Development and Validation of a Modified Contois Kinetics Model for Microalgae *Chlorella Kessleri*

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Abstract. Microalgae is a promising source for renewable biofuels, medicines, healthy foods, animal feeds and many more. Microalgae have to be produced on a large scale in order to give a significant impact for the benefit of the society. Consequently, an understanding of the kinetic growth model of particular microalgae becomes necessary for the purpose of scaling up the production system. This study explores the development of an algae growth model for the *Chlorella kessleri* culture. The proposed growth model for *Chlorella kessleri* was developed from the original Contois growth model, which later modified to include the second substrate as the influencing factor for the growth of cell biomass. Differential equations resulted from mathematical development and modification of Contois model were numerically solved using the Runge-Kutta method. Values of model parameters are optimized using the Levenberg-Marquardt algorithm method. All computation for the above modeling purpose was done with the aid Matlab 7.1 software. The results of the study show that validation of the original Contois model produced predictions of cell and substrate concentrations which deviate from experimental data. When the Contois model was modified to include the effect of the second substrate for the *Chlorella kessleri* cell growth, in addition to N-NO_3 as the first substrate, predictions produced an excellent agreement with the experimental data. Further validation against other experimental data of *Chlorella kessleri* culture is required to allow the generality of the proposed modified Contois model.

Keywords: *Growth Model, Microalgae, Contois Model, Chlorella kessleri*

1 Introduction

Microalgae or microscopic algae have high economic and industrial potentials as sources for producing medicines, healthy foods, ingredients, chemicals, biofuels, electricity, animal feeds and many more. In addition, microalgae also offer a solution to current environmental problems, such as the greenhouse effect and wastewater treatment. Microalgae can bind carbon dioxide through photosynthesis and eliminating excess nutrients with minimal costs. They are also able to utilize various types of nitrogen compounds and absorb heavy metals. As a consequent, many studies have been done to seek possibilities of microalgae as a source of foods [1], energy production [2], medicines [3], and other valuable products.

Although in general algae are some of the most robust organisms on earth able to grow in wide range conditions, in nature they grow in a completely submerged aquatic environment having low-density cellular suspension. For the purpose of having a significant impact on social, economic and environment, microalgae have to be produced on a large scale in order to increase the production volume. In this relation, a knowledge on microalgae kinetics growth model

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becomes an important issue for scaling up an algae processing system for the intention of commercial production. Unfortunately, studies on growth kinetics on specific microalgae, with particular attention to Chlorella kessleri are still very rare and consequently, information about the dynamics of the particular microalgae growth is still very limited. A kinetics growth model obtained from a laboratory kinetic study would be very beneficial for the design of a microalgae production system, whether it is continuous or batch ones. A number of general growth models, both structured and non-structured, are available elsewhere [4].

The development of a growth model requires consideration on several factors so that the mathematical model becomes relatively simple to be implemented, without losing its biological characteristics. Growth models are often developed on the basis of a steady state condition by only considering a single substrate that limits cell growth [5]. However, the growth phenomena are the result of a number of very complex biochemical reactions that occur in the cells. For engineering purposes, such phenomena are described by chemical reactions and measured by a parameter termed as yield [6].

A number of growth models for various types of algae have been developed. Davidson & Gurney [5] used the Droop growth model [7] to describe the growth of Thalassiosira pseudonana microalgae. One of the objectives of their study was to develop a model which is able to include the influences of nutrients competition in the model. Filali et al [8] developed kinetics growth models for microalgae Chlorella vulgaris in a photo-bioreactor. The model took into account the combined influences of light intensity and the total inorganic carbon available per cell. It was capable of providing a good result when validated against experimental data. Although microalgae Chlorella kessleri has attracted attention to a number of investigators due to its ability to produce biofuel and at the same time capable of minimizing organic and nitrogen compounds in wastewater [9], [10], limited information is available on its kinetics growth model. It is, therefore, a requirement to develop a kinetics growth model that is suitable for the growth of microalgae Chlorella kessleri. The present study reported the development of a growth model for Chlorella kessleri on the basis of Contois model and validated against the experimental data of [10].

2 Microbial Kinetics Growth

Microbial kinetics growth describes the relationship between the specific growth rate (μ) and the substrate concentration (S). It can be categorized into non-structured and structured models, depending on the level of complexity. The former models perceive cells as a unity with a fixed and clear nature and are used to represent the overall microbial response. Even though it was introduced for more than seventy years ago, such models are still mostly used at the present time among investigators. Unfortunately, these models still possess shortcomings since they do not account for changes in the biological characteristics of the microorganisms, while the structured model considers various cellular properties that influence the process.

