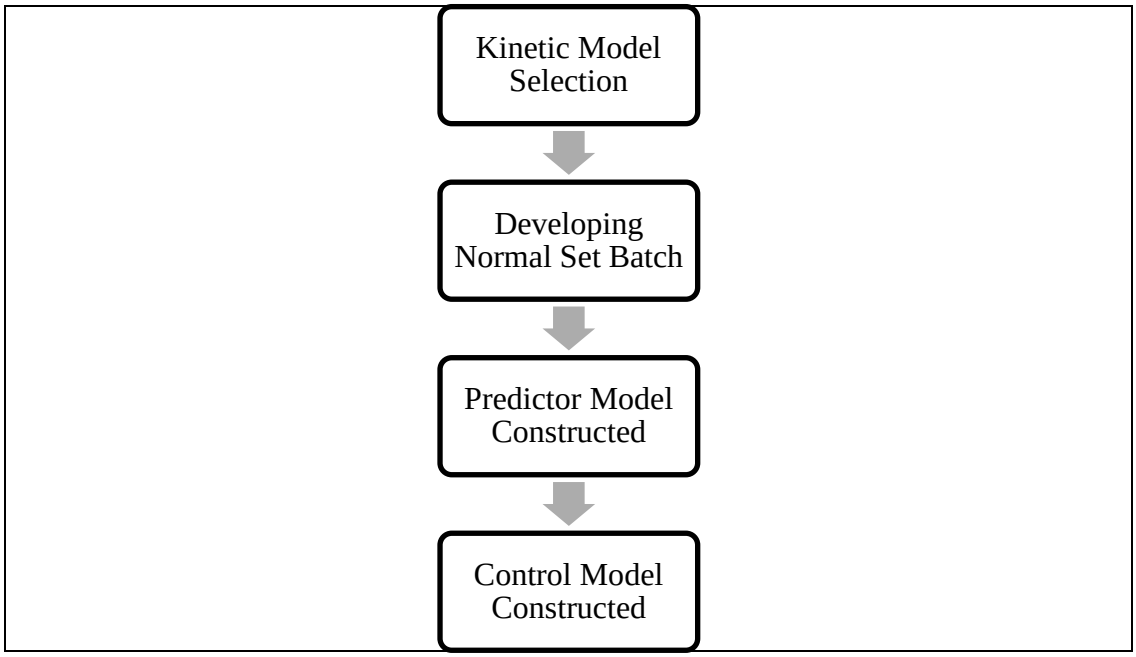

Figure 3.1: Methodology Flow Chart for Phycocyanin Production

3.2 Kinetic Model Selection

Kinetic models for cell growth, substrate utilization and product formation is selected to be used in developing a steady state model for phycocyanin production. The kinetic models selected were derived by Zhang et al (1998) as shown in equation 3.1, 3.2 and 3.3.

For cell concentration

$$\frac{dC_x}{dt} = \frac{0.8831 C_S C_X}{0.0972 + C_S + \frac{C_S^2}{64.3891}} \frac{I}{45.2466 + I} \left(1 - \frac{C_X}{13.7520}\right) \left(1 - \frac{C_P}{0.7386}\right) \quad (3.1)$$

For product concentration

$$\frac{C_P}{dt} = \left(1.8533\frac{dC_x}{dt} + 0.0062C_X\right)\frac{I}{129.8042+I}\ \frac{C_S C_P}{0.048+C_S+\frac{C_S^2}{40.3680}}\left(1-\frac{C_P}{0.7386}\right) \quad (3.2)$$

For substrate concentration

$$-\frac{dC_S}{dt} = \frac{C_S}{0.2982+C_S}\left(\frac{1}{4.0210}\frac{dC_X}{dt} + \frac{1}{0.7692}\frac{dC_P}{dt} + 0.0014C_X\right) \quad (3.3)$$

where C_x, C_p and C_s are cell concentration (g/L), phycocyanin concentration(g/L) and substrate concentration(g/L) respectively whereas I indicates to light intensity ($\mu Em^{-2}s^{-1}$). $\frac{dC_x}{dt}$ is the rate of cell growth, $\frac{dC_P}{dt}$ is the rate of product formation while $\frac{dC_S}{dt}$ is rate of glucose utilization.

By using Matlab R2009b, a steady state model of phycocyanin production has been constructed as shown in Figure 3.2 and Figure 3.3 while Figure 3.4 is the function block parameter. Then in the function block parameter, the initial value of cell (2g/L), phycocyanin (0.01g/L) and glucose (g/L) concentration (Zhang et al., 1998) are inserted and button OK is clicked.

The value of light intensity is adjusted starting at 80 at day 1 to 4 and increased to 100 at day 5 and 6, 120 (day 7 and 80), 140 (day 9 and 10) and 160 (day 11 to 13) as suggested by Zhang et al. The stepwise increase of light intensity is shown in Figure 3.5. Finally the simulation model is run by clicking the start simulation button.

