Development of Design Advisor Tools for Upstream Process of Biopharmaceutical Production

Nurmala Idayu binti Othman and Syazana binti Mohamad Pauzi,

Faculty of Chemical Engineering, Universiti Teknologi Mara

***Abstract*—Unstructured and structured kinetic model is used in introducing the production of antibody starting from cell growth, substrate uptake until the antibody is produce. Basically, the production of antibody are varies depending on the cell used. Thus, take time in the conformation of the production rate by the experimental data. This research is to develop a design advisor tool that focuses on upstream processing of biopharmaceutical production. The upstream process of monoclonal antibody production will be used as reference model and it is limited to batch processing only. The unstructured model of kinetic equation is used in the growth model. The developed tool should be able to assess the design capacity as well as the economics of the process. MATLAB program was used to produce the command setting as well as the graphical user interface. The design advisor tool was tested and verified through a case study of conceptual design of monoclonal antibody production using SuperPro Designer software. The developed advisor tool was able to predict the progress of cell growth, product formation, and the substrate uptake.**

***Keywords*— Advisor tools; Upstream processing, Unstructured model, Monoclonal antibody**

I. Introduction

The demands for biopharmaceutical products seem to be increased year by year. There are several corporations which have been manufactured large scale of industrial plants that contain numerous 10,000 L or larger cell culture bioreactors in response to sturdy the demand [8]. Nowadays, hundreds of biopharmaceuticals products have been approved and a lot more are in late stages of clinical progress as the global biopharmaceutical industry has raise since the first drug Humulin was approved in 1982 [9]. The important aspect which is most desired from the output of biopharmaceutical industry is maintaining high quality of product but still at low cost [1].

The fast growing market of biopharmaceutical products have caused in demanding for more research and activities involving the production process which can be marketed, thus resulting to the requirement of rapid data access towards the large scale process [3]. A simulation which acts as advisor in the production is really helpful to fulfill the demand. The used of simulation in the industries is slow at early stage of introduction, however, nowadays the employment of simulation in the biopharmaceutical has become a priority as it is one of the ways to improve the production.

There is simulation software available such as SuperPro Designer and advisor tool such as Simbiopharma that are already developed for biopharmaceutical industry. Although the tools could boost knowledge in the manufacturing view of the industry, but there are certain limitations to these tools. SuperPro Designer is expensive while Simbiopharma which is a prototype decision-support tools has limited access. So, both computer-aided programs have giving inspiration for this research in developing a design advisor tool.

A design advisor tool for upstream process is a system that follows a consistent knowledge-based that slanted for all tasks or assignments that are required to build an excellent and highly efficient process specifically for upstream process. These tools act as preliminary information to help in the decision-making when starting a process of biopharmaceutical products so that product development can improve tremendously. Similar concept to the simulation, advisor tools help in pre-design the process to predict the outcome of the process which is the yield of the process before it is going to be tested in the real plant production [11]. This is because some process is too sensitive, too large and costly to be directly tested on the real plant production. So, the advisor tools will help to predict the yield of process and thus scaling-up to the required product.

Basically, this research is to develop design advisor tool for the upstream process of biopharmaceutical productions and to verify its reliability against already established simulation which is SuperPro Designer.

II. METHODOLOGY

A. Equations for biomass growth and parameters for cell growth

The model used in this research is kinetic equations which involved growth, substrate and product kinetic. The production of mAb includes the uses of both unstructured and structured kinetic model. The unstructured kinetic model comprises extracellular antibody production which incorporated the cell growth, death kinetics and nutrient uptake while the structured kinetic model includes intracellular antibody production.

The structured models which describe cell activities more closer required more equation solved than unstructured models. It might be easier and faster to develop, simulate and optimize unstructured models of cell culture since there are several equations are required to describe cell growth, consumption of glucose and glutamine, formation of lactate and ammonia, and monoclonal antibody.

Antibody production is expected to be less favored by fast growing cells when compared to the production of cellular protein as it is not an essential product for cell growth.

