Modelling of Hybridoma Cell Growth Profile

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***Abstract*— this research project is basically a study to develop a model equation for the hybridoma cell growth by mathematical modeling. The mathematical modeling is a tool that specially used for simulates, optimize and control any process. Thus, this method is really suitable to produce an overall model of hybridoma cell growth profile that can predict the characteristics of batch system. Thus, revolution has been made in order to develop the model equations that take into account the factors such as unwanted decline in cell viability, growth rate, culture condition and others that lead to the decreasing of MAB production. In order to develop the model equation, the kinetic of hybridoma cell is being studied. Based on the kinetic study, the growth of hybridoma cell is disturbing by several factors that inhibit the growth. The major factors that affecting the growth cell is substrate limitation and inhibitor production. In this simulation, the hybridoma cell is supplied with glucose and glutamine as the substrate. These substrate act as a carbon source for the growing of the cell. Substrate consumption by the cell during its growth is leading to the formation of inhibitor. However, only glutamine exhaustion being the main factor of growth cessation. The constructed of hybridoma cell growth profile is including all the growth phase except death phase. Based on this simulation, the concentration of ammonia increase proportionally to the decreasing of glutamine concentration. The growth profile of hybridoma cell culture were simulated using an unstructured model in batch mode. All the model equation that involved in the growth of hybridoma has been used and solve by ode45 in Matlab software.**

Keywords*— *Hybridoma cell; Unstructured model; Mathematical modeling; Kinetic study; Cell growth profile

I. INTRODUCTION

Currently, the biopharmaceuticals product which produced from mammalian cell culture processes has been an extraordinary increase mainly because of their application in diagnostics and curative treatments. The most popular biopharmaceutical product from mammalian cell is monoclonal antibody. This product has been produce by hybridoma cell as a product formation during its growth. The growth of hybridoma cell has been widely studied in order to optimize the production of monoclonal antibody. The culture of this cell should be done in the optimum condition as allowing the cell growth rapidly hence producing a large amount of desired product. During the cultivation of hybridoma cell, the surrounding and its medium play the important role to enhance the growth. All this factor should be cooperate well with the kinetic characteristics of the cell.

In pharmaceutical field, this hybridoma cell has been widely study through it optimization condition for its growth in order to increase the production of monoclonal antibody. However, the growth of hybridoma cell is only done in small scale in order to maintain its quality. Thus, the revolution has been made to produce an overall model of hybridoma cell growth profile that can predict the characteristics of batch system. Through this revolution, the growth of hybridoma cell is determined limited due to unwanted decline in cell viability, growth rate, culture condition and other affecting factors that may disturb the growth of hybridoma cell.

In traditional way, the determination of hybridoma cell growth is done in laboratory through experimental technique. Nowadays, the technology has been developed well where the growth of hybridoma cell can be simulate by mathematical modeling. This model is very useful in predicting the behavior animal cell culture and optimizing culture conditions. Mathematical model is dividing into two types which are structured and unstructured. One of the earliest and most widely used mathematical modeling in attempting to describe cell growth is that of Monod. This model is categorize as unstructured where it is deterministic and distributed model, which describing the rate of cell growth based on the availability of a single substrate [1].

Some limited research has been conducted on hybridoma cell in order to study the effect of culture condition towards its growth. A methodology is proposed to evaluate which components of the culture medium limit the growth of hybridoma cell during the culture. The growth and metabolism of hybridoma cell in bioreactor can be limited either by the depletion of nutrients (glucose, amino acids, vitamins, oxygen) or by the accumulation of inhibitory metabolites (lactate and ammonia) [2]. However, even several of these factors are simultaneously limiting, any one may play a dominant role. Hybridoma cell has been used in this research in order to study the factors that affecting its growth in a batch culture mode. The modeling of hybridoma cell growth profile is developed by using Matlab software.