The simplest non-structural growth model is the one proposed by Jacques Monod in 1942 based on observations of the growth of E. coli at various glucose concentrations [4]. In the mathematical form, it is shown in Equation 1.

$$u = u_{\max} \frac{S}{K_s + S} \qquad [1]$$

In Equation 1, S represents the concentration of the nutrient in the media at time t and K_s is the half-saturation constant. Symbols μ and μ_{max} are specific and maximum growth rate, respectively. The latter is unique for every microbial culture [11].

The Monod model is very suitable for single culture systems and simple substrates, but its accuracy is poor for systems with mixed cultures and complex substrates which most probably due to its inadequate theoretical understanding of parameters in the equation [12]. To consider these weakness factors, many researchers have tried to further develop the growth model developed by Monod. The Contois model is one model that is often used if biomass is considered to inhibit microbial growth. If the biomass concentration becomes very high, the biotic phase will meet the reactor volume, so that substrate utilization is prevented by the presence of biomass [13]. In the mathematical form, the Contois model is described as in Equation 2.

$$u = u_{\max} \frac{S}{S + K_s X} \qquad [2]$$

Here, X denotes the biomass concentration in the reactor. Of course, it is difficult to imagine how the concentration of cells can hamper its own growth. But in many cases, the Contois model fits experimental data. It could be explained that the Contois kinetics considers the influence of substrates or metabolite products that are not taken into account in the Monod Model.

Although some researchers emphasize environmental influences as a starting point to commence the microbial kinetics growth, many of them ignore the fact that in the real condition, microalgae grow in environments consisting many substrates, and growth is not only controlled by a single nutrient, but also by two or more limiting nutrients [14]. In such conditions, there exists a very complex interaction. Consequently, it would be very difficult to explain the microalgae growth using a non-structured growth model. For the growth which is influenced by the existence of two substrates, Tsao & Hanson [15] has compiled a non-structured growth model with many growth parameters, as shown in Equation 3.

$$u = \frac{u_{\max,1} u_{\max,2} S_1 S_2}{(S_1 + K_1)(S_2 + K_2)} \qquad [3]$$

Equation 3, for example, can be used to model the growth of methanotrophic bacteria with two substrates of O_2 and CH_4. This is one of the processes used to produce single cell proteins.

3 Methodology

Experimental data for the validation of the modified Contois model under this study were taken from the experimental works of Lee & Lee [10]. They conducted a study of the growth of microalgae *Chlorella kessleri* in media containing nitrate and glucose substrates. Mathematical model development to represent the microalgae growth is presented in Section 2.1 and the modification of Contois model is further described in Equation 9 and 10. Differential equations resulted from mathematical development and modification of Contois model were numerically solved using the Runge-Kutta method. The value of model parameters is optimized using the Levenberg-Marquardt algorithm method. The solution to the non-linear equations using the Levenberg-Marquardt approach was performed by Matlab application software version 7.1 [16].

Matlab software was run on laptops that use Intel Core processors at a speed of 2.60 GHz and 4.0 GB of memory access.

3.1 Mathematical Model Development

State variables commonly employed in the non-structured models include substrate concentration, biomass and metabolite products concentrations, and culture growth volume [17]. In the present study, the experimental data of Lee & Lee (2002) provide substrate and microalgae cell concentrations. For the purpose of modifying the growth model, it is started from the mass balance for microalgae growth and substrate reduction which can be described as Equations 4 and 5

$$\frac{dX}{dt} = u \cdot X \qquad [4]$$

$$\frac{dS}{dt} = -(\sigma \cdot X) \qquad [5]$$

where μ is the growth rate indicated by Equation 2 and S is the concentration of the limiting substrate for growth and X is the concentration of microalgae. The rate of the substrate reduction σ can be calculated using Equation 6.

$$\sigma = (\mu \cdot Y_{X/S}) \qquad [6]$$

where the $Y_{X/S}$ cell yield coefficient is equal to the amount of cell mass produced divided by the amount of the substrate utilized. By assuming that at the growth rate (μ) = 0, cells still need a substrate to maintain the cell metabolism, a "maintenance" coefficient, m_s is added to Equation 6, to obtain Equation 7.

$$\sigma = (\mu \cdot Y_{X/S}) + m_S \qquad [7]$$

Equation 8 is obtained by substituting Equation 7 to Equation 5.

$$\frac{dS}{dt} = -(\mu \cdot Y_{X/S} + m_S) \cdot X \qquad [8]$$

Equation 8 demonstrates the change of the substrate concentration over time.

