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Integrating Flux Balance Analysis into Microalgae Growth Kinetics for Dynamic Simulation

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Abstract: Most of the microalgae growth models are based on modified Monod kinetics, which often involve many parameters to identify. Some fundamental questions about the validity of such empirical growth rate still remain. On the other hand, flux balance analysis (FBA) can compute a steady-state flux distribution of metabolic networks within a feasible flux space constrained by fundamental laws including mass balances. This work proposes how to set up various constraints as boundary conditions in FBA, relate the resulting flux distribution to the growth rate, and dynamically simulate the microalgae growth kinetics. In order to relate mass balances of the bioreactor to the FBA solution, accumulation rates as well as uptake and production rates are used. Dynamic simulations were performed by modifying pseudo-steady state assumption for FBA and integrating the ordinary differential equations for bioreactor model over time, leading to a two-time scale description. The proposed scheme can reduce the number of parameters and explain adaptation to the changing environment. A Chlamydomonas reinhardtii culture system is illustrated to present the applicability of the proposed scheme.

Keywords: Mathematical modeling, Bioreactor, Flux balance analysis, Chlamydomonas reinhardtii

1. INTRODUCTION

As the global warming has become a major issue, energy resources from biomass are receiving more attentions. In particular, biodiesel production using microalgae is considered promising because of their local utilization and production efficiency. Although the biodiesel from microalgae is not economically more attractive than biodiesel from conventional sources at present, its competitiveness can be improved by increasing the growth rate and oil accumulation capability (Suriesetty, 2010). There have been a large number of studies to improve the yield of oil production from microalgae from the perspective of both genetic manipulation and operational optimization. Operational optimization include optimal control of nutrient feeding, geometrical shaping of reactor, light regulation etc.

As an effort for operational optimization, many growth models for various kinds of algal species have been suggested until recently. For example a fed-batch bioreactor model for the fresh-water green microalgae *Auxenochlorella protothecoides* is proposed (Suriesetty, 2010). The model consists of 12 parameters, 11 equations and 9 state variables. The 11 equations involve 6 differential equations of mass balance and 5 constitutive equations including substrate uptake rates and the growth rate. In these equations, especially growth rate equation is of an empirical nature. It is known that the microalgal growth rate depends on the intracellular nitrogen concentration (Q) or nitrogen cell quota (q) and external carbon source concentration (S2) (Suriesetty, 2010). A general hyperbolic function for the growth rate on Q and S2 can be written as:

$$\mu = \mu_m \left(\frac{q - q_m}{K_q + q}\right)\left(\frac{S_2}{K_s + S_2}\right)$$

where K_q is the half saturation constant of nitrogen quota for growth, K_s is the half saturation constant of carbon source for growth, q_m is minimum cell quota for supporting growth, and μ_m is the maximum growth rate of biomass.

Such empirical growth rate equation has some limitations. First, it involves many parameters to estimate; K_q, K_s, q_m, μ_m. Second, it cannot explain various biological phenomena such as sudden growth phase change or influence of light, because it is just an empirical equation for explaining data within the experimental range.

On the other hand, flux balance analysis (FBA) can compute a steady-state flux distribution of metabolic networks using mass balance equations and constraints of whole metabolites of the organism. These flux distributions also include growth rate information as a flux or reaction rate per dry biomass weight. Thus if the constraints of each flux can be set up from concentration information, the growth rate can be computed without any empirical equation. This study proposes an integrated dynamic simulation framework that computes the growth rate from FBA and integrate it into the microalgal growth kinetics for dynamic simulation. This simulation is performed by modifying the conventional FBA, which assumes a steady-state, and integrating the ordinary differential equations for bioreactor model over time, leading to a two-time scale description.

2. MICROALGAE GROWTH MODEL

2.1 Monod and Droop model

There are various types of bioreactor models. Their mass balance equations are identical; however, growth rates or nutrient uptake and releasing rates are different. For example, the Monod equation relates the growth rate to the concentration of limiting nutrient in medium:

$$\mu = \mu_m * \frac{S}{S + k_s}$$

Here, μ_m is the theoretical maximal growth rate of the species, k_s is the half maximal concentration of nutrient, and S is the concentration of limiting nutrient in medium. It seems a quite simple expression of growth rate; however, the maximal growth rate is a function of other variables like pH, temperature or the length of day.