Unstructured growth model of cell cultures

The main model used in the estimating the growth of cells is unstructured model that describes cell proliferation in batch cultures based on the consumption of two main nutrients which is glucose and glutamine and also the production of corresponding

byproducts of the cells metabolism; lactate and ammonia, based on the nutrients uptakes [7]. The model used is adapted from Kontoravdi et al. [14] based on the work of Jang and Barford [15] which consists of a total of 6 ordinary differential equations and 16 model parameters. For the purposes of our study, the model has been operated in its batch mode.

The material balance for the total cell concentration is given by:

$$\frac{dX}{dt} = \mu X \tag{1}$$

Where, X denotes the total cell concentration measured in cells/L.

The specific growth rate that appears in the equation is estimated through the following formula:

$$\mu = \mu_{max}\left(\frac{[GLC]}{K_{glc} + [GLC]}\right)\left(\frac{[GLN]}{K_{gln} + [GLN]}\right). \left(\frac{K_{lac}}{K_{lac} + [LAC]}\right)\left(\frac{K_{amm}}{K_{amm} + [AMM]}\right) \tag{2}$$

Where, the $K_{glc}$and K_{gln}parameters are the Monod constants for the primary nutrients; glucose and glutamine. Similarly, the K_{lac} and K_{amm} parameters are the inhibition constants of the primary products of metabolisms, namely lactate and ammonium. All of the parameters constant, K are measured in mM. $[GLC], [GLN], [LAC]$ and $[AMM]$ represent the extracellular concentrations of the mentioned nutrients and also products and measured in mM.

Since the model is unsegregated, it only represents the overall concentrations of nutrients and by-products of cellular metabolism within the bioreactor. Therefore, by performing material balances on each compound, 4 ordinary differential equations yielding the temporal concentration of metabolites are obtained. Specifically, the material balance for the concentration of glucose can be formulated as shown below:

$$\frac{d[GLC]}{dt} = -Q_{glc}X \tag{3}$$

Where, Q_{glc} is the specific glucose consumption rate (mM/cell/h) and is defined as:

$$Q_{glc} = \frac{1}{Y_{x,glc}}\mu + m_{glc} \tag{4}$$

The parameters $Y_{x,glc}$ and m_{glc} which appear in equation above are the cell yield on glucose (cell/mM) and maintenance energy of glucose (mM/cell/h), respectively.

The material balance for glutamine is described by the following equation:

$$\frac{d[GLN]}{dt} = -Q_{gln}X - K_{d,gln}[GLN] \tag{5}$$

The only difference is the term containing glutamine degradation, $K_{d,gln}$.

$$Q_{gln} = \frac{1}{Y_{x,gln}}\mu + m_{gln} \tag{6}$$

Where,

$$m_{gln} = \frac{\alpha_1[GLN]}{\alpha_2[GLN]} \tag{7}$$

with α_1 (dimensionless) and α_2 (mM) being the relevant kinetic constants.

The material balance for glutamine is described by the following equation:

$$\frac{d[GLN]}{dt} = -Q_{gln}X - K_{d,gln}[GLN] \tag{8}$$

The only difference is the term containing glutamine degradation, $K_{d,gln}$.

$$Q_{gln} = \frac{1}{Y_{x,gln}}\mu + m_{gln} \tag{9}$$

Where,

$$m_{gln} = \frac{\alpha_1[GLN]}{\alpha_2[GLN]} \tag{10}$$

with α_1 (dimensionless) and α_2 (mM) being the relevant kinetic constants.

Similarly, mass balances can be formulated to describe the temporal evolution of the concentrations of the primary by-products of cell metabolism. More specifically, for lactate:

$$\frac{d[LAC]}{dt} = Q_{lac}X \tag{11}$$

With

$$Q_{lac} = Y_{lac,glc}Q_{glc} \tag{12}$$

Similarly for ammonia:

$$\frac{d[AMM]}{dt} = Q_{amm}X + K_{d,gln}[GLN] \tag{13}$$

With

$$Q_{amm} = Y_{amm,gln}Q_{gln} \tag{14}$$

Where Q_{lac} and Q_{amm}, represent the specific production rate (mM/cell/h) while $Y_{lac,glc}$ and $Y_{amm,gln}$ represent the yield of the particular product on its primary nutrient (mM/mM).