II. METHODOLOGY

A. Kinetic study of hybridoma cell

In the knowledge of the kinetics of hybridoma culture, the keys factors are the concentration and evolution of substrates and products. In the growth of hybridoma cell, glucose and glutamine are fundamental nutrients. Glucose is one of the main carbon and energy sources. Part of this glucose is used for the synthesis of biomass through pentose pathway even it is mostly transformed into pyruvate. In the process, pyruvate is partially converted into water (H_2O) and carbon dioxide (CO_2) through The Citric Acid (TCA) cycle, partially converted into fatty acids as well as partially into lactic acid. Not only limited to glucose, glutamine is also another important substrate for the growth of hybridoma cells. However, part of the glutamine is deaminated and other also enters into TCA cycle. Through TCA cycle, the glutamine is yielding adenosine triphosphate (ATP), CO_2 and H_2O. The other part of glutamine that being deaminated are producing ammonium and glutamate as a by-product and next being transformed into amino acids for biosynthesis purposes. Unfortunately, the production of ammonium and lactate as a cell metabolism can act as inhibitors when their concentration is high enough [3]. The main metabolic route of hybridoma cell is described in Figure 1.

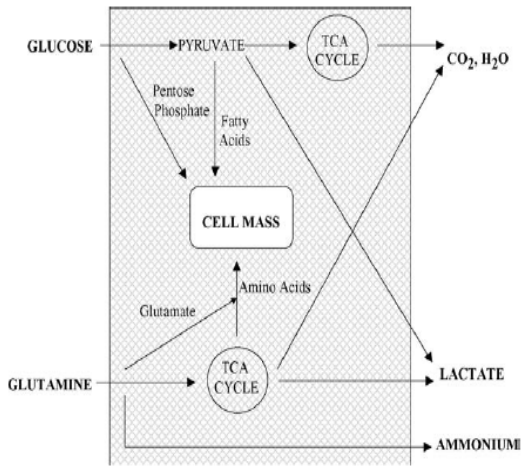

Figure 1: The main metabolic route of hybridoma cell [3]

B. Modeling equation

The model equation has been developed based on the kinetic study of hybridoma cell growth. The basic component that involved in the culture of hybridoma cell is glucose and glutamine. These components that being the carbon sources for the growth of the cell. However, the consumption of this glucose and glutamine by the cell may leading to the formation of inhibitory product. Thus, in order to simulate the growth of hybridoma cell, all the model equation regarding to the cell growth, substrate consumption and inhibitory product formation should be taken into consideration. The model equation of each components are shown as below:

Equation of cell growth:

In the cell growth, the viable cell grow by multiplying the viable cell mass (X_v) at a particular specific growth rate (μ). Therefore, the concentration of viable cell in the batch culture is expressed as below equation:

$$\frac{dVX_t}{dt} = \mu VX_v$$

However, under usual condition, the growth of cell is exaggerated by many restriction such as nutrient limitation and inhibitory product accumulation, which may affecting the maximum specific growth rate. Thus, by taking all this matter into consideration, the specific growth rate of hybridoma cell is modeled as below:

$$\mu = \mu_{max} f_{lim} f_{inh}$$

Where f_{lim} and f_{inh} represent nutrient limitation and inhibitory product formation. In hybridoma cell culture, glucose and glutamine were assumed to be the major limiting nutrient, while ammonia and lactate as main inhibitors. Thus, the equation for specific growth may be described as:

$$f_{lim} = \left(\frac{[GLC]}{K_{glc} + [GLC]}\right)\left(\frac{[GLN]}{K_{glc} + [GLN]}\right)$$

$$f_{inh} = \left(\frac{KI_{amm}}{KI_{amm} + [AMM]}\right)\left(\frac{KI_{lac}}{KI_{lac} + [LAC]}\right)$$

Where GLC, GLN, AMM and AMM are the extracellular concentrations of glucose, glutamine, ammonia and lactate. Each of these major components are defined into the model equation.

Equation of glucose concentration:

$$\frac{d(V[GLC])}{dt} = -Q_{glc} V X_v$$

$$Q_{glc} = \frac{\mu}{Y_{x,glc}} + m_{glc}$$

Equation of glutamine:

$$\frac{d(V[GLN])}{dt} = -Q_{gln} V X_v - K_{d,gln} V[GLN]$$

$$Q_{gln} = \frac{\mu}{Y_{x,gln}} + m_{gln}$$

$$m_{gln} = \frac{a_1[GLN]}{a_2 + [GLN]}$$

Equation of lactate:

The model equation of lactate is expressed in term of glucose. This is due to the formation of lactate regarding to the consumption of glucose by the cell. However, the glutamine consumption during the cell growth also lead to the formation of lactate. However, the amount is small and uncountable.

$$\frac{d(V[LAC])}{dt} = Q_{lac} V X_v$$

$$Q_{lac} = Y_{lac,glc} Q_{glc}$$

Equation of ammonium:

