DEVELOPMENT OF COMPETITIVE ALGAL GROWTH MODEL APPLICABLE TO VARIOUS NUTRIENT CONCENTRATIONS FOR CYANOBACTERIAL BLOOMS SUPPRESSION

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Abstract - The algal blooms outbreak in lakes has caused severe water safe problems worldwide due to eutrophication. Though there has been an algae growth model developed by our team, the overestimation of the amount of nutrient absorb for algae was always observed in this previous model, especially under low temperatures, due to the use of the Michaelis-Menten model for nutrient uptake term. In this study, monoculture experiments of Microcystis sp. and its competitor C. meneghiniana were conducted to examine their growth and estimate their nutrient uptake parameters under low temperature (20^{o}C). Competition experiments were also conducted under various nitrogen concentrations and dilution rates at 20^{o}C to explore the different nutrient uptake characteristics of the two species. Based on the experimental results, the nutrient uptake term was further derived. The result showed that the overestimation was reduced, and the accuracy of the model prediction was improved. The determination coefficient increased mostly from $R^2 = 0.26$ to $R^2 = 0.84$.

Keywords - Cyanobacteria, Growth Model, Michaelis-Menten Model, Nutrient Uptake

I. INTRODUCTION

The occurrence of harmful cyanobacterial blooms recently rising with the water eutrophication problem globally [1],[2]. Cyanobacterial blooms can cause various water quantity problems, including unpleasant odor and taste in potable water and toxin productions [3]. Among coexisting species in phytoplankton community, the dominated growth of beneficial algae can suppress the outbreak of harmful cyanobacterial blooms [4]. It is therefore vital to understand the growth characteristics of algae to predict their proliferation in advance, there by develop efficient treatment to suppress the harmful cyanobacterial blooms by promoting the algal species successions. There are many environmental factors affecting the successions of dominant species. As one of them, nutrient concentrations were considered to be an important factor [5],[6]. Various nutrient uptake tendencies, which will affect interspecific competition and ultimately lead to species succession [7]-[9]. To understand the patterns of nutrient uptake by algae is essential to predict the proliferation of cyanobacteria and its co-existing species.

The relationship between nutrient concentrations and algal cell growth is commonly characterized by Monod model, Michaelis-Menten model and Droop model. These models describe the relationship between dissolved nutrient concentrations and growth rate [10],[11] and between intracellular nutrient concentrations and growth rate [12]. Meanwhile, the applicability limitation of these models has also been frequently noticed by several studies. There also have been some studies focused on the revision of these classical models to improve the accuracy of algal prediction and species succession [13]-[15]. However, these revisions were derived depending on the growth and absorption patterns of one and/or some certain algal species measured in experiments.

In Lake Tega (35° 51′ N, 140° 02′ E), Japan, the water quality was the worst from 1974 to 2000. The lake water was always plagued by cyanobacterial blooms (mainly the genus *Microcystis*). After a large amount of water transfer from Tone River, whose water quality was better than Lake Tega, since 2000, the genus *Cyclotella* (Thalassiosirales) has become the dominant algal species instead of *Microcystis*. In order to predict the algal species succession and investigate the appropriate environmental condition for our team [3] developed a competition growth model combined with the Droop and the Lotka-Volterra model by estimating the growth patterns of *Microcystis* sp. Nevertheless, the predicted value of nutrient uptake amounts always displayed overestimation compared with experimental results.

In order to improve this deficiency, in this study we aimed to explore a new nutrient uptake term and introduce it to the previous model, to inhibit the overestimation of cyanobacteria at low temperature during a long term cultivation. Batch monoculture experiments were conducted for *Microcystis* sp. and *C. meneghiniana* at low temperature of 20oC in laboratory to investigate the growth and nutrient absorption patterns. Based on the patterns obtained from monoculture experiments the relationship between dissolved nutrient concentrations and growth rate in long term cultivation was estimated. The applicability of the developed model's applicability was tested by comparing the new prediction values with experimental values. And the new coefficient of

determination (R^2) for prediction was calculated and compared with to the previous one which was calculated from the previous model, to test the prediction fitting accuracy improvement of the new prediction values.