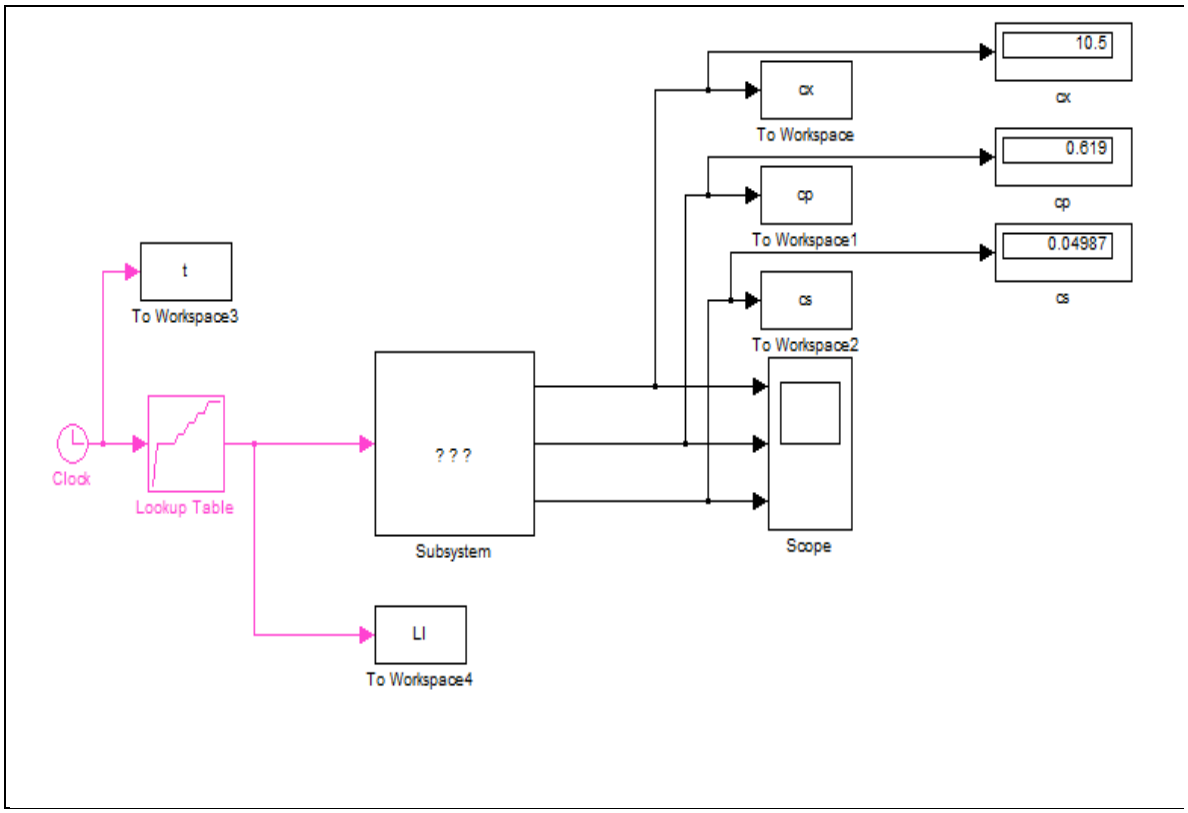

Figure 3.2: Overview of Model Build In Matlab-Simulink

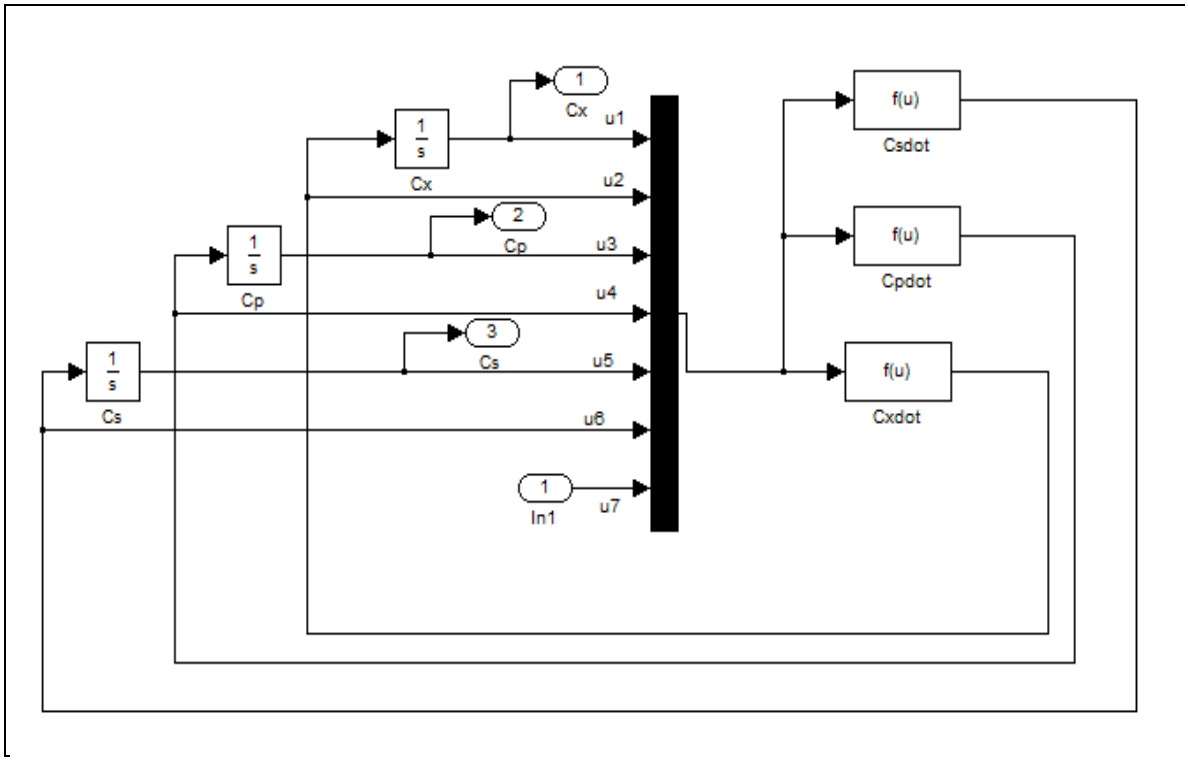

Figure 3.3: Overview of Subsystem Model Built In Matlab-Simulink

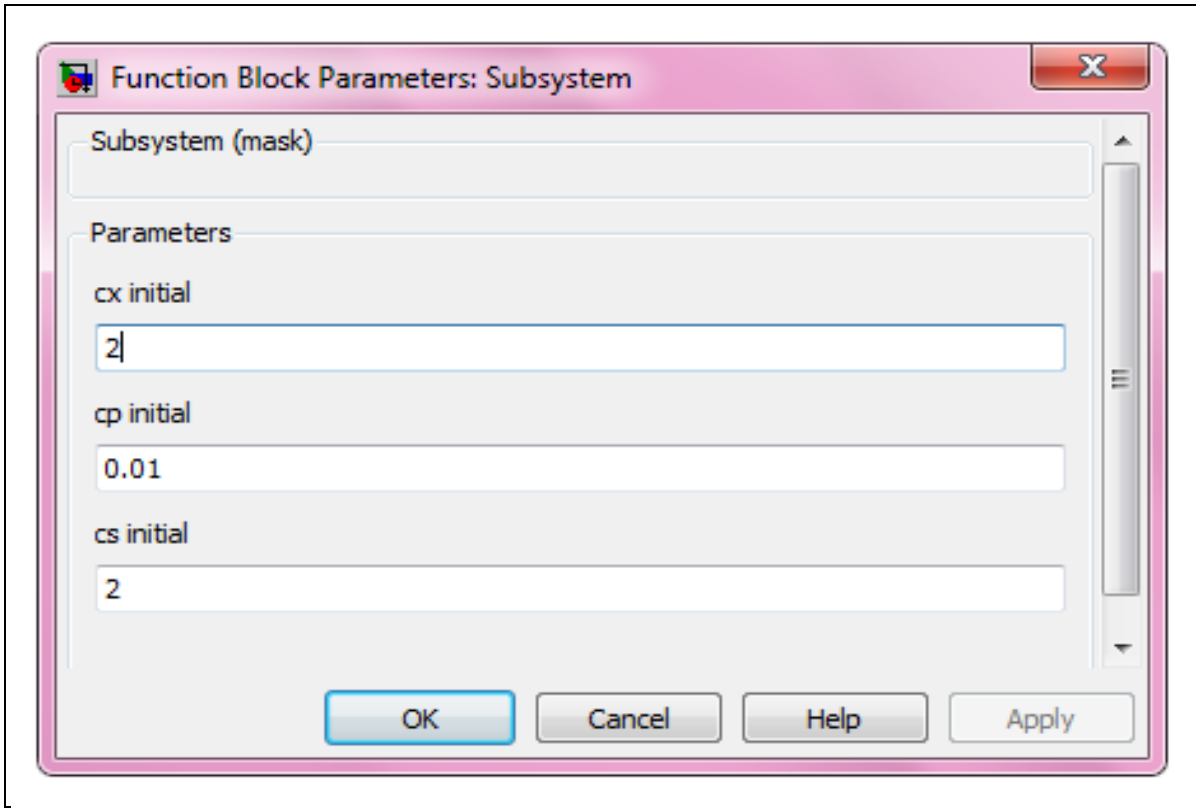

Figure 3.4: Function Block Parameter

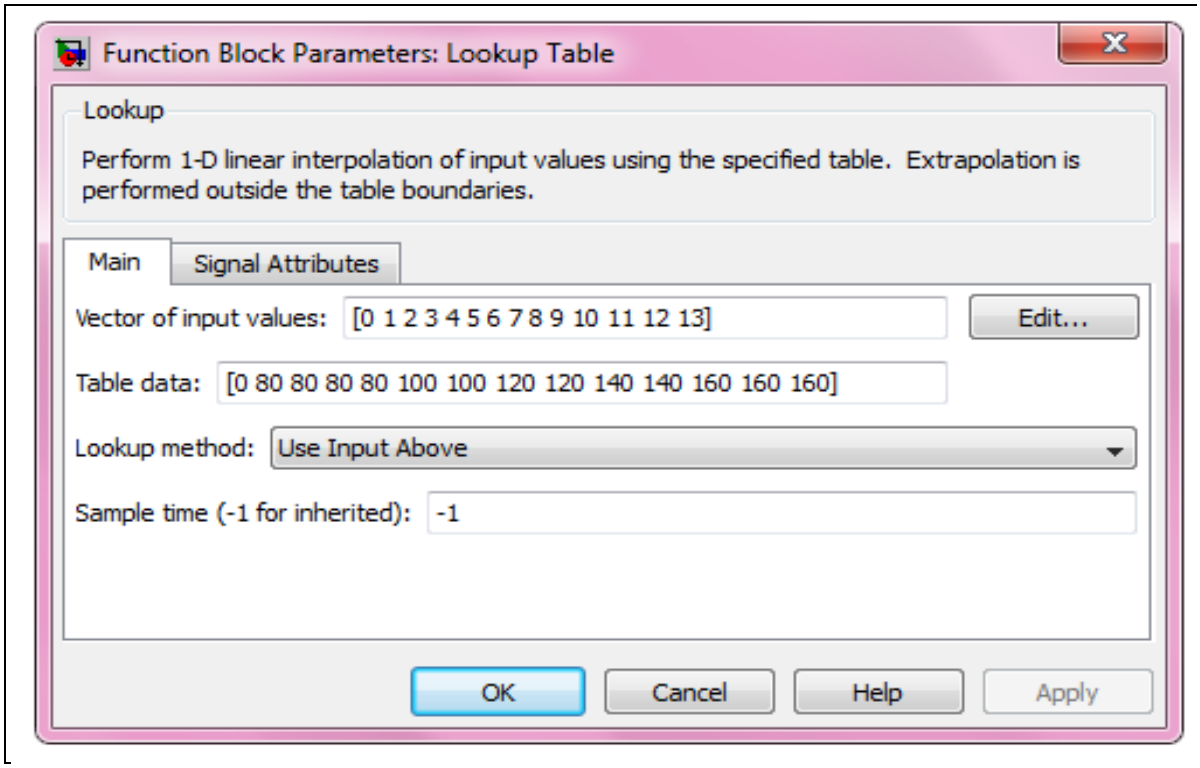

Figure 3.5: Adjusting of Light Intensity

3.3 Developing Normal Set Batch

A normal set batch is developed by using variation of initial cell and product concentration. An assumption is made where the initial variation is based on $\pm 20\%$ of initial value of cell and product concentration that used in part 3.1 before. However, the substrate concentration and light intensity are remained constant. The control variable selected for this part is phycocyanin concentration at day 13 while phycocyanin concentration at day 3 is chosen as manipulated variable. Data gathered from the simulations is used in plotting a graph of normal set batch. The data also will be used in part 3.3 later.

The light intensity is also being manipulated later where during each batch of simulation light intensity is increased as shown in table 3.1 while cell and product concentration remained constant. Table 3.1 shows the value of light intensity adjusted. Data collected from the simulations are then plotted and compared with graph from the previous study by Zhang t al. (1998).

Table 3.1: Light Intensity Adjusted

Batch Simulation	**Light Intensity ($\mu Em^{-2}s^{-1}$)**
1	40
2	80
3	160
4	320
5	40+10t
6	80+10t
7	160+10t
8	640+10t

3.4 Predictor Model Constructed

A predictor model is built based on the previous data collected at part 3.2. Then, the predictor model is verified its validity throughout a validation process where a few random values of initial cell and phycocyanin concentration is simulated and final concentration value for each of them is recorded. Table 3.2 represents the validation process which referred to the value of phycocyanin concentration at initial, day 3 and day 13.

Table 3.2: Summary of test for predictor model

$X_{P,\ initial}$ (g/L)	$X_{P,\ day\ 3}$ (g/L)	$X_{P,\ predicted,\ final}$ (g/L)	$X_{P,\ actual,\ final}$ (g/L)	% error

In the validation process, both the actual and predicted value of phycocyanin concentration at day 13 is compared and the percentage error of them is calculated and recorded. The actual value is measured when the initial value is simulated while the predicted value is get using equation of predictor model.

3.5 Control Model Constructed