Spontaneously, the production of ammonia mostly from glutamine metabolism and degradation. The glutamine is deaminated thus leading to the formation of ammonium as a by-product. Production of ammonia was shown to follow closely the glutamine consumption [4]. Thus, the model equation for ammonium is described in the term of glutamine.

$$\frac{d(V[AMM])}{dt} = Q_{amm} V X_v + K_{d,gln} V[GLN]$$

$$Q_{amm} = Y_{amm,gln} Q_{gln}$$

All this model equation will be used for simulation of hybridoma cell growth. This simulation is done by using Matlab software. Since there are a few model equations, this simulation is solved by ode45 command.

III. RESULTS AND DISCUSSION

A. Simulation of batch culture of hybridoma cell

The hybridoma cell growth was simulated by using the unstructured model. The culture was supplied with a high initial level of glucose (20mM) and glutamine (4mM) as a carbon sources. The modeling of hybridoma cell profile we well predicted by the simulation as seen in Figure 2.

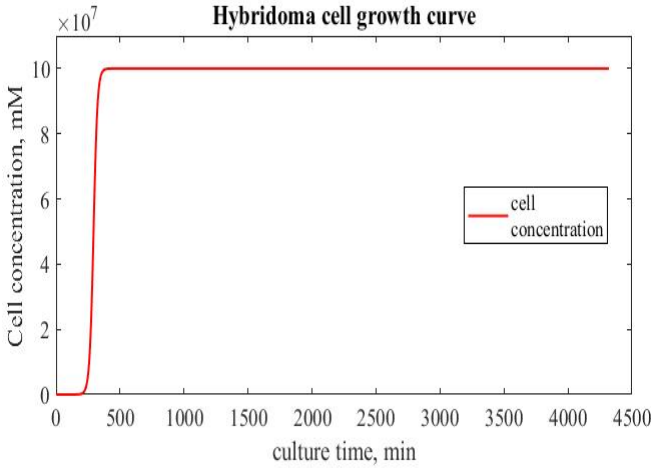

Figure 2: Hybridoma cell growth profile by Matlab software

From Figure 2, it shows that the concentration of viable cells were increasing with the culture time. However, at the some point, the cell concentration has reached its maximum value, and it became constant. This is starting point where the cell stop to growth due to the substrate limitation and accumulation of inhibitors by-product. To be accurate, the cell growth stopped at about 500 min at which indicate that the glutamine has started to be exhausted and ammonia and lactate accumulated. However, the glucose was not the main factor of the growth termination since it was not limited during the culture. This is because glucose were supplied with a higher amount compared to glutamine. Unfortunately, it is still not clear about the precise reason of growth cessation. This simulation does not include the model equation for dead cells. Thus, the cell growth profile only include until the stationary phase. The simulation of glutamine exhaustion and ammonia accumulation is described in figure 3.

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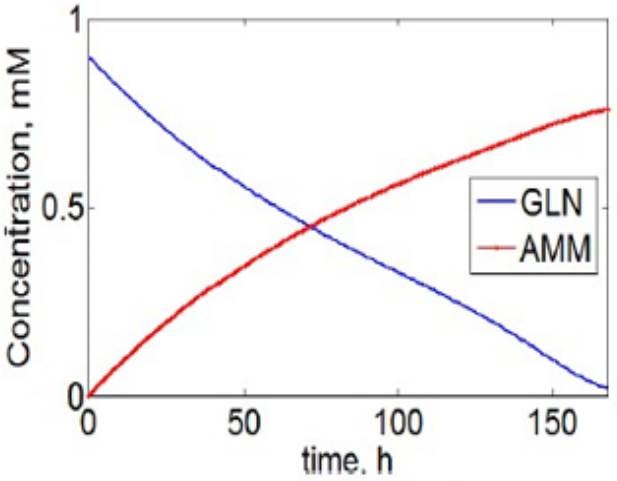

Figure 3: Glutamine and ammonia concentration by Matlab software

From Figure 4.2, it can be concluded that decreasing of glutamine concentration almost directly proportional to the increasing of ammonia production. This is due to the degradation of glutamine that led to the production of ammonia as a by-product. The exhaustion of glutamine was shown as a major factor in growth termination in this simulation. The accumulation of ammonia affected the specific growth rate in the deceleration phase. Thus, once the specific growth rate reduces, the cell growth is reduce gradually until the substrate become limited. In this simulation, the glucose concentration was not being the main factor for the growth of cells. Hence, the graph of lactate concentration and glucose concentration were did not constructed. Model parameters of constant numerical value used in the simulation are summarized in Table 1.