II. MATERIALS and METHODS

Experimental algae and culture conditions

The algal species used in this study were *Microcystis* sp. and *C. meneghiniana* as test algae, which were collected and isolated from Lake Tega, Japan [3]. The size of both species was remarkably different, and the average cell volumes for *Microcystis* sp. and *C. meneghiniana* were 9.73 μm^3 and 243.35 μm^3, respectively. And the changes in individual algal cell's size during cultivation did not change the dominant species, which took larger total biovolume, between co-cultivated algal species. Therefore, the single-cell volume for both species were assumed to be invariant. The Wright's cryptophyte (WC) medium was used as cultivated medium in this study. The WC medium used here was all sterilized by autoclaving at 121°C for 20 minutes before inoculation, and 0.5 M hydrochloric acid and/or sodium hydroxide was used to accommodate the pH of medium to 8.0. The sodium nitrate ($NaNO_3$) and dipotassium hydrogen phosphate (K_2HPO_4) were dissolved to adjust the initial N and P concentrations to 14.0 mg-N/L and 1.55 mg-P/L, respectively. Sodium hydrogen carbonate ($NaHCO_3$) and sodium metasilicate (Na_2SiO_3) were also added as the carbon source and silicon source. As the necessary element for proliferation of *C. meneghiniana*, the silicon (Si) concentration in media was adjusted to 11 mg-Si/L, referring to the silicon (Si) concentration in Tone River water, Japan.

Growth characteristics and nutrient uptake patterns Microcystis *sp. and* C. meneghiniana

Batch monoculture experiments for *Microcystis* sp. and *C. meneghiniana* at the low temperature of 20°C were conducted to explore the nutrient absorption and growth characteristics for both two species in at the low temperature of 20°C.

Before the monoculture experiment, both species were precultured at 20°C for 7 days to adapt them to the cultivation temperature. In the preculture, nitrogen- and phosphorus- free WC medium was used to deplete the intracellular N and P. The two species were separately inoculated in 100 mL WC medium in a 300 mL Erlenmeyer flask. The initial cell density for both species were 1.0×10^4 cells/mL. Cultivation conditions were the same as those in subculture.

In the monoculture experiment, the precultured *Microcystis* sp. and *C. meneghiniana* were inoculated into 200 mL WC medium in a 300 mL Erlenmeyer flask at 20°C. The initial cell densities of *Microcystis* sp. and *C. meneghiniana* were adjusted to 1.0×10^4 and 4.0×10^2 cells /mL, respectively. The inoculation with the different cell density was needed to adjust the same biovolume of around 1.0×10^5 μm^3/mL, which could eliminate the effect of initial biovolume on the growth of each species. Each experimental group was conducted. in triplicates. Other experimental conditions were the same as those in the subculture experiment.

In order to measure the growth characteristics of the two species, the cell densities in each flask were measured every 3 or 5 days. Batch monoculture experiments were continued conducted until the cell densities were saturated and/or constant within 3-time consecutive measurements. And the dissolved N and P concentrations of medium in each flask were measured every 7 days to obtain the nutrient adsorption characteristics for both two species. The measurement of nutrient concentration also continued until the cell densities caught saturation.

Growth characteristics and nutrient absorption of the two algal species were obtained by measuring cell densities and dissolved nutrient concentrations change in monoculture experiments with the following equations. The growth rate (μ, /day) was calculated from (1), which is an important indicator to evaluate the algal growth characteristics.

$$\mu = \frac{\ln C_2 - \ln C_1}{t_2 - t_1} \quad (1)$$

where, C_2 and C_1 are the cell densities (cells/mL) at measured day, and t_2, t_1 (days) are the days when the measurement was done.

The maximum specific growth rate μ_{max} (/day) and the nutrient concentration half saturation constant K_μ (mg/L) was obtained following the Monod equation with various nutrient concentrations S (mg/L) in (2):

$$\mu = \mu_{max}\frac{S}{K_\mu + S} \quad (2)$$

The assimilated nutrients by algae cell defined as cell quota Q (pg/cell) was determined by the nutrient concentration quotient change and relating cell density C as shown in (3):

$$Q = \frac{S_0 - S}{C} \quad (3)$$

where, S_0 (mg/L) is the initial nutrient concentrations.