In order to build a control model of phycocyanin production, new manipulated variable is introduced while control variable is remained as phycocyanin concentration at day 13. The new manipulated variable introduced as light intensity. An assumption is

made in order to meet with the suitability of the control model. At day 3 of fed batch process, a sample is taken to be analyzed. It is assumed that the time taken to finish the analysis is one day considering the recovery and purification process. The phycocyanin concentration is measured and together with online measurements, the final phycocyanin concentration is predicted. If the value falls outside the control rgion, a control action is taken at day 4 by adjusting the light intensity needed to get the desired final concentration.

In this part, a control region is also defined where upper and lower limit is calculated from the equation 3.4 (easycalculation.com). UPL is the upper and lower limit, $\ddot{X}_n$ is the sample mean, A is the t-distribution value, Sn is variance and n is number of sample.

$$UPL = \ddot{X}_n \pm A\frac{S_n}{\sqrt{n}} \qquad (3.4)$$

Table 3.3 shows the measurement values to build a control model.

Table 3.3: The measurement values for control model development

$X_{P,\ day\ 3}$	$X_{P,\ predicted,\ final}$ (Y'_f)	**Light intensity adjustment at day 4 onwards (ΔLI)**	$X_{P,\ actual,\ final}$ **after LI adjustment,** Y_f	$\Delta=Y_f-Y'_f$

Data gathered from table is used to construct a control model by fitting a graph of $\Delta=Y_f-Y'_f$ vs ΔLI. Then the control model is validated by adjusting the light intensity needed by increasing or decreasing the light intensity according to its needed to identify its final value.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Kinetic Model Selection

From the kinetic models selected, a simulation of phycocyanin production from Spirulina platensis is simulated. The optimum values are 2g/L for both *Spirulina platensis* and glucose concentration and 0.01g/L for phycocyanin concentration which extracted from Zhang et al (1998). The result obtained is shown through Figure 4.1. Figure 4.1 shows that there are three graphs which referred to *Spirulina platensis* concentration, phycocyanin concentration and glucose concentration respectively. 10.59g/L, 0.6085g/L and 0.04894g/L are the concentration of Spirulina platensis, phycocyanin and glucose after 13 days of production time. From the figure, the graphs show that as time increased, *Spirulina platensis* and phycocyanin concentration increased but glucose concentration decreased.