Unstructured model of monoclonal antibody producing cell cultures

Specifically, the unstructured model describing cellular growth is coupled to a structured model describing the process of antibody formation in the cell. The model assumes no intracellular accumulation of the species involved in the mAb production pathway and no proteolytic or other degradation of the antibody chains in the cell. The model consists of an intracellular heavy- and light- chain mRNA balance and also includes the Endosplasmic Reticulum (ER) heavy and light chain balances. Basically, the unstructured model involved all the intracellular reaction inside the cell. Finally, the expression for antibody production is:

The kinetic parameters chosen to be used in the development of the model is based on model presented by Kontoravdi et al. [14]. The kinetic parameters are shown in Table 1.

$$\frac{d[mAb]}{dt} = (\gamma_2 - \gamma_1\mu)Q_{mAb}X \tag{15}$$

Where

$$Q_{mAb} = \varepsilon_2\lambda K_G[H_2L_2]_G \tag{16}$$

Where Q_{mAb} is the specific mAb production rate (mg/cell/h), λ is the molecular weight of IgG1 (g/mol), and ε_2 is the Golgi glycosylation efficiency factor. In equation above, [mAb] is the concentration of mAb secreted in the culture, and $\gamma_2, \gamma_1$are constants. K_G is the rate constant for Golgi-to-extracellular medium antibody transport (h^{-1}).

Table 1: Nominal values of model parameters (Kontoravdi et al., 2010).

Symbol	Units	Nominal Value
μ_{max}	h^{-1}	5.8*10^{-3}
K_{amm}	mM	28.484
K_{lac}	mM	171.756
K_{glc}	mM	0.75
K_{gln}	mM	0.075
$Y_{lac,glc}$	dimensionless	1.399
m_{glc}	mM/cell/h	4.853*10^{-14}
$Y_{x,glc}$	cell/mM	1.061*10^{8}
$Y_{x,gln}$	cell/mM	5.565*10^{8}
$K_{d,gln}$	h^{-1}	9.6*10^{-3}
α_1	mM L/cell/h	3.4*10^{-13}
α_2	mM	4
$Y_{amm,gln}$	dimensionless	0.4269
γ_1	dimensionless	0.1
γ_2	dimensionless	2
ε_2	dimensionless	1
λ	g/mol	146
K_G	h^{-1}	0.1386

B. Models fitted to equations found and solved by using ODE in MATLAB.

As the production of mAbs is involving the biological growth, its environmental condition which is hard to control may give different products since it varies in conditions. A mathematical model which includes the all the cell culture growth kinetic such as Monod equation to express the growth rate is used. A mathematical model can be distinct as a formulation or equation that shows necessary features of a physical system or process in mathematical terms [13]. Thus, the growth and production profile of mAb can be represented by mathematical models. The kinetic equations also include the kinetic of substrate and product.

Ordinary differential equations or ODE in MATLAB is used to solve the equations used. MATLAB popular among user for its ability to solve typical engineering problems which includes the solving of a standard second order ODE involving constant coefficients with initial conditions and also nonhomogeneous. The most important thing is the ODE only can solve first order ODE. So, for the second order and above, the ODE should be set to the first order of ODE before being converted to another. As stated, MATLAB solve equations need by using functions give in the command window.

There are many type of ODE and one of it is ODE45. ODE45 solve the stiff equations by employing variable step Runge-Kutta integration methods and uses a 4th and 5th order Dormand-Prince pair for higher accuracy. ODE45 is used to solve the equations in this research for it higher accuracy in solving the equations.

