Comparison of the modified Monod and Droop function combined with Logistic function for describing algae growth

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Abstract. Microcystis aeruginosa (\textit{M. aeruginosa}) are algae found in common freshwater blooms in China, and Dunaliella tertiolecta (\textit{D. tertiolecta}) are economically important marine algae. Understanding of the microbial growth kinetics plays a significant role in the management of \textit{M. aeruginosa}'s blooms and biodiesel production by \textit{D. tertiolecta}. This study has shown that the combination of mechanistic models (Logistic and Monod) proved to be efficient in describing relationship between \textit{M. aeruginosa} growth rates and specific concentrations of total dissolved phosphorus (TDP), orthophosphate (PO\textsubscript{3}\textsuperscript{3}-P), total dissolved nitrogen (TDN) and ammonia (NH\textsubscript{3}-N) reasonably with $R^2=0.28$-$0.93$. Meanwhile, results also show that both PO\textsubscript{3}\textsuperscript{3}-P and NH\textsubscript{3}-N are important forms of TDP and TDN in influencing \textit{M. aeruginosa} growth. It was also noted that the combination of modified Monod and Logistic functions is suitable for describing specific growth rates of \textit{D. tertiolecta} versus extracellular nitrate concentrations ($R^2=0.24$-$0.72$). In terms of the combination of Droop and Logistic functions, it was analysed to better explain the relationships between \textit{M. aeruginosa} specific growth rates and cellular P and N concentrations ($R^2=0.41$-$0.86$) as compared to the application of Droop function alone. It is also observed that the relationship between \textit{D. tertiolecta} specific growth rates and intracellular nitrate concentrations also can be well described by the combination of Logistic and Droop functions. In addition, \textit{M. aeruginosa} growth was affected by less intracellular P concentrations than intracellular N concentrations. In sum, the combination of modified Monod and Logistic functions and the combination of Droop and Logistic functions all can predict algae growth reasonably well, while the combination of Droop and Logistic functions is slightly better. Meanwhile, it is through these two combinations that two sets of better parameters in modified Monod and Droop functions can be respectively obtained to characterize algal population kinetics with changing nutrient concentrations.

Abbreviation table

| Abbreviation | Description |
|--------------|-------------|
| $N$          | the algae density or algae biomass, $1\times 10^7$ cells·mL$^{-1}$ or mg C·L$^{-1}$ |
| $N_{\max}$  | the maximal algae density or maximal algae biomass, $10^8$ cells·mL$^{-1}$ or mg C·L$^{-1}$ |
The intrinsic growth rate, $d^{-1}$

time, d

the specific growth rate, $d^{-1}$

the computed specific growth rate, $d^{-1}$

the maximal specific growth rate in Monod function, $d^{-1}$

the maximal specific growth rate in Droop function, $d^{-1}$

the nutrient concentration, mg·L$^{-1}$

the residual concentration of nutrients when the algae density reach equilibrium, mg·L$^{-1}$

the half saturation coefficient in Monod function, mg·L$^{-1}$

cell quota, mg·(10$^7$ cells)$^{-1}$ or g N·(g C)$^{-1}$

the minimal cell maximal specific growth rate, mg·(10$^7$ cells)$^{-1}$ or g N·(g C)$^{-1}$

orthophosphate

total dissolved phosphorus

total phosphorus

particulate phosphorus

particulate nitrogen

ammonia

total nitrogen

total dissolved nitrogen

$M. aeruginosa$ + HT fish feed

$M. aeruginosa$ + HP fish feed

$M. aeruginosa$ + ZT fish feed

first experiment;

second experiment;

third experiment;

fourth experiment

1. Introduction

Mathematical functions are useful tools for predicting responses of freshwater and marine microalgae in response to their changes in nutrient intake [1, 2]. Recently, eutrophication which is badly affected by global changes [3-5] as well as the development of microalgal biofuels [6, 7] have heightened our demand for predictive functions of planktonic ecosystem dynamics [8, 9]. At their core, the functions can simulate and predict the relationships among important controlling factors and rate processes, such as concentrations of limiting nutrients, rates of nutrients uptake and associated population growth of phytoplankton species and so on [10]. In fact, relationships between algal specific growth rates and nutrients uptake rates are key elements in most process-based functions [9]. Two functions in current study were selected to characterize algal growth kinetics in relation to their changing nutrient intake rates: 1. Monod’s extracellular nutrient and algae growth function [11]; 2. Droop’s intracellular nutrient and algae growth function [12].