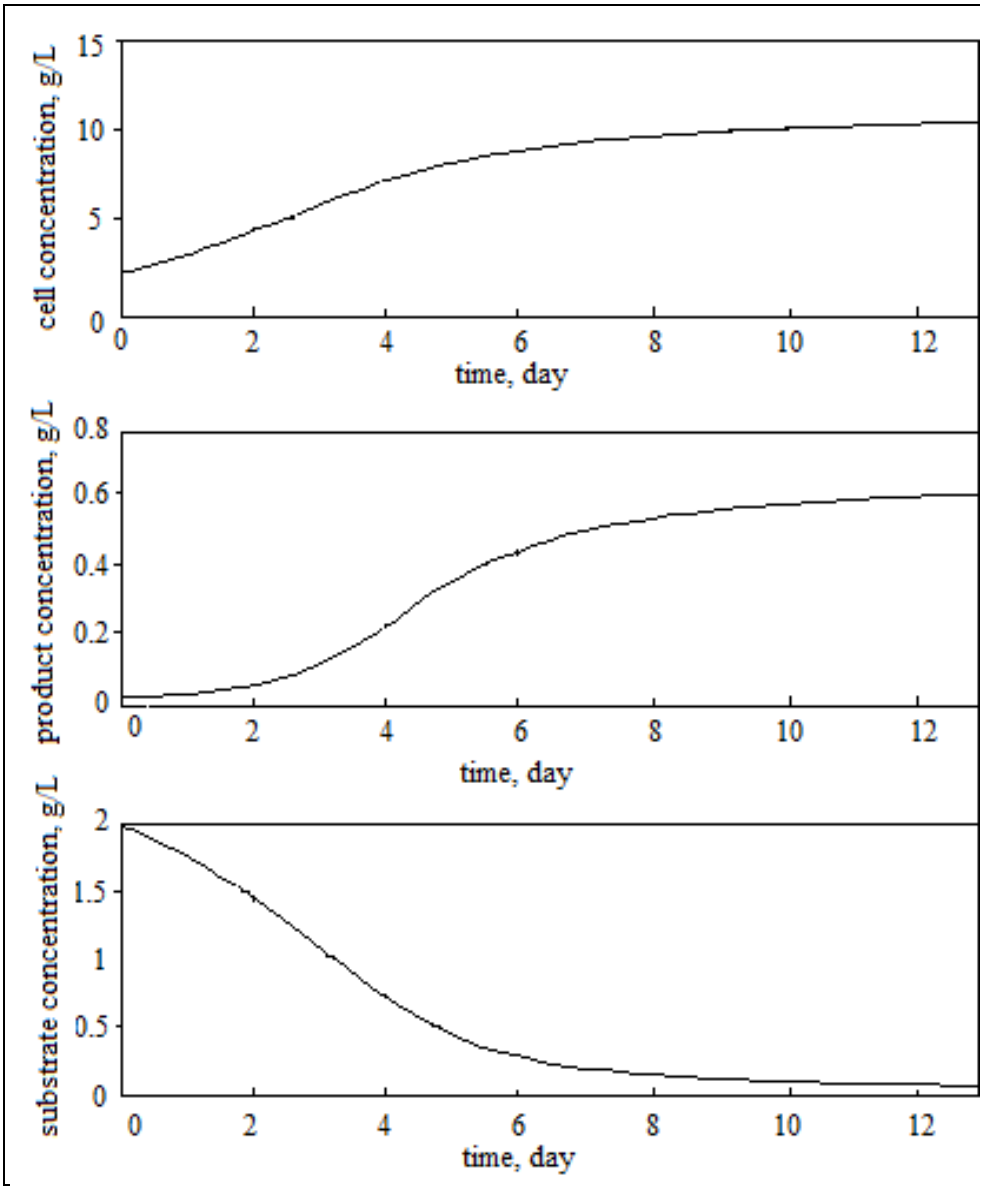

Figure 4.1: Graph of *Spirulina platensis*, phycocyanin and glucose concentration at optimum initial value for each parameter.

4.2 Developing Normal Set Batch

Simulation is continued with the 50 batches of the production are simulated based on the data extracted from previous study and also estimation of $\pm 20\%$ deviation of initial concentration for *Spirulina platensis* and phycocyanin. These simulations are going to be the normal reference batch set for this study. Figure 4.2 and 4.3 are the simulation of 50 batches of *Spirulina platensis* for 13 days production for cell and

product concentration. Cell concentration and product concentration are depended on production time. The graph pattern shows that as time increase, the cell and product concentration will also increase. Moreover, the final concentration for each batch in normal reference set also increase.