C. Advisor tools are developed using GUI in MATLAB.

Advisor tools are developed by using the information collected, with the aided of Graphical User Interfaces (GUI) in MATLAB. To make an advisor tools, the uses of Graphical User Interfaces or GUI in MATLAB is needed. GUIs provide point-and-click control of software applications, removing the necessity to learn a language or type commands in order to run the application [10]. It has the ability which only needs simple steps to build windows like applications by using MATLAB software with its programming language. In MATLAB, the two crucial tasks of application building which are laying out of the pictorial components and programming behavior and allows to immediately move between graphical design in the canvas and code development in an integrated version of MATLAB Editor.

The GUIs development is started with differentiate of values needed by the kinetic model which are constant and varies. Dividing the layout into two parts, one part for kinetic parameters of a sample tested and one part for the values that user varies for an experiment. The examples for kinetic parameters are maximum specific growth rate, saturation constant, constant related to product, maintenance coefficient and yield of cells on substrate. The examples for varies values are substrate concentration, cells concentration and product concentration. Values that are varies are the values that will be resulted from the solvation of the kinetic model by using the constant of kinetic parameters.

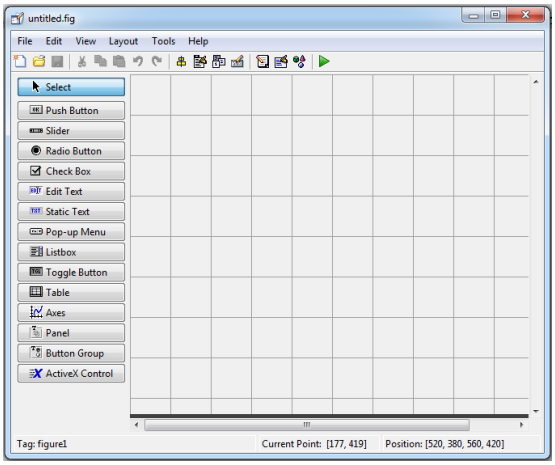

Figure 1: Graphical User Interfaces (GUI)

III. RESULTS AND DISCUSSION

All of the parameters have contained in the model and provides details of their nominal values as they have been recorded in the work of Kontoravdi et al. [14]. Based on the graph, there is increase in cell concentration which means the cell is growing. If compare with the model represented by Kyparissides [7] which using the same equation and parameter from the base case of Kontoravdi [14], the cell is growing and did not have the phase that cell should have which are lag phase, exponential phase, stationery phase and death phase. The resulted graph from this model only shows that the cell is suddenly increase in concentration which means it only have lag phase and stationery phase.

A. Graphical User Interface (GUI)

The following GUI in Figure 2 was developed using MATLAB GUIDE. The GUI will calls a set of .m files which in turn call s subset of .m files.

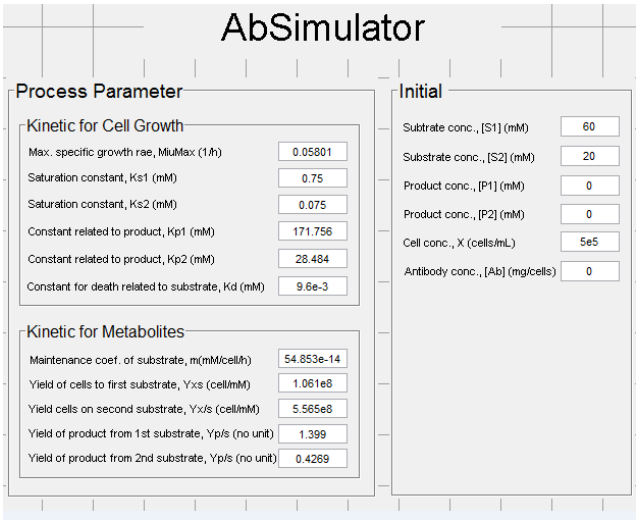

Figure 2: GUI Interface

For most animal cells, glucose and L-glutamine are the major sources of carbon and nitrogen, respectively. The metabolism of both glucose and L-glutamine is interrelated, and the proportion of each nutrient consumed by the different cellular pathways depends on the metabolic state of the cells [12]. Glucose [GLC] and L-glutamine [GLN] are the major carbon and energy sources in most cell culture media and both nutrients are essential for cell growth. The metabolism of both glucose and L-glutamine is interrelated and the proportion of each nutrient consumed by the different cellular pathways depends on the metabolic state of the cells. Glucose and L-glutamine influence the specific growth rate of cells while Lactate [LAC] and ammonia [AMM] are the inhibitory products impacting specific growth rate.