Monod found that the relationship between the substrate availability and bacterial growth in reactor system with a single growth-limiting substrate could empirically be expressed by Monod function [11]. In follow-up research, Monod function has been applied to mathematically model nutrient limitation and primary productivity [3, 7, 13-15], which is an important ecological process in aquatic systems [13]. Monod function provides a simple and effective method to apply in aquatic systems. However, it did not consider the intracellular nutrients and the algal growth on the intracellular nutrients, and thus could not provide realistic prediction when intracellular nutrients support the main productivity during periods of low water nutrient levels. Cerucci et al. and Flynn also found that Monod function cannot represent the luxury uptake of nutrients by algae [13, 16], which includes excessive nutrient uptake when the nutrient utilization rate in the water is high.
Droop used a continuous culture method to describe phytoplankton growth related to its internal nutrient concentration under vitamin B$_{12}$ and phosphorus limited conditions. Since the original description [17], other nutrients also have been subjected to Droop’s treatment, and these include P, N, Fe, Si and even light [16, 18, 19]. The algal specific growth rate is linked to the internal nutrient pool size or cell nutrient reserve, and the famous Droop function that taking into account the luxury uptake of nutrients is proposed [12, 13, 17, 20]. Droop function is an important structural attribute [21]. It is because the microbial growth process rate can be calibrated independently of the determined nutrient absorption and storage, and indicates that the function is not limited by external environmental conditions [21]. Bernard also believed that in actual systems using the internally stored nutrients, the Droop function can better describe growth in the absence of external nutrients [22]. However, Droop function has no clear biological interpretation; it accounts not only for the internal nutrient pool but also for the internal metabolized nutrient [23]. For example, in Droop’s study the measurement method of intracellular vitamin B$_{12}$ does not make the distinction between organic and inorganic intracellular vitamin [12]. In addition, Sunda et al. argues if both Monod and Droop functions are valid, then Monod function is clearly preferred as it is simpler and required less computing time and measurement of model parameters [10].

In addition, Logistic function, a purely empirical function, describes the kinetics of population that is affected by density-dependence [24]. It is also applied in various single algae such as *M. aeruginosa* [10, 25]. But Logistic function describes only the number of organisms and it does not include the consumption of substrate.

Some studies ignore considering that (modified) Monod function is actually an implicit function of time ($t$), but in our recent research studies it was proposed that combinations of Logistic function with modified Monod function or Monod function is related to function of time ($t$) [10]. The combinations can well fit relationships between external nutrients (e.g. nitrogen and phosphorus) concentrations and algae specific growth rates. Droop function is also an implicit function of time ($t$). However, whether and how Logistic function can be combined with Droop function has not been discussed. Meanwhile, Droop function and (modified) Monod function have their respective advantages and disadvantages. Thus, this has also prompted another question that which combination of (modified) Monod function with Logistic function or of Droop function with Logistic function is better to study algae growth kinetics.

In fact, algal growth kinetics by (modified) Monod, Droop and other functions were analyzed and discussed in many research studies [7, 9, 26-30]. Lee et al. reviewed 42 different kinetic functions for biomass productivity [7], and most functions are either based on external nutrient concentrations using Monod’s concept [11, 31, 32] or internal nutrient storage in cell under nutrient and co-nutrient limitations using Droop’s concept [12, 22, 33, 34]. Among these 42 functions, both (modified) Monod and Droop functions are not combined with Logistic function. Additionally, according to Sunda et al.’s experimental results, (modified) Monod and Droop function approach similarly yielded good trajectories for marine algae [10], but the comparison between (modified) Monod and Droop function in combination with Logistic function were also not conducted for both freshwater and marine algae. Thus, the application of (modified) Monod function and Droop function each combined with Logistic function is necessary to be explored, compared and studied for both freshwater and marine algae.