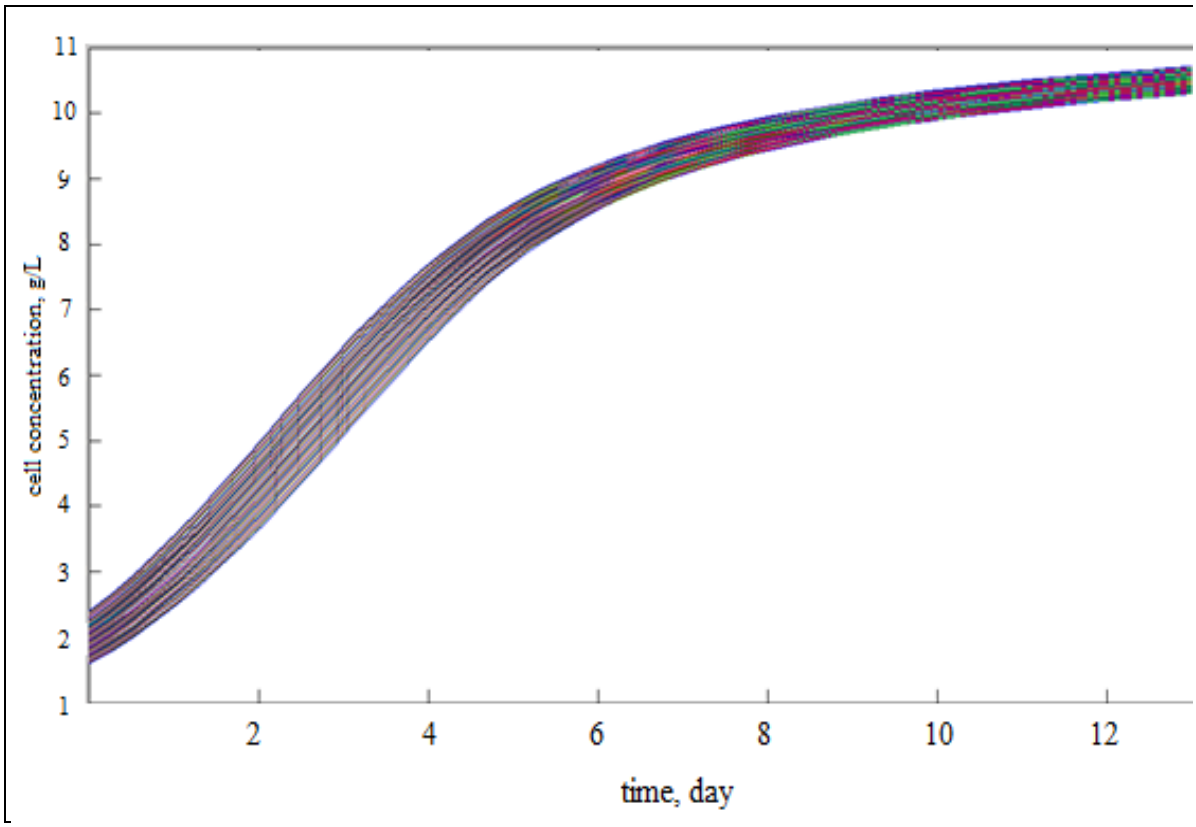

Figure 4.2: Normal batch set for cell concentration

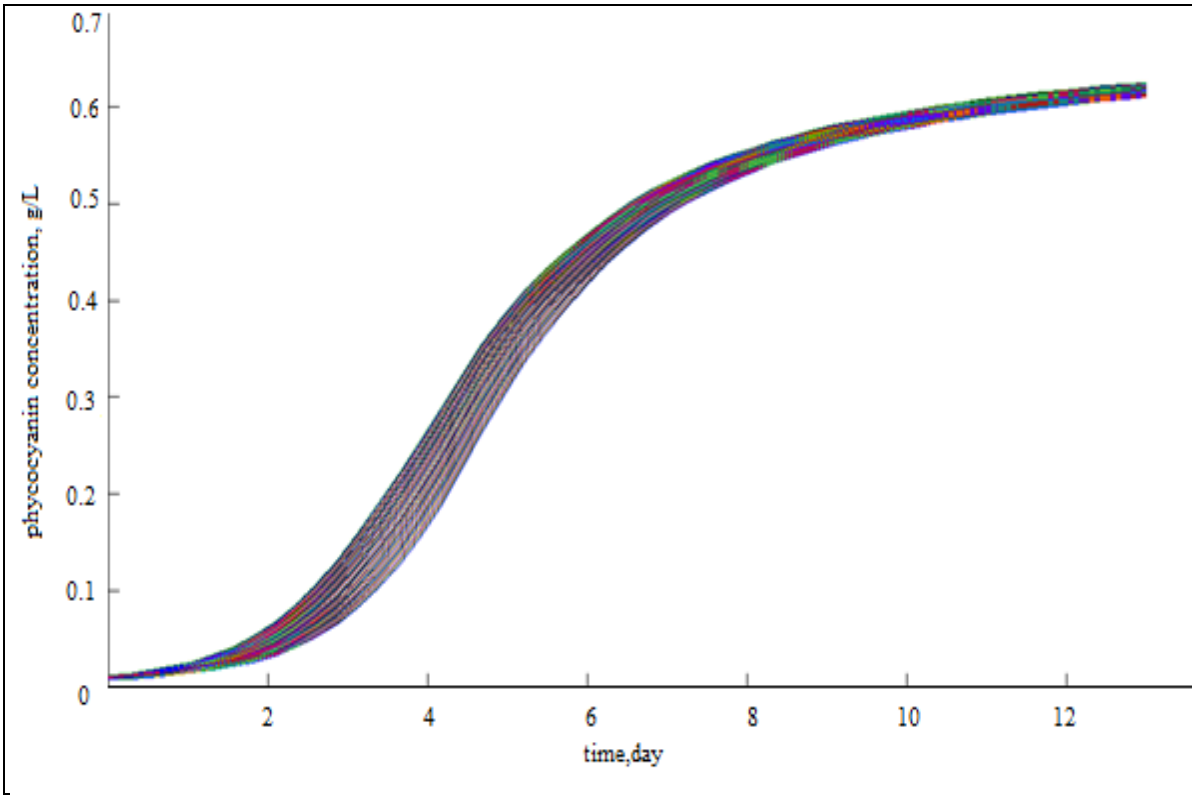

Figure 4.3: Normal batch set for product concentration

Figure 4.4 shows the effect of light intensity to the phycocyanin formation in fed-batch reactor. During 13 days of cultivation, an increasing of phycocyanin concentration is due to the increasing of light intensity. It is clearly shown when different value of light intensity (40, 80, 160 and 320$\mu Em^{-2}s^{-1}$) is used in the cultivation of *Spirulina platensis* plus when light intensity is adjusted by increase it day by day (40+10t, 80+10t, 60+10t and 640+10t) with the highest phycocyanin concentration is at 640+10t of light intensity adjustment.