B. *Cell density*

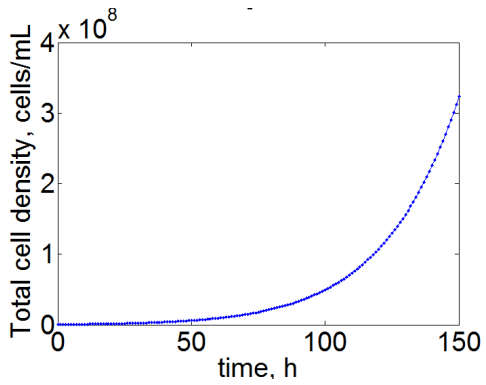

Figure 3: Total cell concentration by simulator

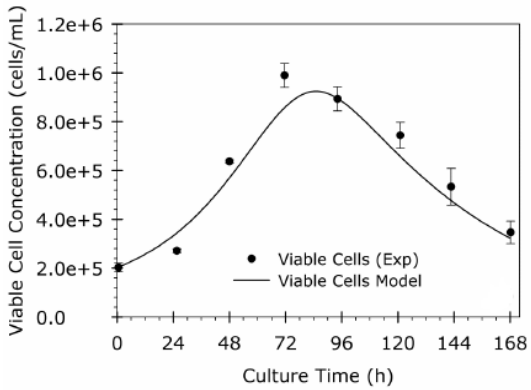

Figure 4: Total cell concentration base case study

All of the parameters have contained in the model and provides details of their nominal values as they have been recorded in the work of Kontoravdi et al. [14]. Based on the graph, there is increase in cell concentration which means the cell is growing. If compare with the model represented by Kyparissides [7] which using the same equation and parameter from the base case of Kontoravdi [14] the cell is growing and did not have the phase that cell should have which are lag phase, exponential phase, stationery phase and death phase. The resulted graph from this model only shows that the cell is suddenly increase in concentration and only have lag phase and stationery phase.

C. *Glucose consumption and lactate production*

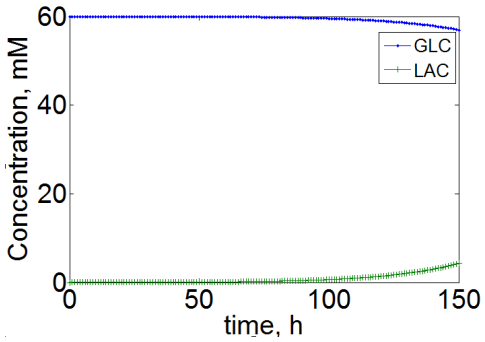

Figure 5: Glucose and lactate concentration by simulator

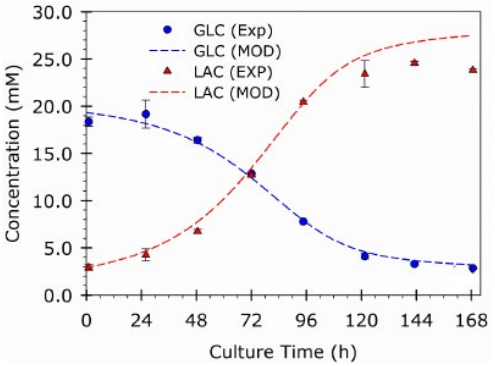

Figure 6: Glucose and lactate concentration of base case study

Glucose consumption

Based on Figure 4, for glucose consumption, this model shows slightly declining of glucose concentration. Comparing the result from this model with base case studied, there is large different for the glucose consumption by this model with the base case study result. Since the glucose consumption is too low and are slightly increase only during the final culture time which are at 100 to 150 h, it can be related to the cell concentration that also have suddenly increase in between the time. Which means that the cell had consume the glucose more at that time for their growth. But, the glucose is not fully consumed as they should which the glucose is usually consumed more during the lag phase and more than that during the exponential phase. This also indicates that it is not a growth limiting nutrient. A gradual decrease, resulting eventually in glucose uptake was observed which indicates that the growth is limited by another nutrient, possibly glutamate examined below.