The minimum cell quota amounts Q_{min} (pg/cell) was calculated by the conventional quota type control developed from Droop equation by [16]:

$$\mu = \mu'_{max}\frac{Q - Q_{min}}{(Q - Q_{min}) + K_Q} \quad (4)$$

where μ'_{max} (/day) is the cell quota depending maximum growth rate, and the K_Q is the cell quota concentration half saturations constant. Similarly, the maximum cell quota amounts Q_{max} (pg/cell) could be derived with μ_{max} from (4), which is also obey the following equation [17]:

$$\mu_{max} = \mu'_{max}\frac{Q_{max} - Q_{min}}{(Q_{max} - Q_{min}) + K_Q} \quad (5)$$

The nutrient uptake rate from cultivation nutrient (ρ, pg/cell/day) can be estimated by the nutrient concentrations change ΔS (mg/L) caused by cell growth with elapsed time Δt like shown in (6):

$$\rho = \frac{\Delta S}{C\Delta t} \quad (6)$$

In some certain short term cultivation period, such as the experiments starts, the relationship between nutrient uptake rate and nutrient concentrations can be simulated using the Michaelis-Menten equation:

$$\rho = \rho_{max}\frac{S}{S + K_\rho} \quad (7)$$

where K_ρ is the half-saturation constant for nutrient uptake. ${\rho_{max}}^{lo}$ was estimated at the period of growth rate decreasing and ${K_\rho}^{lo}$ is the corresponding half-saturation constant, while ${\rho_{max}}^{hi}$ and ${K_\rho}^{hi}$ were obtained at the start of experiment.

Sunda, Shertzer and Hardison [14] have proved the nutrient uptake rate was not only affected by the dissolved nutrient concentrations in long term cultivation.The nutrient uptake rate of cell will change to zero at a certain nutrient concentration, but the concentration not always be zero. Meanwhile, from the experiments results, it can be observed when growth rate μ trends to decrease, the nutrient uptake rate ρ will decrease with it. And Lim, Jeong, Jang, Jang and Franks [17] have derived the growth rate change with various cell density follow (8):

$$\frac{dC}{dt} = \mu_c C[1 - \frac{C}{K_C}] \quad (8)$$

where K_C is the saturated cell density limited by environmental capacity. Therefore, combine the effect of cell density on the nutrient uptake rate, the long-term cultivation nutrient uptake rate can be limited by cell density as (9):

$$\rho = \rho_{max}\frac{S}{S + K_\rho}(1 - \frac{C}{K_C}) \quad (9)$$

Furthermore, Morel [18] indicated that the maximum nutrient uptake rate in long-term cultivation is related to intracellular nutrient contents (cell quota), and the ρ_{max} and K_ρ are decided follow (10) and (11):

$${\rho_{max}}^{hi} = {\rho_{max}}^{hi} - ({\rho_{max}}^{hi} - {\rho_{max}}^{lo})\frac{Q - Q_{min}}{(Q_{max} - Q_{min})} \quad (10)$$

$${K_\rho}^{hi} = {K_\rho}^{hi} - ({K_\rho}^{hi} - {K_\rho}^{lo})\frac{Q - Q_{min}}{(Q_{max} - Q_{min})} \quad (11)$$

Growth competition model

Based on the previous developed model [3], a new growth competition model was established by the nutrient uptake term improvement shown in (12), which limits the excessive nutrient uptake prediction of algal cell in long term cultivation.

The calculation of the model was followed a fourth-order form of the Runge-Kutta method, and the time step was set as Δt = 0.01 day. The prediction value was predicted under the same condition as experiments. After the verification of the model, the growth of *Microcystis* sp. was simulated under various N concentrations and daily renewal rate with a constant and sufficient P concentration, based on which the effective treatments to control *Microcystis* blooms were discussed.

$$\frac{dQ_{NA}}{dt} = \frac{\rho_{NA,max}N}{K_{NA}+N}\left(1 - \frac{C_A}{K_{CA}}\right) - \left[{\mu'}_{NA,max}\left(\frac{Q_{NA} - Q_{NA,min}}{Q_{NA} - Q_{NA,min} + K_{QNA}}\right), {\mu'}_{PA,max}\left(\frac{Q_{PA} - Q_{PA,min}}{Q_{PA} - Q_{PA,min} + K_{QPA}}\right)\right]^* \left(1 - \frac{{C_A}' - \alpha_{AB}C_B'}{K_A}\right)Q_{NA} \quad (12)$$

The biovolume was obtained by multiplying cell numbers with the average single-cell volume. The results of triplicate for the same experimental group were presented as [average value] ± [standard deviation].