Nitrogen and phosphorus availabilities are major driving factors in water ecosystem dynamics [10, 35, 36], but the available forms of nutrients are different for freshwater and marine algae. *Microcystis aeruginosa* (*M. aeruginosa*), being the major species of freshwater blooms in China, are prokaryotic single-celled organisms and are commonly used in biological experiments. Wu et al. reported that ammonia (NH$_4^+$/N) and orthophosphate (PO$_4^{3-}$/P) are the main available form of nitrogen and phosphorus, respectively, which could be easily absorbed by *M. aeruginosa* [37]. Due to fish feed are vital factors affecting aquatic environment, the impact of fish feed on the aquaculture water environment has attracted a lot of attention [25, 38]. In order to study the effects of uneaten fish feed on algae growth, three types of commercial fish feeds are selected as nitrogen and phosphorus sources in culture medium. Abundant nutrients released from fish feed could promote *M. aeruginosa* growth.
effectively [37]. *Dunaliella tertiolecta* (*D. tertiolecta*) is an economically important marine algal species because it is rich in carotenoids, glycerol, lipids, vitamins, minerals and protein with unique commercial value in food, medicine, health care, chemical and aquaculture [39]. Understanding relationships between *D. tertiolecta* growth and nitrate is beneficial to producing lipids for biodiesel production (e.g., 20-50 % dry cell weight). Different from the adsorption of NH$_4^+$-N by *M. aeruginosa*, nitrates are mainly the source of N for *D. tertiolecta*’s growth in studies of Kumar et al.’s [39] and Rizwan et al.’s [40]. Byrd and Burkholder’s experiments were also conducted using NH$_4^+$ as the N source, but NH$_4^+$ resulted in poor growth of *D. tertiolecta*, so the experiments of culturing *D. tertiolecta* included nitrate as the inorganic N source [41].

In the present study, experimental data of *M. aeruginosa* (freshwater algae) and *D. tertiolecta* (marine algae) are selected to apply and compare modified Monod and Droop functions combined with Logistic function respectively. The specific objectives of this study are: 1) to construct modified Monod function in combination with Logistic function with respect to their external nutrient concentrations and specific growth rates for *M. aeruginosa* and *D. tertiolecta* growth; 2) to construct Droop function combined with Logistic function in relation to their cellular nutrient concentrations and specific growth rates for *M. aeruginosa* and *D. tertiolecta*; and 3) further evaluation of the comparison of the applicability of two constructed combinations.

2. Materials and Methods

2.1. Freshwater algae experiment Sample Collection

2.1.1. Experimental material. *M. aeruginosa* (FACHB-905), a kind of blue green algae as well as a kind of cyanobacterium, was obtained from the Freshwater Algae Culture Collection, Institution of Hydrobiology, Chinese Academy of Sciences. They were grown in an incubator.

The fish feed were purchased from Huaian Tongwei Company Limited, Hebei Panda Feed Company Limited and Zhongshan City Taishan Feed Company Limited respectively. They are named HT fish feed, HP fish feed and ZT fish feed respectively in the present study.

2.1.2. Experimental methods. *M. aeruginosa* were cultured in M-II culture medium for 15 days before doing experiment. The M-II culture medium consists of the following components: NaNO$_3$ (100 mg·L$^{-1}$), K$_2$HPO$_4$ (10 mg·L$^{-1}$), MgSO$_4$×7H$_2$O (75 mg·L$^{-1}$), CaCl$_2$×2H$_2$O (40 mg·L$^{-1}$), Na$_2$CO$_3$ (20 mg·L$^{-1}$), Fe·citrate×H$_2$O (6 mg·L$^{-1}$) and Na$_2$EDTA×2H$_2$O (1 mg·L$^{-1}$). The initial pH of culture medium was adjusted to 8.0 by 0.5 mol·L$^{-1}$ NaOH and 0.5 mol·L$^{-1}$ HCl. The culture was maintained at 28 ℃ and 3000 lx under 12 h light and maintained at 20 ℃ and 0 lx under 12 h dark in incubator. The methods used to deplete the intracellular nutrients stores were described in Wu et al.’s study [37].