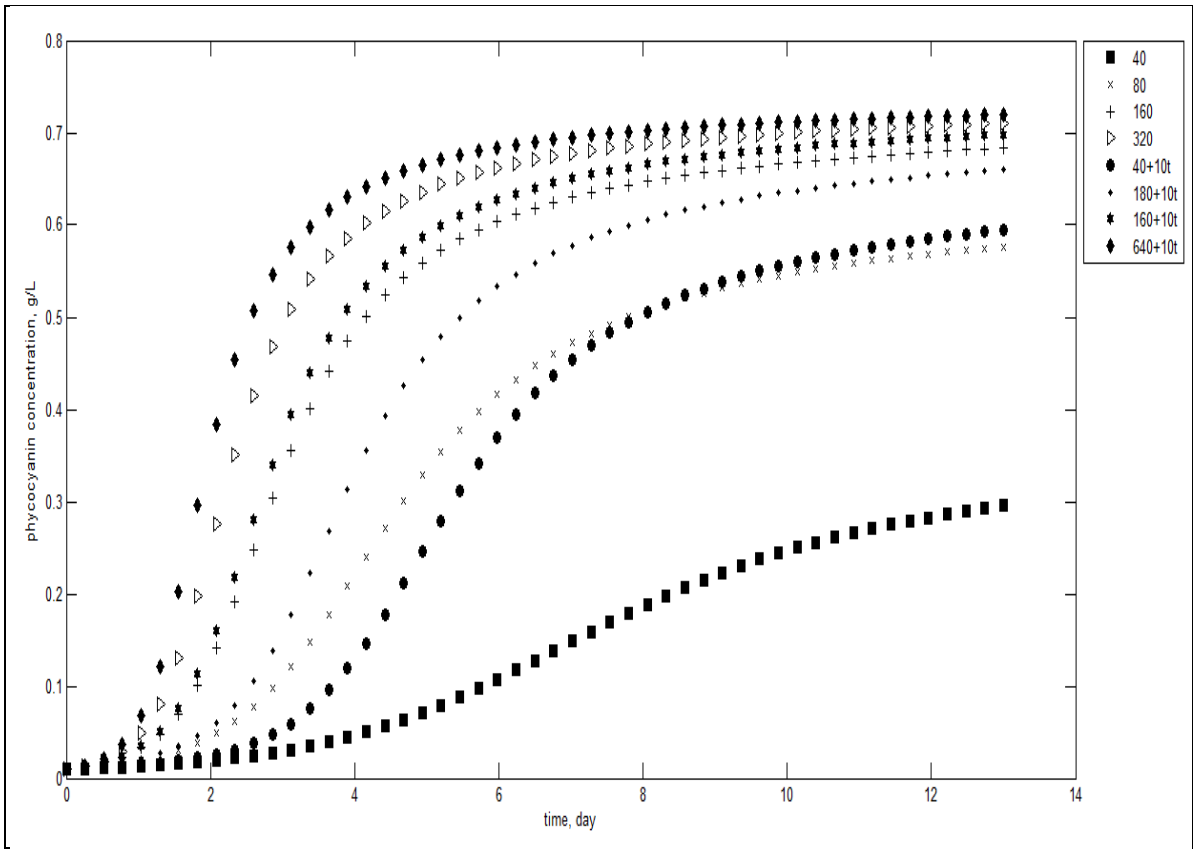

Figure 4.4: Product concentration with various light intensity

4.3 Prediction Model and Its Validation

A model for prediction of the final concentration of phycocyanin is shown in figure 4.5. This prediction model will predict the final concentration of *Spirulina platensis* based on the equation below:

$y = -0.62x^2 + 0.34x + 0.59$, where x is the concentration of Spirulina platensis at day 3 while y represents the concentration of Spirulina platensis at day 13 of fed-batch process.

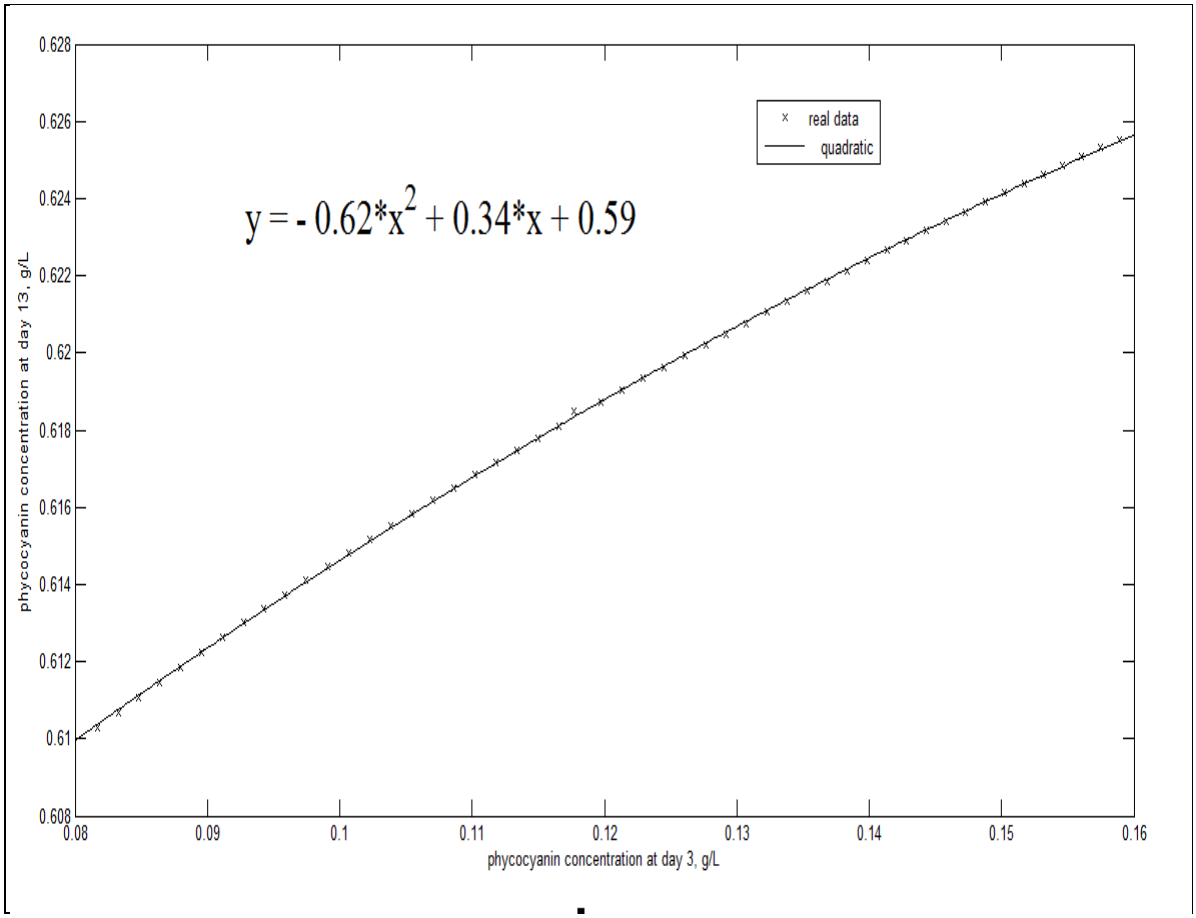

Figure 2.5: Prediction model for phycocyanin production in *Spirulina platensis*

After predictor model is constructed, it undergoes validation step to justify its ability to predict the final concentration. Table 4.1 shows the values of final product concentration when random initial cells and products concentration are simulated. The actual and predicted values are still can be accepted since the percentage error for each batch is below 1%. However, at -80% deviations this prediction model cannot be used seeing that the percentage error calculated at this value is greater than 1%. The mean value for actual and predicted product concentration also quite similar means this prediction model is feasible for the phycocyanin production. To be more understanding, Figure 4.6 is the visual version of predictor model validation shows the values of phycocyanin concentration that lay outside the control region.

Table 4.1: Evaluation of predictor model

Random %	**C_p(day 3)**	**C_{pf} (actual)**	**C_{pf} (predicted)**	**% error**
±10	0.1014	0.615	0.6181	0.48
	0.1406	0.6225	0.6255	0.48
±20	0.0817	0.6103	0.6136	0.54
	0.1589	0.6256	0.6284	0.43
±30	0.0629	0.6048	0.6089	0.67
	0.1761	0.6282	0.6306	0.38
±40	0.0458	0.5982	0.6043	0.99
	0.1920	0.6302	0.6324	0.35
±50	0.0311	0.5903	0.6000	1.62
	0.2067	0.6321	0.6338	0.27
±80	0.005	0.5479	0.5917	7.4
	0.2428	0.6359	0.6360	0.016
	Mean	0.61175	0.6186	