Another well-known equation for growth rate is the Droop model. This equation relates the growth rate to *the cell quota of the limiting nutrient*.

$$\mu = \mu'_m * (1 - \frac{q_0}{q})$$

Here, μ'_m is the maximal growth rate which is different from the above maximal growth rate expression of Monod equation. q is the intracellular nutrient quota; that is, if X is a total biomass concentration and Q is an intracellular nutrient concentration,

$$q = \frac{Q}{X}$$

q_0 is the minimal necessary cell quota of the nutrient for growth. If the cell quota of limiting nutrient or q is lower than q_0, the growth rate becomes zero. Besides the growth rates, the uptake and releasing and producing rates of nutrient are expressed similarly according to the types of equation. There are many experimental evidences that verify each of equations. However, in the case of microalgae the Droop equation is recognized as the best fitted model.

2.2 Flux Balance Analysis

Flux balance analysis (FBA) is a method for searching for the growth rate or other intracellular reaction rates by using metabolic information of the organism and the scheme of it is summarized as follows:

- Set the mass balance equations of each metabolites
- Assume the steady state
- Combine the whole equations as matrix form like S*v=0
- Set the upper and lower bounds of fluxes(reaction rates)
- Set an objective function and solve the above equation

A popular objective function as a hypothesis is maximizing active biomass (organelles of cell) production rate, because the organism changes the intracellular metabolisms for increasing its offspring. Although there are various objective functions like minimizing ATP or maximizing carbon source yield, the most commonly used one is maximizing active biomass production rate. If the objective function is set as maximizing active biomass production rate, it becomes a linear program, which can be easily solved. The resulting output is a set of whole fluxes or reaction rates of the organism under a particular boundary condition.

Because nutrient uptake rates are dominant factors for determining the growth rate, the uptake rate equations as boundary conditions are needed. These equations are expressed as either Monod or Droop equation form. We incorporate these into the flux balance analysis as constraints as well.

2.3 Integrating FBA into the bioreactor model

As mentioned above, the growth rate can be computed by using flux balance analysis. Using the equations that are used in previous work (Suriesetty, 2010), the equations of uptake/producing rate and mass balance equations can be constructed as in Table 1.

Table 1. Continuous bioreactor model

$X = x + Q + I_p$
$\rho = \rho_m \left(1 - \frac{S_0}{S_1}\right)^{1+\varepsilon} * \frac{x}{X}$
$\pi = \pi_m \frac{S_2}{K_\pi + S_2}\left(1 - \frac{I_p}{X}\right) * \frac{x}{X}$
$\frac{dx}{dt} = \mu * \varphi_x * X$
$\frac{dS_1}{dt} = -\rho * \varphi_1 * X$
$\frac{dS_2}{dt} = \left(-\frac{1}{Y_{xs}} * \mu - k_m - \frac{1}{Y_{ps}} * \pi\right) * \varphi_2 * X$
$\frac{dQ}{dt} = \left(\rho - \frac{1}{Y_{xq}} * \mu\right) * \varphi_1 * X$
$\frac{dI_p}{dt} = \pi * \varphi_3 * X$

In the above proposed equations, X is the total biomass weight concentration, ρ is the nitrogen source uptake rate per dry weight of biomass, π is the lipid production rate per dry weight of biomass, x is the active biomass weight concentration, S_1 is the nitrogen source weight concentration in media, S_2 is the carbon source weight concentration in media, Q is the intracellular nitrogen source weight concentration, and I_p is the intracellular lipid weight concentration. φ_i is the molar weight of each substrate for changing the unit of equations from mmol to gram.

Whereas conventional FBA uses steady state hypothesis, our proposed scheme allows for accumulation of metabolites like

lipid and nitrogen source within the cell. This is reflected as steady state assumptions for metabolites *except for the accumulated metabolites*. The accumulation rate equations of intracellular nitrogen source and lipid can be derived from several established equations easily. First the intracellular nitrogen source accumulation rate is expressed as:

$$N = \rho - \frac{1}{Y_{xq}}\mu$$

The lipid accumulation rate is more difficult to set up than the first one, because there are many variants of triacylglycerol (TAG) in the organism. We use the information of fatty acid compositions and compute the relative amounts of TAGs (Siaut, 2011). These relative amounts of TAGs are combined together as accumulation rate of each TAG:

$$G_i = \sigma_i * \pi$$

In this equation σ_i represents the relative quota of each TAG species and the summation of σ_i equals to 1. Thus by using these equations and modified flux balance analysis, the growth rate of organism can be calculated and it can be used to solve other differential equations above.