Regression analyses were conducted by software Python 3.10.6. Kruskal-Wallis test was performed via SPSS Statistics (IBM, America), to measure the differences of experimental data at different experimental groups. And coefficient of determination (R^2) was calculated to measure the prediction accuracy. When $p < 0.05$, the results were determined to be significantly different.

III. RESULT

Growth and nutrient adsorption characteristics of Microcystis *sp. and* C. meneghiniana *at low temperature*

The change of biovolume and dissolved nutrient (N and P) concentrations of the two species cultivation were shown in Fig.1. The different growth and nutrient adsorption characteristics of *Microcystis* sp. and *C. meneghiniana* at low temperature can be observed from the curves in this figure.

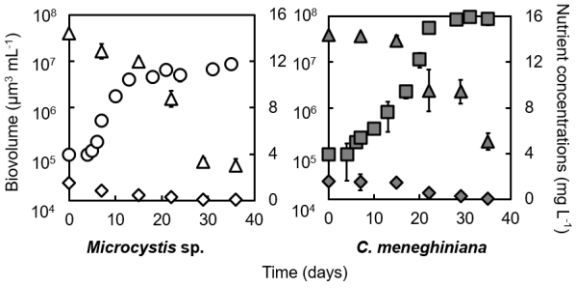


Fig.1 Growth curves of *Microcystis* sp. and *C. meneghiniana* and nutrient concentrations change in monoculture experiments. The open circle presents the cell densities for Microcystis sp. and the filled square presents that for *C. meneghiniana*. The open and filled triangle presents the nitrate concentrations in the monoculture experiment for *Microcystis* sp. and *C. meneghiniana*, respectively. The open and filled diamond presents the phosphate concentrations in the

monoculture experiment for *Microcystis* sp. and *Cyclotella meneghiniana*, respectively.

At 20°C, the *Microcystis* sp. can grow with WC medium and the growth of cell entered into the exponential growth phase from day 3 and lasted until day 13 with the highest growth rate μ of 0.882 ± 0.03 /day during this phase. The cell density finally reached saturation at day 35 occupying the biovolume of 1.013×10^7 ± 1.21×10^6 μm^3/mL which determined as K_A. The concentration of P decreased faster at the first measured time point (day 7 and day 14) than the following phase, and the P concentration decreased to 0.05 ± 0.002 mg/mL at the end of experiment in day 36. Meanwhile, the N concentrations continued decreased to the final concentration of 3.02 ± 0.001 mg/mL at day 36.

The C. meneghiniana entered into the exponential growth phase from day 4 at the same conditions, and the phase lasted longer to day 20 during when the highest growth rate μ up to 0.681 ± 0.12/day. At day 31, the cell density reached saturation with the saturated biovolume of 9.734×10^7 ± 1.09×10^6 μm^3/mL, that is K_B, the environmental capacity of C. meneghiniana. Therefore, the interaction coefficient α_{AB} in 20°C was calculated to be 0.175, while α_{BA} was 1.334. Both the concentrations of N and P decreased with cell growth to 5.05 ± 0.17 mg/mL and 0.06 ± 0.0035 mg/mL, respectively, at the end of experiment.

Other growth and uptake parameters for *Microcystis* sp. and *C. meneghiniana* were then calculated and summarized in Table . The μ_{max} related to dissolved N concentration of *Microcystis* sp. is 6 times as high as that of *C. meneghiniana*, while the cell quota limiting growth rate of *Microcystis* sp. is nearly the same as that of *C. meneghiniana.* The low maximum N uptake rate for *Microcystis* sp. ρmaxlo is only one-tenth of that of *C. meneghiniana*, but the one obtained from experimental beginning ρ_{max}^{hi} for the two species were not much different.

Parameter	*Microcystis* sp.	*C. meneghiniana*	*Microcystis* sp.	*C. meneghiniana*
	Nitrogen		Phosphorus	
Growth parameters				
μ_{max} (day^{-1})	0.267	0.38	0.383	1.17
K_μ (mg L^{-1})	7.623	1.22	4.645	2.59
Cell quota parameters				
μ'_{max} (day^{-1})	0.426	0.65	1.76	1.32
Q_{max} (pg cell^{-1})	2.483	1.399	1.878	3.61
Q_{min} (pg cell^{-1})	0.425	0.1206	1.57	0.357
K_Q (mg L^{-1})	1.225	0.908	1.107	0.417
Uptake parameters				
ρ_{max}^{hi} (pg cell^{-1} day^{-1})	2.95	4.68	1.107	0.74
K_ρ^{hi} (mg L^{-1})	18.88	14.282	0.134	1.63
ρ_{max}^{lo} (pg cell^{-1} day^{-1})	0.35	3.216	0.267	0.232
K_ρ^{lo} (mg L^{-1})	10.489	14.176	0.393	0.039