Weights of 0.1000 g of the three fish feed were added into the sterilized M-II culture medium without nitrogen and phosphorus, the three fish feed served as P and N sources in medium. Treatments with algae containing HT, HP and ZT fish feed were named “MHT”, “MHP” and “MZT”, respectively; treatments without algae containing HT, HP and ZT fish feed were named “HT”, “HP” and “ZT”, respectively. Each treatment sets two duplicates. All of experimental flasks were shaken three times a day, and their positions were changed randomly. The algae density at experimental beginning was 10×10$^4$ cells·mL$^{-1}$.

2.1.3. Extracellular and intracellular nutrient analysis. In experimental period with 37 days, algae density, TP, PO$_4^{3-}$-P, TDP, TN, NH$_4^+$-N, and TDN were monitored [15, 25]. Meanwhile, PP equals TP minus TDP, and PN equals TN minus TDN.

The measurement of intracellular nutrients is essential to explore Droop function’s application in this experiment. PP and PN in treatments with algae are mainly from algae and undissolved fish feed, and undissolved fish feed is same in all treatments with and without algae. Thus, the amount of internal P (or N) per unit of algae density (mg·(10$^7$ cells)$^{-1}$) i.e. cellular P (N) quota, can be calculated.
as the difference between PP (or PN) (mg·L\(^{-1}\)) with and without algae in the group to the corresponding algae density (10\(^4\) cells·mL\(^{-1}\)). The measurement conforms to Droop’s measurement of intracellular nutrient in general [12].

2.2. Marine algae experiment

*D. tertiolecta* biomass, nitrate concentrations and cellular nitrogen quota in experiment of *D. tertiolecta* (a kind of marine algae) are collected from Benavides et al.’s study used for constructing both modified Monod function and Droop function separately combined with Logistic function in the study [42], and their experiments can be briefly explained as follows. *D. tertiolecta* is cultivated in the photobioreactor (PBR), which has a culture volume of 13 L and is illuminated from one side by a set of six fluorescent tubes placed vertically and parallel to the front side of the reactor, with the same height and width as the reactor [42]. Data (biomass, external nitrite concentration and cellular nitrogen quota) collected from four batch cultures were considered and denoted in the following as the first experiment, second experiment, third experiment and fourth experiment, respectively. Each culture has duration of about five days, but differs in their initial conditions. KNO\(_3\) is the nitrogen source in culture medium, and TN is detected utilizing a chemiluminescence detector. Meanwhile, different from the present experiment, although the biomass concentrations are C (total organic carbon) based instead of cell based, the two methods measuring biomass concentrations are acceptable. Most theoretical studies ignore the differences between cell- and C nutrient-based [16].

2.3. Growth Kinetics

2.3.1. The Logistic function. The form of Logistic function is

\[ N = \frac{N_{max}}{1 + e^{-r t}} \]  

(*M. aeruginosa* density and specific growth rates can be well described by equation (1) i.e. Logistic function and equation (2) that derived from Logistic function, respectively [25]:

\[ \mu_c = \frac{d(ln N)}{dt} = \frac{r e^{-r t}}{1 + e^{-r t}} \]  

Parameters of \(N_{max}, a\) and \(r\) could be obtained by fitting equation (1) to experimental data. \(\mu_c\) (d\(^{-1}\)) is defined as the computed specific growth rate.

2.3.2. Modified Monod function combined with Logistic function. Monod function posits that the specific growth rate (\(\mu\)) is a function of the limiting nutrient concentrations (\(C\)), and the functional relationship used most frequently is a growth-saturation function referred to as Monod function [11]:

\[ \mu = \frac{dN}{Nd} = \frac{\mu_{m-M}C}{K_{c-M}+C} \]  

The modified Monod function combined with Logistic function could well fit the relationship between algal specific growth rate and external nutrients concentration in fish feed culture medium [25]. We also take the combined application of modified Monod and Logistic functions into account:

\[ \mu_c = \frac{r e^{-r t}}{1 + e^{-r t}} = \frac{\mu_{m-M}(C-c_0)}{K_{c-M}+(C-c_0)} \]  

which is applied to fit the experimental data.

