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

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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

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

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.

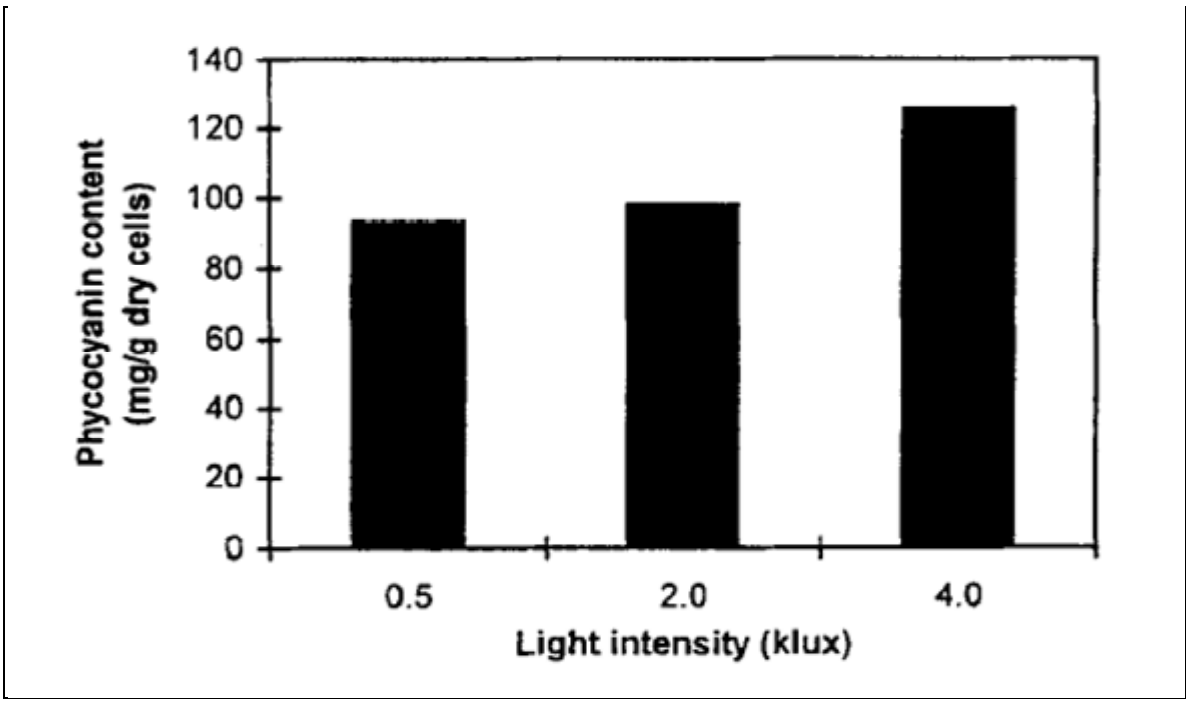

Figure 2.1: Phycoyanin content versus light intensity (Chen et al., 1996)