4 Results and Discussion

Experimental data of Lee & Lee[10] show the trend of the concentration of N-NO_3 substrate which declined over time until it reached below 2 mg/L. Therefore, in the present study, for the purpose of growth simulation, it is assumed that N-NO_3 is the limiting substrate for growth. In addition, the experimental data also show that the growth rate decreases over time allowing to assume the growth rate experiences inhibition due to the increase of microalgae biomass concentration. On the basis of these two assumptions, the simulation was carried out using the Contois growth model as shown by Equation 2.

Figures 1 and 2 presented the comparison of predicted biomass and substrate concentrations against experimental data, respectively. Solid lines are prediction resulted from simultaneous solutions of Equations 2, 4 and 7 while symbols are experimental data provided by Lee & Lee[10]. The values of the model parameter appeared in Equations 2, 4 and 7 are first obtained through optimization with the Levenberg-Marquardt algorithm. Detailed methodology for such an optimization process can be consulted through Matlab manual [16]. The resulted optimization values for the model parameter are presented in Table 1.

Table 1. Values of growth parameters for Contois model on the basis of nitrogen as the limiting substrate

Parameter	Definition	Unit	Value
μ_{max}	the maximum specific growth rate	day^{-1}	0.834
K_s	half saturation constant	mg/L	0.025
$Y_{X/S}$	cell yield coefficient	mg cell/mg substrate	0.024
m_s	maintenance coefficient	mg substrate/mg cell	0.0009

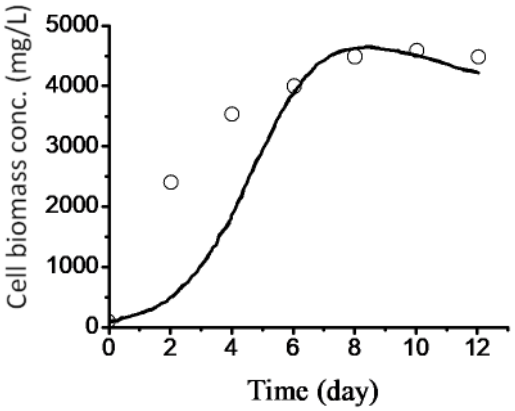

Figure 1: Comparison of predicted biomass concentration by Contois model (line) and experimental data (symbol)

Figure 1 compares predictions of cell biomass concentration using the Contois model with experimental data of by Lee & Lee[10]. Under- predictions of cell biomass concentrations are observed during the first six days of algal culture in the bioreactor. However, predictions are in good agreement in the later period. Such results indicate that the original Contois model is not capable of providing a good prediction of algae biomass concentration at the initial stage of its growth. Similar results were also evident in the prediction of substrate concentration as shown in Figure 2. Experimental data clearly illustrated that a rapid decrease of substrate concentrations was seen in the first few days of the operation and followed by a slow decrease of the substrate concentration in the later days of operation. Unfortunately, the prediction by the original Contois model was unable to capture the whole trend of experimental data.

Although the predictions are in good agreement with experimental data for the substrate concentrations in the later period of bioreactor operation, predictions in the first few days of operation significantly deviated from the experimental data. With these discrepancies, it can be concluded that the assumption of nitrogen as the only single substrate which controls the growth is not appropriate. Consequently, in an attempt to produce better predictions, in agreement with the experimental data, it is necessary to modify the Contois growth model.

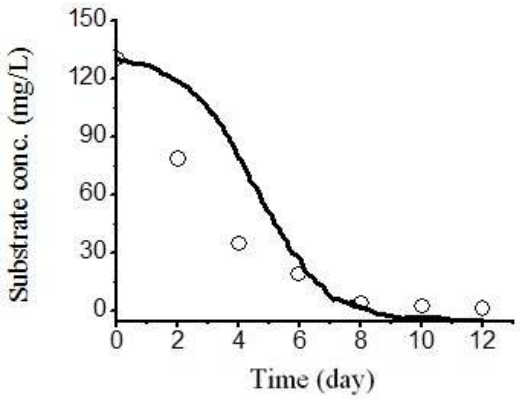

Figure 2: Comparison of predicted substrate concentration by Contois model (line) and experimental data (symbol)

The modification made to the Contois model was started by assuming that the growth is also influenced by the concentration of glucose as a carbon source for the growth of microalgae. This means that glucose is the second substrate, in addition, nitrogen nitrate. The Modification of the Contois model can be developed by combining the original Contois model from Equation 2 with a non-structural growth model proposed by Tsao & Hanson [15], to give Equation 9.