2.3.3. Droop function combined with Logistic function. In the Droop function, the cellular nutrient content \(Q\) varies, and the growth rate is modelled as a function of \(Q\) using the Droop equation [12]:

\[ \mu = \frac{dN}{Nd} = \frac{\mu_{m-D}(Q-Q_{min})}{Q} \]  

(5)
The combination of Logistic and Droop functions is written as equation (6), considering the existence of implicit function t in Droop function. The combination is beneficial to compare the modified Monod and Droop functions for description of algae growth.

\[
\frac{re^{a-rt}}{1+e^{a-rt}} = \left( \mu_c \right) = \frac{\mu_m-p(Q-Q_{min})}{Q}
\]

which is applied to fit the experimental data.

2.4. Statistical Analysis

All of functions were tested for their fit to experimental data by the SPSS 19.0 or Origin 8.6. Data in Benavides et al.’s study is obtained by Digitizer for Origin 8.6 [42]. Xu et al. also use Plot Digitizer software to extract data in their study to model maximal lipid productivity of microalgae [6].

3. Results and discussions

3.1. The growth of M. aeruginosa

Understanding of M. aeruginosa growth kinetics is beneficial for the management of freshwater algal blooms. This section shows our experimental results of M. aeruginosa growth, and justifies modified Monod function and Droop function combined with Logistic function respectively.

3.1.1. Monitoring indicators. Uneaten fish feed in aquaculture water can effectively simulating algae growth. From Figure 1, three phases of algae growth are observed including lag phase (days 0-10), exponential phase (days 10-30) and stationary phase (days 30-34). And from Figure 1(c), mainly influenced by algae utilization, both TDP and PO\textsubscript{4}\textsuperscript{3-}-P concentrations decrease gradually to minimal values in general, which agrees with Wu et al.’s study [37]. PO\textsubscript{4}\textsuperscript{3-}-P concentrations are lower than TDP concentrations obviously because it is only a part of it. Meanwhile, in Figure 1(d), either NH\textsubscript{4}+\textsuperscript{-N} or TDN concentrations increased to their maximal concentrations firstly and then followed by a decrease which is influenced by the release of nitrogen from fish feed and the algal nutrients utilization. TDN is composed of ammonium, nitrate, nitrite and so on, thus NH\textsubscript{4}+\textsuperscript{-N} concentrations are also lower than TDN concentrations. Moreover, the order of nutrients (TDP, PO\textsubscript{4}\textsuperscript{3-}-P, TDN and NH\textsubscript{4}+\textsuperscript{-N}) in water in all treatments conforms to MHT>MHP>MZT. Thus, nutrients level of water will be affected by fish feed as well as their types.
Figure 1. Variations of algae densities, cellular P quota, cellular N quota, P and N concentrations with time stimulated by three different fish feed. (Dots experimental data).

Figure 1(a) and 1(b) also exhibit variations of cellular P and N quota respectively. Both cellular P and N quota increase rapidly first and then decrease in the lag phase, and then they keep stable values close to $0 \text{mg} \cdot (10^7 \text{cells})^{-1}$ thereafter. The maximal values of cellular N quota in all treatments (0.033-0.076 mg·(10^7 cells)^{-1}) are obviously higher than those of cellular P quota (0.0070-0.024 mg·(10^7 cells)^{-1}), and this may be because *M. aeruginosa* need more N to store in cell than P.

It is well known that these algae can uncouple the uptake of nutrients from the growth [43], that is, algae can grow some more days after the nutrients are exhausted as shown in Figure 1(a) and 1(b). Algae can absorb and store nutrients at higher rates than those necessary for growth at times of excess nutrient availability in the water [13, 20]. Moreover, the stored nutrients in algae cells are able to support them at times of low nutrient availability in water [13, 20], which is known as nutrient luxury uptake [20]. It conforms to our results in Figure 1(a) and 1(b).