Table 1: Summary of constants used in the model equation

Constant	Value
μ_{max}	0.065 h^{-1}
K_{glc}	0.75 mM
K_{gln}	0.075 mM
KI_{lac}	90 mM
KI_{amm}	15 mM
K_{dgln}	$9.6x10^{-3}$ mM
m_{glc}	$2.0x10^{-12}$ mmol $cell^{-1}$ h^{-1}
$Y_{lac,glc}$	2.0 mol mol^{-1}
$Y_{x,glc}$	$2.37x10^{8}$ cells mol^{-1}
$Y_{x,gln}$	$8.0x10^{8}$ cells mol^{-1}
$Y_{amm,gln}$	0.7 mol mol^{-1}

IV. CONCLUSION

The profile of hybridoma cell growth were constructed. In this simulation, the growth curve profile including all the phase such as lag, exponential, decreased and stationary. However, the death phase is excluded since the model equation for dead cell is not taken into account. Along the growth of cell, there are a few factors that affecting the cell growth. The consumption of glucose and glutamine as carbon sources leading to the production of inhibitory by-product which are lactate and ammonia. The increasing of ammonia concentration is proportionally to the decreasing of glutamine concentration. This has proved that termination of cell growth is due to the substrate exhaustion and inhibitor limitation. The cessation step start at decreased phase and leading to the stationary phase where the cell concentration become constant. This has been conclude that cell growth is affected by the raising of inhibitory products. However, there are still other environment factors that do affect the hybridoma cell growth.

V. NOMENCLATURE

X_v	Viable cell mass
V	Culture volume
f_{lim}	Limitation function
f_{inh}	Inhibition function
[GLC]	Glucose concentration
[GLN]	Glutamine concentration
[LAC]	Lactate concentration
[AMM]	Ammonia concentration
K_{glc}	Monod constant of glucose
K_{gln}	Monod constant of glutamine
$K_{d,gln}$	Constant for glutamine
KI_{lac}	Inhibition constant of lactate
KI_{amm}	Inhibition constant of ammonia
Q_{glc}	Specific consumption rate of glucose
Q_{gln}	Specific consumption rate of glutamine
Q_{lac}	Specific production rate of lactate
Q_{amm}	Specific consumption rate of ammonia
$Y_{x,glc}$	Yield of cells on glucose
$Y_{x,gln}$	Yield of cell on glutamine

$Y_{lac,glc}$	Yield of lactate from glucose
$Y_{amm,gln}$	Yield of ammonia from glutamine
m_{glc}	Maintenance coefficient of glucose

ACKNOWLEDGMENT

Thank you to my supervisor, Mrs. Syazana binti mohd Pauzi and co-supervisor, Ir. Nor Madyzah binti Ahmad for their constant guidance, unwavering support and an objective criticism of my work that helped in shaping up my subsequent research efforts. Not to forget, my deepest thanks to Universiti Teknologi Mara for allowing me to conduct this research. I would like to also take this opportunity to express my gratitude to my fellow friends for their support, guidance and valuable information that helped me in completing this research project. Last but not least, a big thank you to my parents their continuous support and encouragement without which this research would not be possible.

References

[1] J. P. B. Jae Deog Jang, "An unstructured kinetic model of macromolecular metabolism in batch and fed-batch cultures of hybridoma cells producing monoclonal antibody," *Biochemical Engineering Journal,* p. 153–168, 2000.

[2] A. M. J. E. J. G. A. Lourenqo da Silva, "Kinetic model of hybridoma cultures for the identification of rate limiting factors and process optimisation," *Mathematics and Computers in Simulation 42,* pp. 197-205, 1996.

[3] J. D. A. L. M. D. Lorea Legazpi, "Kinetic analysis of hybridoma cell culture in a protein-free medium: Substrate and agitation effects," *Biochemical Engineering Journal,* p. 122–130, 2005.

[4] D. C. H. B. G.W. Hiller, "Cell retention-chemostat studies of Hybridoma cells-analysis of Hybridoma growth and metabolism in continuous suspension culture on serum free medium," *Biotechnol. Bioeng.,* vol. 42, pp. 185-195, 1993.