2.4 Two-time-scale dynamic simulation using FBA-based bioreactor model

To use this proposed new bioreactor model, the iterative algorithm is needed because of growth rate. In other words, the carbon source uptake rate equation and intracellular nitrogen accumulation rate equation, which contain the growth rate term, are also needed to get growth rate. So the converged value of growth rate should be computed, and the proposed scheme is summarized as follows:

At time t_i

1. Obtain the value of μ from the previous time t_{i-1}
2. Calculate the nutrient uptake rates and accumulation rates
3. Apply these into flux balance analysis
4. Obtain a new value of μ and compare it with previous one
5. Iterate this algorithm and obtain a converged value of μ

With a computed value of μ at t_i, growth kinetic equation is integrated to estimate macroscopic variables such as concentrations of substrates at t_{i+1} . In this way the concentrations and growth rate can be attained marched forward over time.

We note that the growth rate is considered constant during one time interval of numerical integration . The rationale behind this idea is that the intracellular metabolism reaches a new steady state much faster (fast-time scale phenomena) compared to the overall change in the macroscopic variables, which act as boundary conditions for the intracellular changes in metabolism. In the previous works on *Escherichia coli* or *Shewanella oneidensis*, the time interval for numerical integration is usually order of hour (Varma 1994, Feng 2012). The growth rate change of Clamydomonas reinhardtii is slower than other organism's growth rate changes. Thus it is a reasonable to choose 1 hour for the time interval.

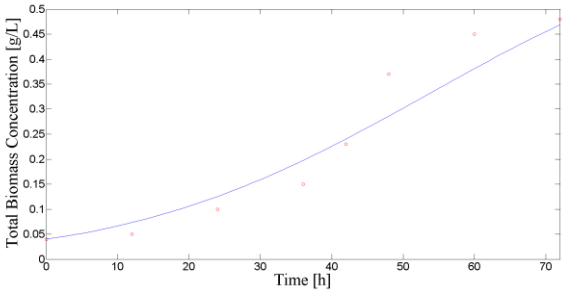
3. CASE STUDY

The model organism is the microalgae, Chlamydomonas reinhardtii. This species is one of the most well studied organism and the only microalgae that has the reconstruction of metabolic networks available (Roger, 2011).

There are also existing experimental data of Chlamydomonas reinhardtii harvesting in batch and fed-batch reactor (Zhang, 1999). In that work the microalgae was harvested in heterotrophic way (without light). The feed consists of acetate as carbon source and nitrate, ammonium and urea as nitrogen source.

For validation of the proposed scheme, we used the data where the nitrogen source is only nitrate and the reactor is run in a batch mode.

The first simulation case (Fig. 1) is that nitrogen source or nitrate in media is not used up during the period of simulation. In this case the total biomass and acetate concentration are linear. In other words, though the initial and final values are matched with experimental data, the other values are not. So in this way, the error beween data and simulation result is too big to assure that the proposed model is correct or not.



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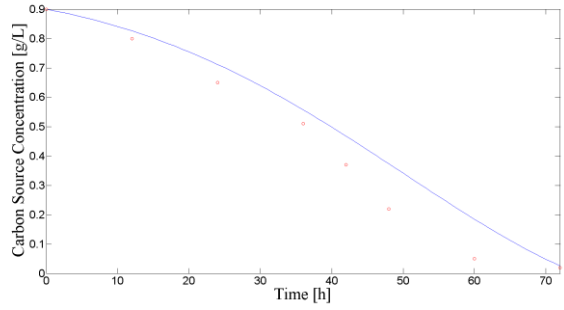

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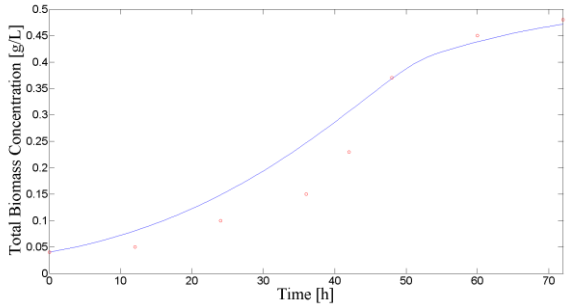
Fig. 1 Case 1: Nitrogen source in media is not used up. The time courses of total biomass concentration (a) and carbon source or acetate concentration (b) in heterotrophic batch reactor. Red dot for experimental data and solid line for simulation result of proposed model.