Lactate production

Referring to the graph of the tested kinetic parameters by this model in Figure 4, there is slightly increased in lactate concentration for lactate production. This might be due to the simple relationship used to describe the formation of lactate.

Comparing the result from this model with base case studied, there is large different for the glucose consumption and lactate production by this model although this model shows that it has follow the right performance which the lactate production should be increase following by the decreasing of glucose concentration. Based on the base case study, there should be sudden decrease of glucose concentration during the time of 48 to 120 h which the cells are consuming more glucose for their growth.

D. Glutamine consumption and ammonia production

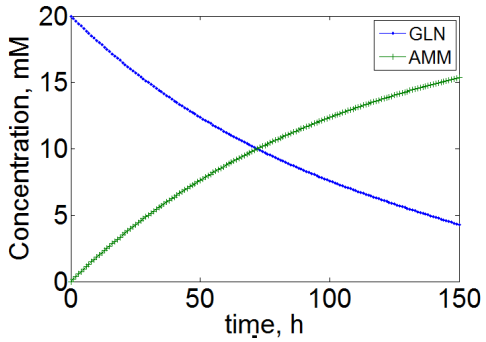

Figure 7: Glutamine and lactate concentration by simulator

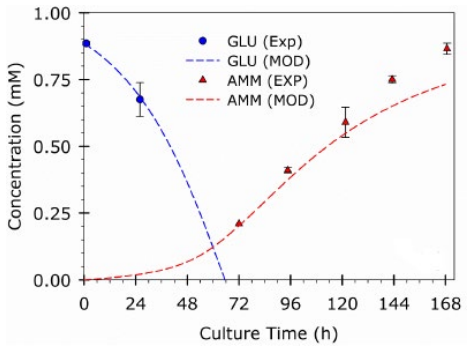

Figure 8: Glutamine and lactate concentration of base case study

Glutamine consumption

Referring to the graph of the tested kinetic parameters by this model in Figure 7, there is decreased of the glutamine concentration. Glutamine is almost completely depleted in the tested model. Same goes to glucose, the glutamine concentration should be decreasing as the cell is consuming glutamine for their growth. Comparing the result from this model and the base case, the results are almost the same. The declining in glutamine concentration is followed by the increasing of ammonia concentration. The glutamine was consumed during the cell growth and as the result; the byproduct form the reaction is produce which is the ammonia. It was quite successful since the result from this model is almost the same with the base case studied.

Ammonia production

There is increased of ammonia concentration by the model used which is the same result as base case study. The results confirm that ammonia appears in the culture media shortly after the depletion of glutamate. The increasing of ammonia concentration is affected by the decreasing of the glutamine concentration. This is because of the relationship of the glutamine and ammonia; ammnoia is the byproduct of the glutamine consumed by the cells. It is clearly that, as the concentration of glutamine is decreased, the concentration of ammonia will be increased.