In addition, according to Fig 1(a) and 1(c), cellular P is nearly exhausted since day 10, then it does not increase even external P (TDP and PO_4^{3-}-P) supplied is excess in groups with algae (MHT, MHP and MZT). John and Flynn observed that the initial nutrient concentrations supplied for all treatments resulted in achieving different nutritional status of algal stationary growth [44].

### 3.1.2. Application of modified Monod function on *M. aeruginosa* growth.

The results show that the joint application of modified Monod and Logistic functions could well describe variations of *M. aeruginosa* specific growth rates ($\mu$) as TDP, PO_4^{3-}-P, TDN, NH_4^+-N concentrations changes caused by algal utilization. The correlation coefficients ($R^2$) are 0.64-0.93, 0.76-0.90, 0.28-0.84 and 0.67-0.82 for TDP, PO_4^{3-}-P, TDN and NH_4^+-N respectively.
Figure 2. *M. aeruginosa* growth described by Monod function combined with Logistic function (equation 4). (Dots computed values based on Figure 1 and lines model prediction).

According to Figure 2, the specific growth rate of each group MHT, MHP and MZT increases rapidly at lower concentrations of nutrients, then increases slowly or keeps stable with increasing concentrations of nutrients (in Figure 2). The curve form of specific growth rates conforms to Monod function [25]. In Figure 2, $C_0$ of nitrogen and phosphorus are consistent with their measured values (in Table 1). Due to $\text{NH}_4^+\text{-N}$ are taken up completely in each group at the experimental ending, $C_0$ of $\text{NH}_4^+\text{-N}$ in MHT, MHP and MZT groups obtained by equation (4) fitting were all close to 0 mg·L$^{-1}$. Meanwhile, from the Table 1, $\mu_m$ and $K_c$ are also reasonable computed from equation (4). In addition, from Figure 2, not only the fitted curves of specific growth rates versus TDP and $\text{PO}_4^{3-}\text{-P}$ concentrations are similar, but also the fitted curves of specific growth rates versus TDN and $\text{NH}_4^+\text{-N}$ concentrations are similar. It indicates that both $\text{PO}_4^{3-}\text{-P}$ and $\text{NH}_4^+\text{-N}$ concentrations are important forms of nitrogen and phosphorus nutrients in determining algal growth.

Table 1. Parameters of modified Monod function combined with Logistic function describing *M. aeruginosa* growth.

| TDP      | Correlation coefficient ($R^2$) | $\mu_m$ | $K_c$ | $C_0$ |
|----------|---------------------------------|---------|-------|-------|
| MHT      | 0.93                            | 0.47    | 0.36  | 0.44  |
| MHP      | 0.90                            | 0.48    | 0.33  | 0.50  |
| MZT      | 0.64                            | 0.34    | 0.18  | 0.32  |

| PO$_4^{3-}\text{-P}$ | Correlation coefficient ($R^2$) | $\mu_m$ | $K_c$ | $C_0$ |
|----------------------|---------------------------------|---------|-------|-------|
| MHT                  | 0.89                            | 0.38    | 0.14  | 0.40  |
3.1.3. Application of Droop function on M. aeruginosa growth. The application of the Droop function to phytoplankton is believed suitable to describe specific growth rates [45]. In Sunda et al.’s study, Droop function could be applied to describe steady-state relationships between intracellular nutrients and microalgal specific growth rates of *Nannochloropsis oculata*, *Thalassiosira pseudonana* and *Thalassiosira weissflogii* [10]. Garcia et al. also fit the Droop function to the *Synechococcus* specific growth rate and elemental (carbon, nitrate and phosphate) quota data in basic [5].

In the present experiment, as shown in Figure 3, the application of Droop function alone, i.e. equation (5), also could be applied in describing the relationships between measured specific growth rates and intracellular nutrients quota generally (in Table 2). Results have shown that specific growth rates increased rapidly with increasing cellular P quota when the cellular P quota were low, and then increase slowly or keep stable when the cellular P quota were high. However, specific growth rates increase gradually with increasing of cellular N quota. The correlation coefficients ($R^2$) are low, and they are 0.24-0.33 and 0.18-0.44 for the cellular P and N quota in Table 2 respectively. Thus, we also take Logistic function into Droop function account.