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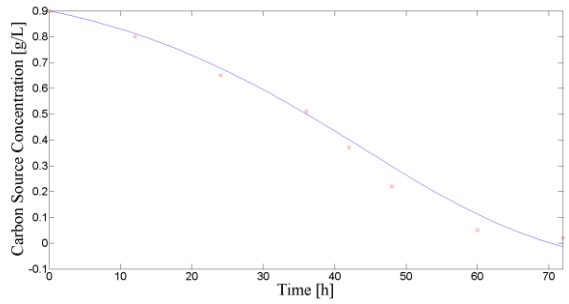

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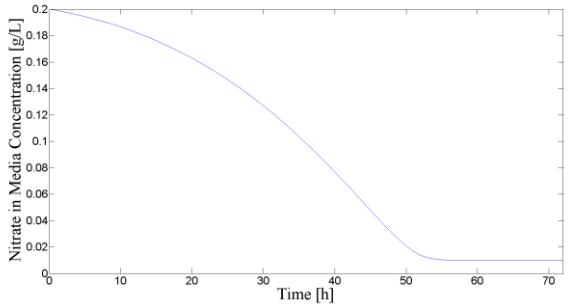
Fig. 2 Case 2: Nitrogen source in media is used up. The time courses of total biomass concentration (a) and carbon source or acetate concentration (b) in heterotrophic batch reactor. Red dot for experimental data and solid line for simulation result of proposed model.

The second simulation case (Fig. 2) is that nitrogen source in media is used up in the middle of the period of simulation. The complete consumption up to minimal limit of nitrate is occurred at 52 hours. In this case the total biomass concentration is similar with the experimental data. In other words the biomass grows exponentially at first and the growth rate decreases gradually then it converges to the steady state at final. The simulation result of total biomass concentration in early phase is not well matched with the experimental data, which has a *capital S form*. This seems to be caused by mismatch in growth rate as shown in Fig. 4.



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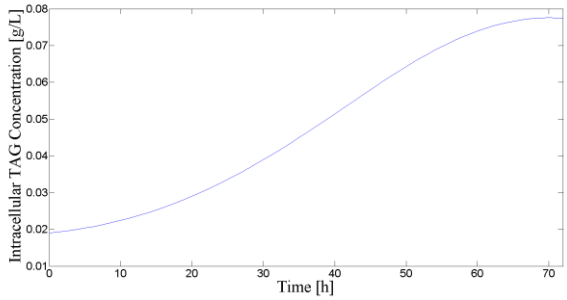

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Fig. 3 The time courses of intracellular nitrogen source or nitrate concentration (a) and triacylglycerol (TAG) concentration (b) in case 2.

On the basis of second simulation result of case 2, the dynamics of other materials can be also simulated. Especially the concentration of intracellular TAG which is the objective product of biodiesel optimization can be obtained from this simulation (Fig. 3. b). According to this proposed model, the intracellular TAG concentration is similar with the one of total biomass concentration.

Table. 2 Parameters for each case

	Case 1	Case 2
ρ_m	0.1	0.8
Y_{xs}	1/35	1/55
k_m	0	0
Y_{ps}	1/35	1/55
π_m	0.075	0.05
K_π	1.00	1.07
Y_{xq}	1/3.073	1/3.055
S_0	0.005	0.01

The meanings of the parameters are as follows; ρ_m for maximum uptake rate of nitrogen source, Y_{xs} for biomass to substrate yield, k_m for maintenance constant, Y_{ps} product to substrate yield, π_m for maximum oil production rate, K_π for half saturation constant for oil production, Y_{xq} for biomass to substrate quota yield and S_0 for threshold nitrogen source concentration.