E. Monoclonal Antibody production

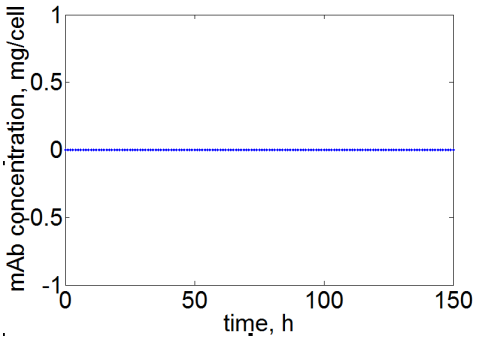

Figure 9: mAb concentration by simulator

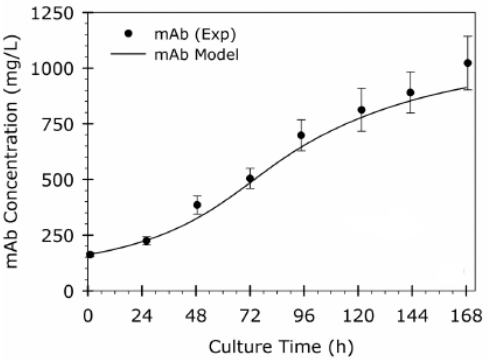

Figure 10: mAb concentration of base case study

Unfortunately, the result for the mAb production is far from the base case study. There is no increase in the mAb concentration which means there is no mAb produce at all by this model. Referring to the base case study, the mAb concentration should be increase following the increasing in the cell concentration and also decreasing in glutamine concentration as the main source for the production of mAb is the glutamine. This unsuccessful result might be affected by the unsuccessful model of glucose and glutamine consumption, and lactate, ammonia and cell production. Besides, the results can be attributed to the lack of mechanistic information which mAb production in the equation is solely related to the specific growth rate whereas antibody production is really more complex process in reality [7]. In addition, the model used for the measurement of the mAb secretion is not fit best for the mAb and proved that inadequate description of antibody production to enable it to capture the right trends such as the base case

IV. Conclusion

The combination of structured and unstructured kinetic models was used to describe cell growth, metabolism of nutrients and antibody synthesis as to get the models predictions in good agreement with the experimental values in all cases for all measured variables especially from the base case study. The main objective of this study is to develop a design advisor tool for upstream process of biopharmaceutical production. A general method for the estimation of output from the kinetic parameters has been demonstrated but for this model it is quite far from the expected results. The approach is general and mathematically straight-forward and suit well towards the estimation of the output. It is conclude that there is minor problem with the kinetic model used as the result for quite the same as base case study for cell concentration, glutamine concentration and ammonia concentration but only shows unsuccessful results for mAb production as te concentration does not changes at all. For glucose concentration and lactate concentration, there are still changes at the concentration even not it is too small.

ACKNOWLEDGMENT

Thank you to my supervisor and Universiti Teknologi Mara.

References

[1] Bayrak, E.S., Tony, W., Jerums, M., Coufal1, M., Goudar, C., Cinar, A., &Undey, C. (2016). In Silico Cell Cycle Predictor for Mammalian Cell Culture Bioreactor Using Agent-Based Modeling Approach. Symposium on Dynamics and Control of Process Systems, Including Biosystem. Trondheim, Norway.

[2] Baughman, A. C. et al. (2009). On the Dynamic Modeling of Mammalian Cell Metabolism and mAb Production. Computers and Chemical Engineering 34 (2010) 210–222

[3] Ding, M. et al. (2014). Innovation and Marketing in the Pharmaceutical Industry. International Series in Quantitative Marketing, 20. DOI 10.10079/978-1-4614-7801-0_1

[4] Farid, S. S. et al. (2006). Modelling Biopharmaceutical Manufacture: Design and Implementation of SimBiopharma, Computers and Chemical Engineering (2006), doi:10.1016/j.compchemeng.2006.10.020

[5] Green, A., J. (2015). Model Based Process design for a Monoclonal Antibody-Producing Cell Line: Optimisation Using Hybrid Modelling and An Agent Based System. Biopharmaceutical & Bioprocessing Technology Centre School of Chemical Engineering and Advanced Materials Newcastle University

[6] Jae, D., J., & Barford, J., P. (2000). An Unstructured Kinetic Model of Macromolecular Metabolism in Batch and Fed-Batch Cultures of Hybridoma Cells Producing Monoclonal Antibody. Biochemical Engineering Journal 4 (2000) 153–168.

[7] Kyparissidis, A., D. (2010). Development of a Combined Mathematical and Experimental Framework for the Control and Optimisation of Mammalian Cell and Chemical Technology Culture Systems. Department of Chemical Engineering. Imperial College London.