Table I Growth characteristics of Microcystis sp. and Cyclotella meneghiniana under low temperature (20°C)

When it come to the P related parameters, the μ_{max} for *C. meneghiniana* was 3 times as that for *Microcystis* sp. And both the maximum and minimum cell quota amount of P for *C. meneghiniana* were twice higher than the values of N for it. However, the Q_{max} of P for Microcystis was nearly the same as that of N. The ρ_{max}^{lo} and ρ_{max}^{hi} of the two species were almost same.

Based on the measured cell densities and nutrients concentrations change, the nutrient uptake rate of N and P for the two species were calculated as shown in Fig.2. The calculated data was displayed as every measured data instead of the average value to show the changing trend more clearly. The nutrient uptake rates for both of *Microcystis* sp. and *C. meneghiniana* decreased with dissolved nutrient reduce. The curves were simulated by the previous growth model [3] under the same conditions as experiment. The coefficient of determination (R2) for experimental N uptake rate and simulated value of *Microcystis* sp. was 0.374, while that for *C. meneghiniana* was 0.457. Compared the experimental data of P uptake rate for the two species with the simulated values, the R^2 for *Microcystis* sp. was 0.181, and for *C. meneghiniana* was 0.076. None of the simulation could be recognized to be accurate.

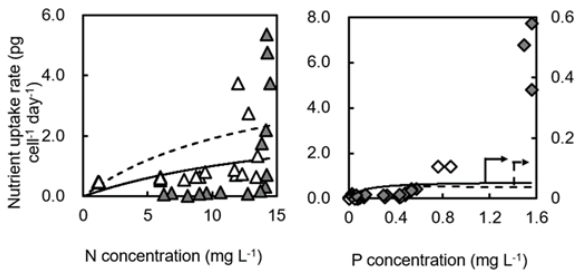


Fig.2 Measured nutrient uptake rate ρ of *Microcystis* sp. and *C. meneghiniana* and Michaelis-Menten model simulated curves. The open and filled triangle presents the measured nitrate uptake rate for *Microcystis* sp. and *C. meneghiniana* respectively. The open and filled diamond presents the measured phosphate uptake rate for *Microcystis* sp. and *C. meneghiniana* respectively. Solid lines are the simulated values for *Microcystis* sp. and dashed lines are those for *C. meneghiniana*.

The accuracy of predicted value

Cell proliferation of *Microcystis* sp. and *C. meneghiniana* under different daily renewal rate and N concentrations was presented in Fig. 3. And the curves were the simulated results from the new improved growth model in this study to test the prediction accuracy.