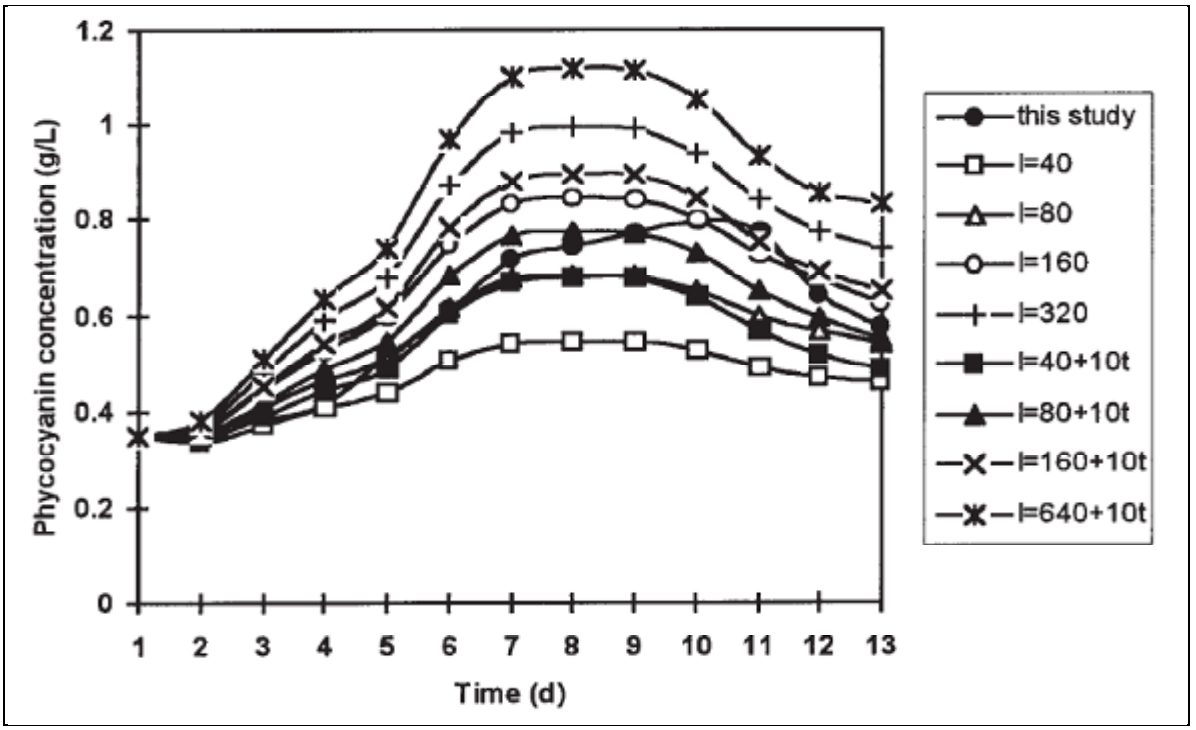

Figure 2.2: Effect of light intensity on phycocyanin concentration (Zheng et al., 1998)

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

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.

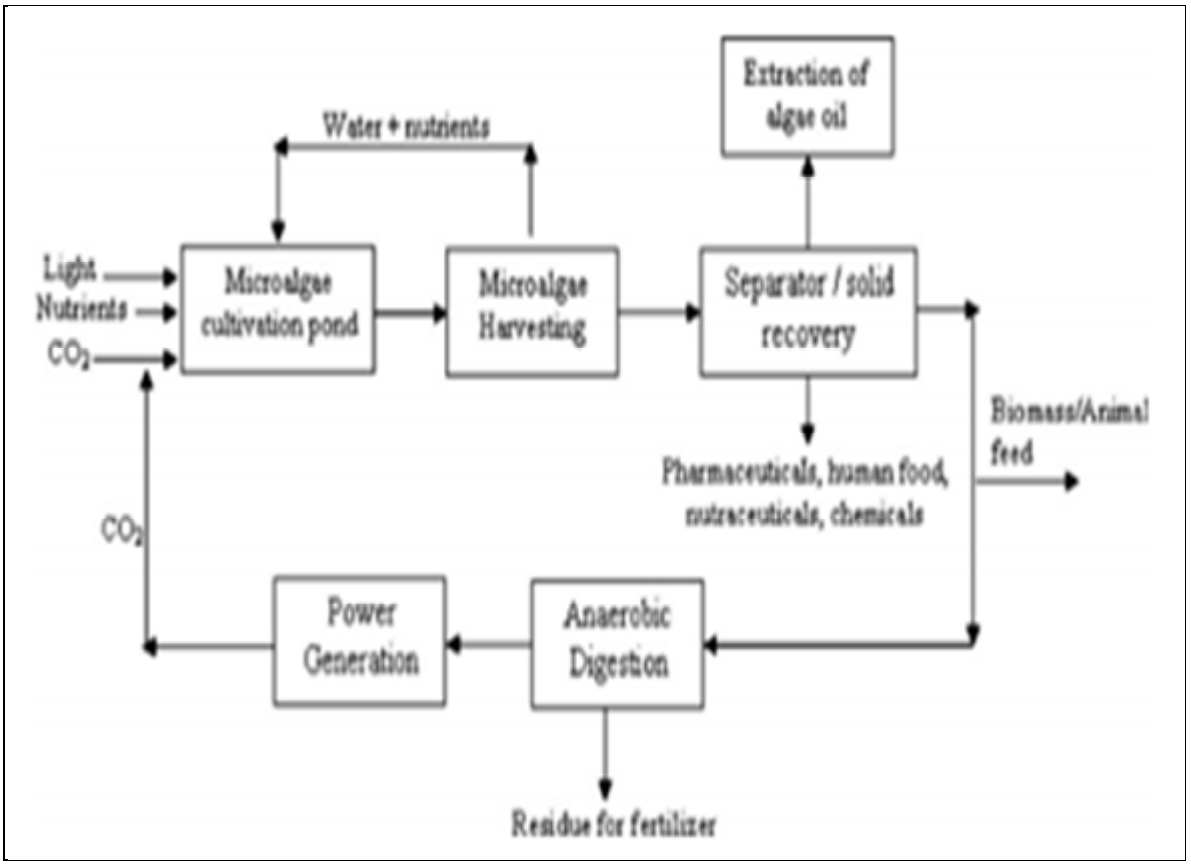

Figure 2.3: Schematic Diagram of Cultivation of Microalgae

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 flocculatio

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.