Moreover, it must be pointed out that intracellular nutrients are difficult to make the distinction between organic and inorganic intracellular nutrients [12, 46]. N and P in forms of NH$_4^+$-N and PO$_4^{3-}$-P respectively are less in *M. aeruginosa* cell and are difficult to detect. Thus, we do not discuss the relationship between specific growth rates and intracellular NH$_4^+$-N (or intracellular PO$_4^{3-}$-P), and we just compare Droop and modified Monod function based on nitrogen and phosphorus ignoring the forms of NH$_4^+$-N and PO$_4^{3-}$-P.

![Graphs showing specific growth rates vs. cellular P and N quotas](image)

**Figure 3.** *M. aeruginosa* growth described by Droop function (equation 5) (Dots experimental data and lines model prediction).
Table 2. Parameters of Droop function describing *M. aeruginosa* growth.

| Parameters of application of Droop function alone | The amount of P per cell | The amount of N per cell |
|------------------------------------------------|--------------------------|-------------------------|
| Group                                           | $R^2$ | $\mu_{m,D}$ | $Q_{min}$ | $R^2$ | $\mu_{m,D}$ | $Q_{min}$ |
| MHT                                             | 0.33  | 0.40       | 7.54×10^-4 | 0.18  | 0.39       | 5.99×10^-3 |
| MHP                                             | 0.24  | 0.32       | 6.00×10^-4 | 0.44  | 0.40       | 5.04×10^-3 |
| MZT                                             | 0.27  | 0.36       | 8.04×10^-4 | 0.35  | 0.39       | 7.34×10^-3 |

| Parameters of combination of Logistic and Droop functions | The amount of P per cell | The amount of N per cell |
|----------------------------------------------------------|--------------------------|-------------------------|
| Group                                                    | $R^2$ | $\mu_{m,D}$ | $Q_{min}$ | $R^2$ | $\mu_{m,D}$ | $Q_{min}$ |
| MHT                                                      | 0.50  | 0.32       | 7.72×10^-4 | 0.84  | 0.37       | 5.91×10^-3 |
| MHP                                                      | 0.41  | 0.36       | 7.43×10^-4 | 0.86  | 0.40       | 4.76×10^-3 |
| MZT                                                      | 0.85  | 0.28       | 7.35×10^-4 | 0.84  | 0.28       | 6.45×10^-3 |

$R^2$, correlation coefficient; $\mu_{m,D}$ (d^-1), the maximal specific growth rate; $Q_{min}$ (mg·(10^7 cells)^-1), the minimal cell quota.

Results show that compared to the applied results of Droop function, Droop function combined with Logistic function could better simulate *M. aeruginosa* growth in their specific growth rate related to their intracellular P and N concentrations. The correlation coefficients ($R^2$) is 0.41-0.85 and 0.84-0.86 for intracellular P and N concentrations respectively (in Table 2). Variations of computed specific growth rates by equation (6) in Figure 4 are similar to variations of computed specific growth rates by equation (5) in Figure 3. According to Droop function, the minimal internal nutrient quota ($Q_{min}$) is gradually reached and the growth rate converges to zero when nutrients are persistently limiting [47].

The cellular P and N quota is a characteristic of algal cells, which is the comprehensive influence of external factors on algal cells themselves and is the direct reflection of the influence of external environmental conditions on algal cells. According to the Droop theory, algal specific growth rates are dependent on the amount of intracellular nutrients. Intracellular nutrients concentrations, $Q$, determine the synthesis reaction rate directly, and the proliferation of cells will stop below $Q_{min}$. $Q_{min}$ for intracellular P concentrations (7.72-7.43×10^-4 mg·(10^7 cells)^-1) is obviously lower than those of intracellular N concentrations (4.76-6.45×10^-3 mg·(10^7 cells)^-1), which indicates that algae growth need less P. In Yamaguchi et al.’s study, minimum cell quota of *Chattonella ovata* obtained for P (0.48 pmol N cell^-1) is also lower than that of N (5.5 pmol N cell^-1) [46], which is consistent with our result. Predicting the specific growth rate by $Q$ can be independent of the differences in external environmental conditions, and it is of great significance for establishing a unified prediction function for water bodies at different times and region areas.