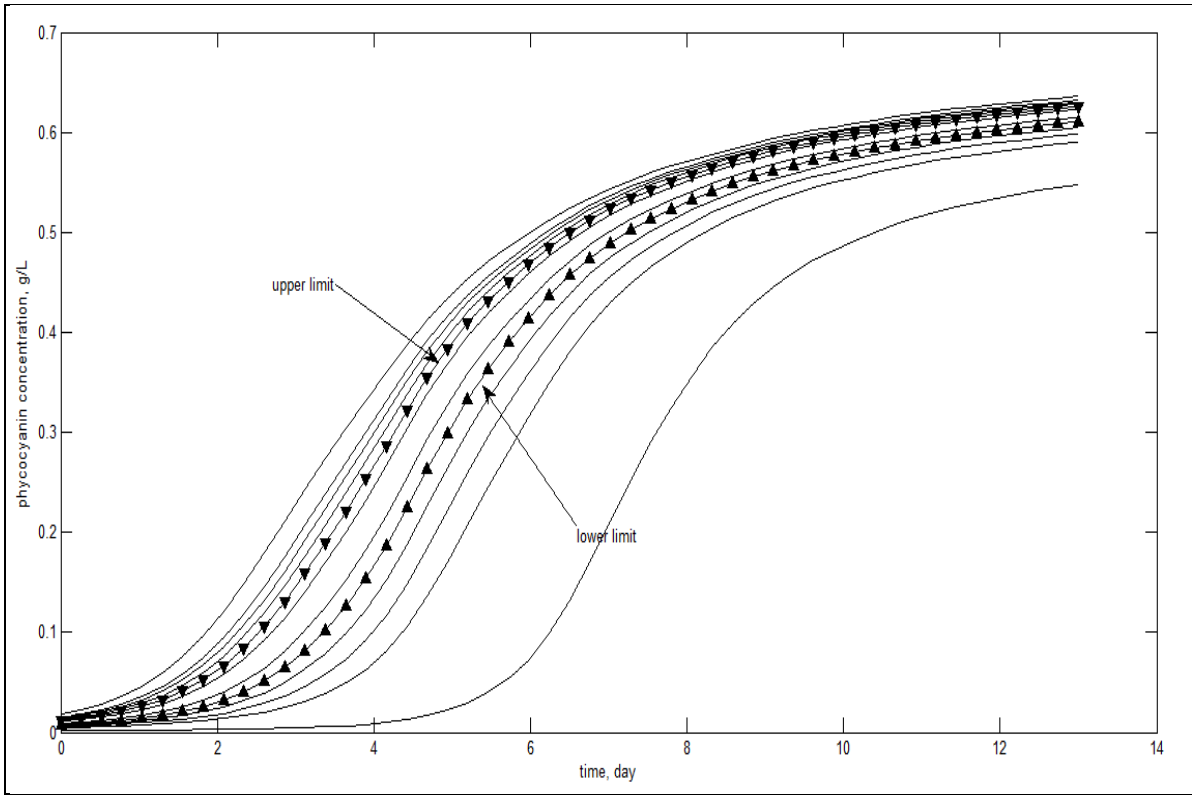

Figure 4.6: Final concentration of phycocyanin with the upper and lower limit defined

4.4 Control Model and MCC Approach

Final concentration of phycocyanin can be control by light intensity because from the previous study, when light intensity increase, phycocyanin concentration will also increase. Algae like *Spirulina platensis* need light to growth. The optimum value of light intensity in the production of phycocyanin by *Spirulina platensis* is 80 to 160 which is suggested by Zhang et al. (1998). Result from previous study also shows that when variant of light intensity is added into the fed-batch system, the concentration of *Spirulina platensis* and phycocyanin will increase until they reach their optimum value.

The control model is developed by using light intensity adjustment to the fed-batch reactor. Furthermore, phycocyanin concentration is greater when light intensity is greater. Therefore, a graph of light intensity vs phycocyanin concentration at day 13 is plotted as shown in Figure 4.7. The mathematical equation obtained from the graph is

$y = 1263.1x + 2.4966$ where y is referred to difference of Y_{mean} and $Y_{predicted}$ and x is referred to light intensity difference from 80µEm^{-2}s^{-1} to ±20080µEm^{-2}s^{-1}. A control region is also defined throughout the upper and lower limit measurement using equation 3.1. The mean value is 0.6186 while standard deviation is 0.00022 and t-distribution is 2.718. The upper and lower limit is 0.617 and 0.619 respectively.