[8] Li, F., Vijayasankaran, N., Shen, A., Kiss, R., & Amanullah, A. (2010). Cell culture processes for monoclonal antibody production. mAbs, 2(5), 466-477. doi: 10.4161/mabs.2.5.12720

[9] Market Research. (2016). Global Biopharmaceutical Market Report & Forecast (2016-2021). International Market Analysis Research and Consult.

[10] MathWorks, T. (1994). Creating a GUI with GUIDE - video - MATLAB & Simulink. Retrieved December 16, 2016, from https://www.mathworks.com/videos/creating-a-gui-with-guide-68979.html?requestedDomain=www.mathworks.com

[11] Sachidananda, M., Erkoyuncu, J., Steenstra, D., & Michalska, S. (2016). Discrete Event Simulation Modelling for Dynamic Decision Making in Biopharmaceutical Manufacturing. Procedia CIRP, 49, 39-44. doi: http://dx.doi.org/10.1016/j.procir.2015.07.026

[12] Sandadi, S. (2009). Mass Transfer, Mixing, Chinese Hamster Ovary Cell Growth and Antibody Production Characterization Using Rushton Turbine and Marine Impellers. Graduate School-New Brunswick Rutgers, The State University of New Jersey.

[13] Chapra, S.C. (2005). Applied Numerical Methods with MATLAB for Engineers and Scientists. McGraw-Hill Education. Singapore. (pg 15).

[14] Kontoravdi C., Pistikopoulos E.N., Mantalaris A. (2010). Systematic development of predictive mathematical models for animal cell cultures. Computers & Chemical Engineering, v.34, pp: 1192-1198.

[15] Jang J.D., Barford J.P. (2000) An unstructured kinetic model of macromolecular metabolsim in batch and fed-batch cultures of hybridoma cells producing monoclonal antibodies. Biochem. Eng. Journal, v.4, pp:153-168.

Nomenclature

μ	Specific growth rate (h^{-1})
μ_{max}	Maximum specific growth rate (h^{-1})
a_1	Constant for the maintenance term of glutamine uptake (dimensionless)
a_2	Constant for the maintenance term of glutamine uptake (mM)
$K_{,Amm}$	Constant for metabolic inhibition from ammonia (mM)
K_{Glc}	Saturation constant for growth based on glucose metabolism (mM)
K_{Gln}	Saturation constant for growth based on glutamine metabolism (mM)
$K_{,Lac}$	Constant for death related to lactate toxicity (mM)
$K_{d,Amm}$	Constant of ammonia for cell death (mM)
$K_{d,gln}$	Constant for spontaneous degradation of glutamine (mM)
m_{glc}	Maintenance coefficient of glucose (mmol $cell^{-1}$ h^{-1})
Q_{ab}	Specific antibody productivity (pg $cell^{-1}$ h^{-1})
Q_{amm}	Specific production rate of ammonia (mmol $cell^{-1}$ h^{-1})
Q_{glc}	Specific consumption rate of glucose (mmol $cell^{-1}$ h^{-1})
Q_{gln}	Specific consumption rate of glutamine (mmol $cell^{-1}$ h^{-1})
Q_{lac}	Specific production rate of lactate (mmol $cell^{-1}$ h^{-1})
X	Total cell concentration (cells l^{-1})
$Y_{amm;gln}$	Yield of ammonia from glutamine (mol mol^{-1})
$Y_{lac;glc}$	Yield of lactate from glucose (mol mol^{-1})
$Y_{X;glc,}$	Yield of cells on glucose (cells mol^{-1})
$Y_{X;gln}$	Yield of cells on glutamine (cells mol^{-1})
K_{ER}	Rate constant for ER-to-Golgi antibody transport (h^{-1})
K_G	Rate constant for Golgi-to-extracellular medium antibody transport (h_{-1})
$\gamma 1$	Constant
$\gamma 2$	Constant
$\varepsilon 2$	Golgi glycosylation efficiency factor.
λ	Molecular weight of IgG_1 (g/mol),