4. DISCUSSION

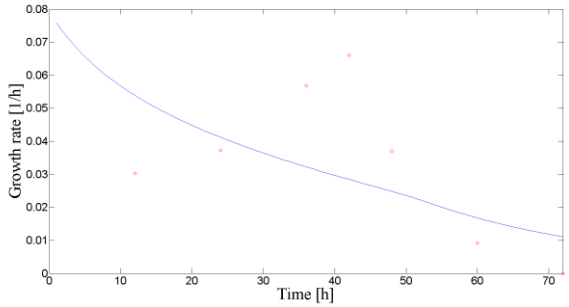
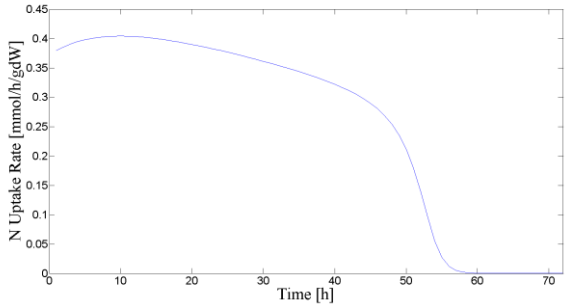


Fig. 4 Time courses of growth rate in case 2. Red dot for experimental data and solid line for simulation result of proposed model.

As mentioned above, the simulation results are well matched with experimental data at initial and end points; however, at the other points they are not well matched. It seems to be caused by computed growth rate from FBA; simulated growth rate is much bigger than the experimental one at early phase, so their exponential growth is exaggerated at this phase (Fig 2. a). And then the simulated growth rate is just decreased continuously to the end. On the other hand, the actual growth rate is smaller than the simulated one at the early phase, but it increased until the middle phase and then decreased to the end (Fig 4).

Then how can this mismatching be improved? First, the objective function of FBA should be changed according to the external conditions. Actually the organisms react to the changes of environmental conditions like substrate concentration or light intensity by changing the metabolic flux distribution. So it is not correct to assume that the organism always changes its metabolic flux distribution for maximizing biomass production; in some conditions it may put maximization of ATP producing fluxes or minimization of carbon source consumption before maximization of biomass production (Schuetz, 2007). Thus to improve the capability of FBA, it is needed to find the best matching objective function according to the external conditions. Second, uptake and production rate equations should be improved. The computed growth rate from FBA is a function of uptake, production and accumulation rates of substrates so if these equations are not matched with experimental data as shown in (Fig 5. b), they may cause the mismatching when calculating the growth rate by FBA. There have been some studies for improvement of these equations (Linkés, 2012), so by using these equations the growth rate can be computed more correctly. Third, the hypothesis for intracellular nitrogen source storage may not be correct. In this work, it is assumed that the nitrogen source stored in the cell maintains its original form by Droop model; nitrate, ammonium or urea. But these nitrogen sources may be stored as different forms like carbon source storage; TAGs. So this nitrogen storage form should be identified first, then the metabolic information of that component can be applied into FBA and other equations above.



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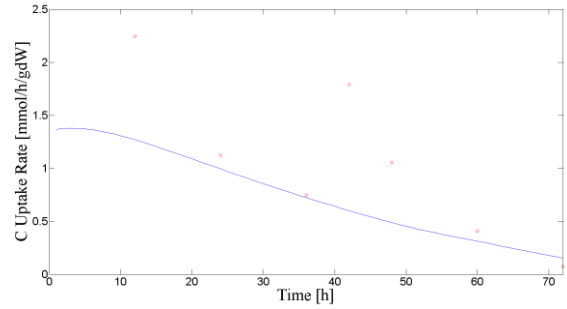

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Fig. 5 Time courses of nitrogen source (a) and carbon source (b) uptake rate. Red dot for experimental data and solid line for simulation result of proposed model.

5. CONCLUSION

In this work a new dynamic modelling for microalgae bioreactor using FBA is proposed. The simulation results are compared with the existing experimental data and the parameters of the model are estimated. By using metabolic flux distribution information, the number of parameters of model to be estimated is decreased and the usefulness of dynamic model is increased. Although there are some shortcomings like necessity of flux distribution information, this method can be utilized in various ways.

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