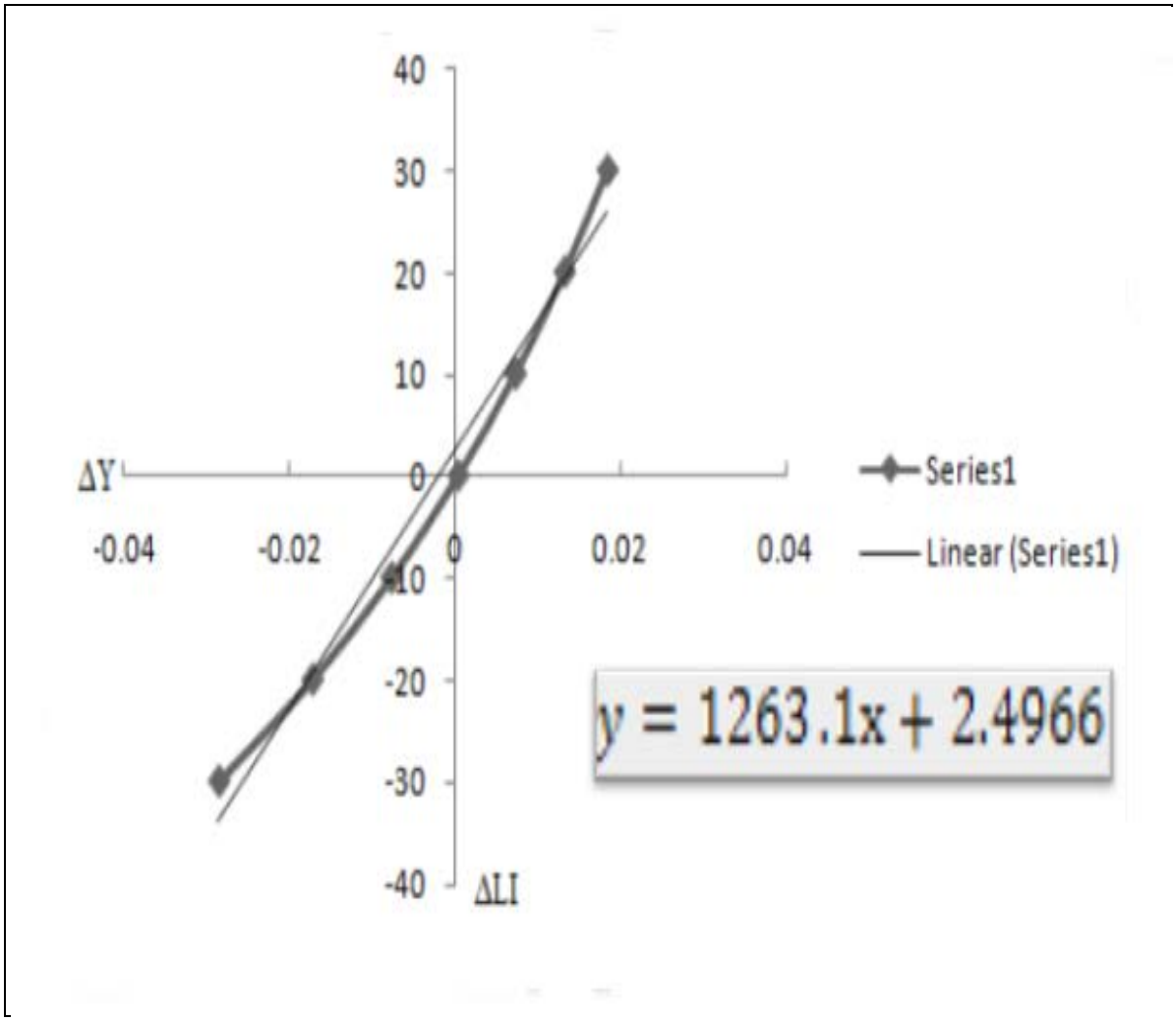

Figure 4.7: Control model of product concentration

A validation of control model is also done by adjusting the light intensity needed to put the final phycocyanin concentration at its desired value. Table 4.2 is the summary of control model validation as well as MCC approach to the control system while Figure 4.8 is the visual version of MCC applied to the control system. Light intensity is adjusted at only ±50% and ±80% and the figure shows that the control model is feasible

for ±50% and +80% of deviation because the final phycocyanin concentrations after MCC approach for each of them lie on the control region but not to -80% deviation.

Table 4.2: Summary of control model validation and MCC applied

Random %	**Product concentration (day 3)**	**Final product concentration (predicted), Y_p**	**Control action**	**Δ Y (Y_{mean}-Y_p)**	**Light intensity adjustment, ΔLI**	**Final product value after MCC applied**
±10	0.1014	0.6181	no	NA	-	-
	0.1406	0.6255	no	NA	-	-
±20	0.0817	0.6136	no	NA	-	-
	0.1589	0.6284	no	NA	-	-
±30	0.0629	0.6089	no	NA	-	-
	0.1761	0.6306	no	NA	-	-
±40	0.0458	0.6043	yes	0.0123	18.03	0.6148
	0.1920	0.6324	no	NA	-	-
±50	0.0311	0.6000	yes	0.0166	23.46	0.6138
	0.2067	0.6338	yes	-0.01719	-19.22	0.6187
±80	0.005	0.5917	yes	0.0249	33.95	0.5970
	0.2428	0.6360	yes	-0.0194	-22	0.6213

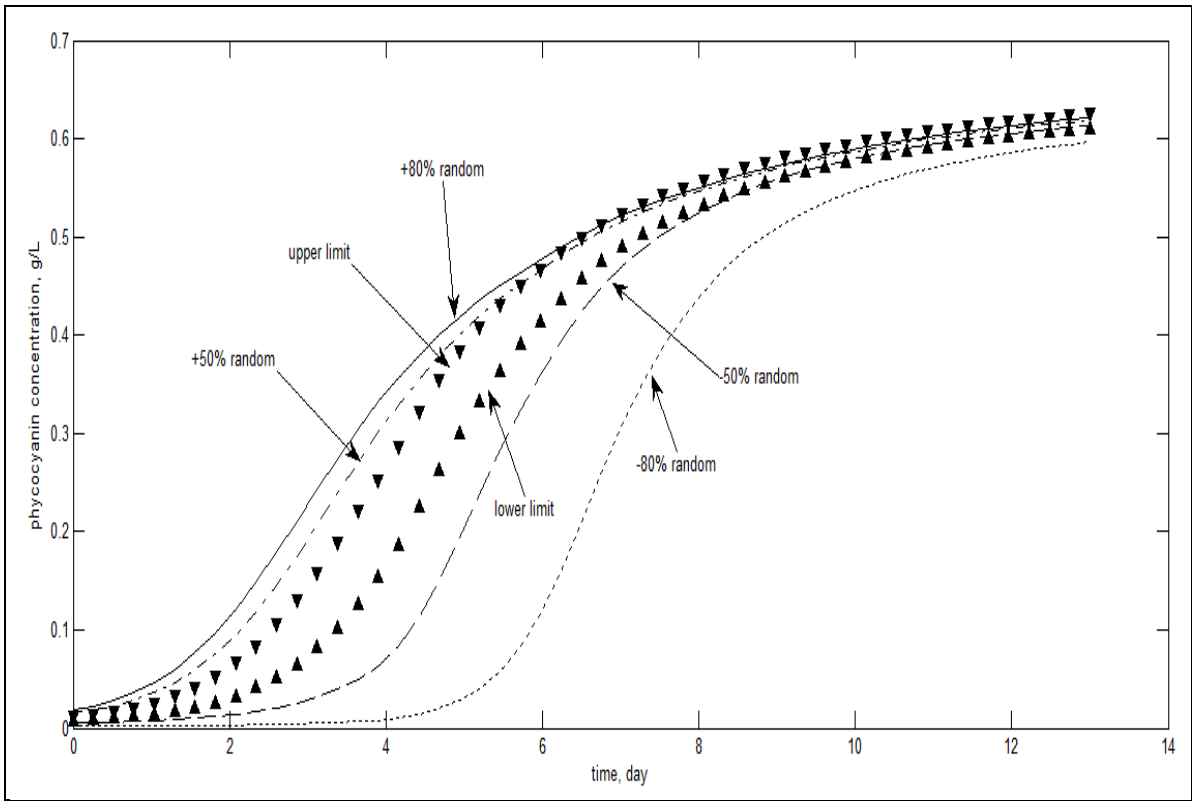

Figure 4.8: Effect of light intensity adjustment on phycocyanin concentration as well as MCC approach

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

In the phycocyanin production from *Spirulina platensis,* the concentration of cell and product are correlated well with substrate utilization and light intensity. When using glucose as the substrate and adjusting the light intensity around its optimum value in the fed-batch reactor, the experimental data from Zhang et al (1998) and Chen et al (1996), the concentration value of cell and phycocyanin is higher. Therefore, by using the data collected from these previous experiments, a control system of cultivation of *Spirulina platensis* to produced phycocyanin in fed-batch reactor is built.

In order to control the quality of a product, the final concentration of phycocyanin is predicted by using predictor model. A test is done to the predictor model to assure its availability to predict the final concentration of phycocyanin. From the test, only at when initial value of cell and phycocyanin is deviated at ±80% from its optimum value the predictor model fail to predict since the difference between actual and predicted value is high and the percentage error is also greater than 1.

Then when the light intensity is adjusted at day 5 to let the prediction value meets the desired value which is inside the control region, all the predicted value fall in the control region except the prediction value that is deviated at -80% from its optimum value does not falls in the control region. Therefore it can be concluded that predictor model and control model are feasible for the deviation of up to ±50%.

5.2 RECOMMENDATION

There are several recommendations that are important to approach MCC and have a good results. Firstly, obtaining any available online measurement can help in predicting a good, fast and accurate final phycocyanin concentration. Other than that, a prediction should be done as early as possible so that more corrective choices can be made to optimize the phycocyanin production.

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