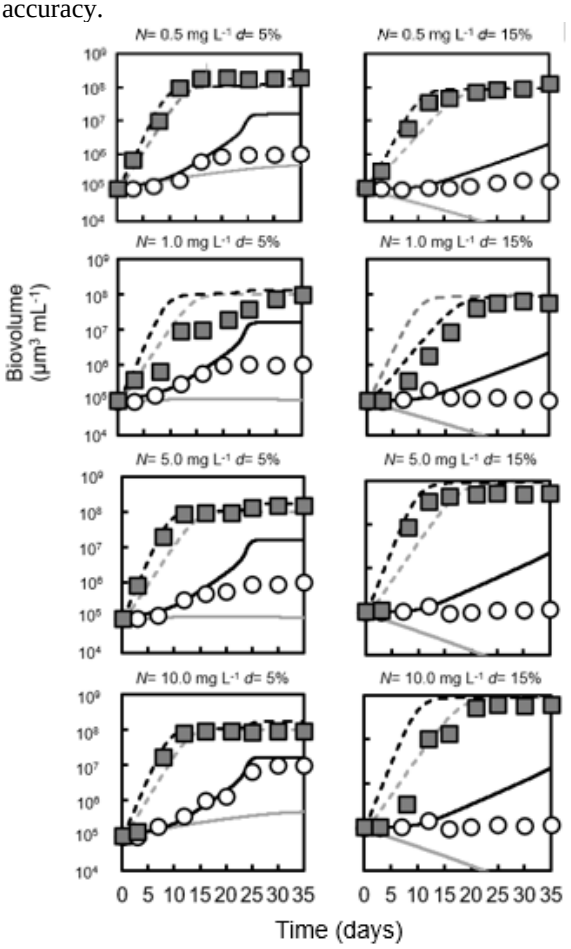


Fig.3 Simulated growth patterns of *Microcystis* sp. and *Cyclotella meneghiniana* under various conditions. The open circles are the experimental measured biovolume of *Microcystis* sp. and the filled squares are those of *Cyclotella meneghiniana.* The grey solid and dashed curves are the predicted biovolume for *Microcystis* sp. and *Cyclotella meneghiniana* by the previous growth model. Meanwhile, the black solid and dashed curves are the predicted biovolume for *Microcystis* sp. and *Cyclotella* by the new growth model.

It was displayed in Fig. 3 that in all the experimental groups under 20°C, the dominant species all were *Cyclotella meneghiniana*. In the experimental group with 0.5 mg-N/L and d = 5%, the highest growth rate of *Microcystis* sp. was only 0.322 ± 0.021 /day and the saturated biovolume of it was 9.87×105 ± 1.04×104 μm^3/mL reached at day 25. When the *d* raised to 15%, the growth of *Microcystis* sp. was relatively inhibited, and the saturated biovolume of it was only 1.69×105 ± 0.92×104 μm^3/mL. Compared with the precious competition model, the new simulated results were closer to the experimental. The R^2 of new simulated curve and experimental data was 0.63 while it of the precious one was 0.88. In the *d* = 15% group, the R^2 for the precious model was 0.53, and it improved in the new prediction up to 0.71. In contrast, the growth of *C. meneghiniana* was more vigorous compared with that of *Microcystis* sp. Even the increase of daily renewal rate also did not cause obvious effect. And compared to the previous simulated results, the R^2 in the two groups were both improved. The R^2 in *d* = 5% raised from 0.81 to 0.90 and raised from 0.87 to 0.94 in *d* = 15%.

The inhibited effect of dilution on the growth of *Microcystis* sp. was portrayed in all the other groups with the daily renewal rate of d = 15%. The *Microcystis* sp. in these groups can almost not proliferate and the cell density only increased less than one order of magnitude. In low renewal rate groups of d = 5%, the growth of *Microcystis* sp. was promoted by the increasing N concentrations. The saturated biovolume of *Microcystis* sp. increased up to 1.02×10^7 ± 0.71×10^6 μm^3/mL in the group with 10 mg-N/mL, which is two orders of magnitude higher compared with the initial biovolume. And the new competition model has predicted the proliferation accurately (R^2 = 0.97).

IV. DISCUSSION

In this study the overestimation of the previous growth model has been ameliorated depend on the growth characteristics and nutrient uptake rate of C. meneghiniana and Microcystis sp. isolated from Lake Tega, Japan.

The inaccurate nutrient concentration prediction by the precious growth model was mainly caused by the nutrient adsorption term ρ. The predicted curves in Fig. 2 displayed obvious deficiency compared with

measured data ($p < 0.05$), especially under the high nutrient conditions. As the cell grows, the cell nutrient uptake activity tend to stabilize, at when the uptake characteristics followed the Michaelis-Menten equation, which was consistent with curve fitting result in Fig. 2. However, at the end of experiment when the nutrient concentration decreasing to 0, the deficiency of prediction result occurred again. Ahn, Chung and Oh [19] have observed the phosphate uptake rate of green algae was inversely proportional to the cell density, and Mallick and Rai [20] also have proved the inversely proportional nitrate uptake rate with increasing cell density. These nutrient adsorb patterns and the nutrient adsorb characteristics in experiments were unanimous. When the cell densities gradually reach the environmental capacity of the incubator, the rate of nutrient adsorb began to decrease. Thus, after adding the cell density relating item into the previous nutrient adsorption term, the overestimation of nutrient adsorption has been refined.

Although the new model can predict the growth and nutrient concentrations change accurately so far, the sources of nitrogen and phosphorus in experiments were only nitrate and phosphate. In actual water environments, there are many types of nitrogen such as ammonium and organic nitrogen, which can affect the cell nutrient uptake [16] [21], the difference in uptake ability of various nutrient source will lead to deficiency in the model prediction. To improve the accuracy of prediction value, these uptake kinetic should be further explored in following study.

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