$$u = \frac{u_{\max,1} u_{\max,2} S_1 S_2}{(K_1 \cdot X + S_1)(K_2 \cdot X + S_2)} \qquad [9]$$

where $\mu_{max,1}$ is the value of the specific growth rate for nitrogen substrate. and $\mu_{max,2}$ is the specific growth rate for the carbon substrate. The S_1 and S_2 notations represent the concentration of the nitrogen and carbon substrates, respectively. Symbols K_1 and K_2 denote the saturation constant for the nitrogen and carbon substrates, respectively. The change of the concentration of the carbon source, glucose, in this case, can be calculated using Equation 10.

$$\frac{dC}{dt} = -\left(\mu \cdot Y_{X/C}\right) \cdot X \qquad [10]$$

where $Y_{X/C}$ is the yield coefficient for the carbon substrate. Again values for growth parameter appeared in Equations 8, 9 and 10 were obtained through optimization with the Levenberg-Marquardt algorithm. Values for those model parameters are shown in Table 2.

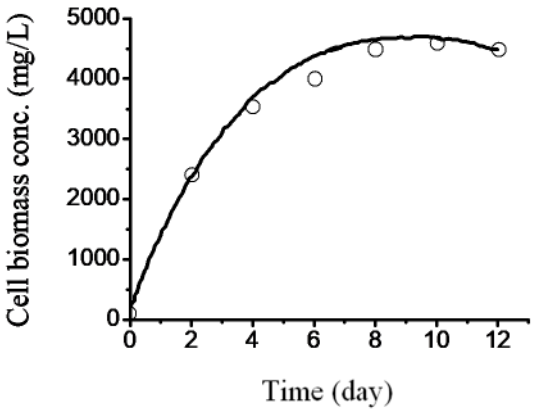

Figure 3: Comparison of predicted biomass concentration by the modified Contois model (line) and experimental data (symbol)

On the basis of the modified Contois model, using Equations 8, 9 and 10 as well as optimized values of model parameters in Table 2, predictions of algal cell biomass and substrate concentrations were validated against experimental data. Figure 3 presents the predictions of cell biomass concentrations on the basis of the modified Contois model, validated against experimental data. It is clearly seen that qualitatively and quantitatively the predictions are in excellent agreement with the experimental data. A drastic improvement in predicted cell and substrate concentrations was evident after the inclusion of the second substrate, glucose, as the carbon source for the growth of the microalgae. This result indicates that the assumption of the cell growth affected by two substrates, N-NO_3 and glucose is valid. Although the validation of the modified Contois model against experimental data of Lee & Lee [10] is in excellent agreement, legitimacy of the modified model needs to be tested against other experimental data of *Chlorella kessleri* culture. However, the lack of experimental data of this specific culture hinders the validation of the modified model.

Table 2. Values of growth parameters for the use with modified Contois model

Parameter	Definition	Unit	Value
$\mu_{max,1}$	maximum specific growth rate for nitrogen substrate	day^{-1}	0.7844
$\mu_{max,2}$	maximum specific growth rate for carbon substrate	day^{-1}	0.2018

K_1	nitrogen substrate affinity coefficient	mg/L	0.0312
K_2	carbon substrate affinity coefficient	mg/L	0.0001
$Y_{X/S}$	cell yield coefficient for nitrogen substrate	mg cell/ mg substrate	0.0204
$Y_{X/C}$	cell yield coefficient for carbon substrate	mg cell/ mg substrate	0.0159
m_s	maintenance coefficient	mg substrate/ mg cell	0.0012

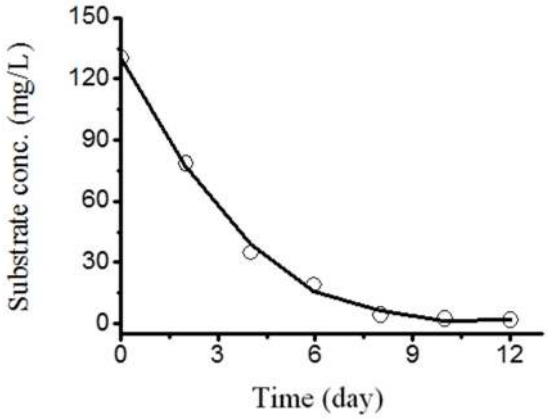

Figure 4: Comparison of predicted substrate concentration by the modified Contois model (line) and experimental data (symbol)

5. CONCLUSIONS

A non-structured kinetic growth model to represent the *Chlorella kessleri* culture has been developed. The new model was developed on the basis of Contois growth model, modified to include the effect of the second substrate for the *Chlorella kessleri* cell growth, in addition to N-NO_3 as the first substrate. Inclusion of the second substrate to the original Contois model produced excellent predictions in terms of cell and substrate concentrations when validated against the experimental data. Further validation against other experimental data of *Chlorella kessleri* culture is required to allow the generality of the modified Contois model.

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