As shown in Figure 1(a) and 1(b) about measured result, *M. aeruginosa* can absorb a lot of nutrients in a short time without causing a rapid increase in algae density, further demonstrating that freshwater algae is available to store nutrients, and its growth should also be a function of intracellular nutrients. Meanwhile, according to the fitting results of combination of Logistic and Droop functions in Figure 4, the Droop function related with intracellular nutrients proves that freshwater algae does have the function of storing nutrients, and freshwater algae uses the nutrients stored in the cells for growth especially when the nutrients concentrations in the medium is very low.
3.2. The growth of *D. tertiolecta*

Proposed functions include Monod function for utilization of essential nutrients into *D. tertiolecta* and Droop function involving internal nutrient cell quota for *D. tertiolecta* growth, assuming algal biomass is composed of sugar, functional-pool and neutral-lipid [39]. In terms of data in Benavides et al.’s study, experimental data points of specific growth rates and their corresponding measuring nitrate concentrations (i.e. (μ, C)) are difficult to determine and obtain by using Plot Digitizer [42], only some of algae biomass is extracted for fitting Logistic function and further calculating specific growth rates by equation (2). This section shows Benavides et al.’s four experimental results of *D. tertiolecta* growth [42], and discusses the combination approaches, i.e. Eq. (4) and Eq. (6), instead of application of modified Monod function alone (Eq. (3)) and application of Droop function alone (Eq. (5)).

3.2.1. Experimental results of *D. tertiolecta*. The optimal cultivation of *D. tertiolecta* can be studied using bio-kinetic algal function for the prediction of algae-biomass and lipid productivity [2]. From Figure 5(a), in Benavides et al.’s experiment, only exponential phase and stationary phase of *D. tertiolecta* growth are observed. The biomass of *D. tertiolecta* increase rapidly and subsequently entered into a stationary phase. Meanwhile, Logistic function (equation (1)) can describe *D. tertiolecta* growth well with correlation coefficients $R^2=0.98-0.99$ in Figure 5(a). The maximal algae biomass, $N_{max}$ are 482.54, 648.27, 496.24 and 621.46 mg C·L$^{-1}$ for Exp 1, Exp 2, Exp 3 and Exp 4 (in Table 3), which are consistent with measured values we extracted. Meanwhile, in Figure 5(b), calculated specific growth rates from equation (2) are also well in agreement with the measured values from the four experiments and $R^2$ is 0.40-0.80. Both calculated and measured specific growth rates decrease gradually as time goes along.
Table 3. Parameters of Logistic function, modified Monod combined with Logistic function, Droop function combined with Logistic function describing *D. tertiolecta* growth.

| Group | $a$   | $r$   | $N_{\text{max}}$  | $R^2$   |
|-------|-------|-------|-------------------|---------|
| Exp 1 | 2.47  | 1.67  | 482.54            | 0.99    |
| Exp 2 | 0.89  | 1.11  | 648.27            | 0.98    |
| Exp 3 | 2.26  | 1.66  | 496.24            | 0.99    |
| Exp 4 | 2.11  | 1.68  | 621.46            | 0.99    |

| Group | $R^2$ | $\mu_{\text{max}}$/d$^{-1}$ | $C_0$ | $K_c$ |
|-------|-------|-----------------------------|-------|-------|
| Exp 1 | 0.72  | 1.61                        | 0.21  | 1.56  |
| Exp 2 | 0.24  | 1.00                        | -1.25 | 5.53  |
| Exp 3 | 0.37  | 2.29                        | -1.05 | 8.17  |
| Exp 4 | 0.68  | 2.74                        | 0.33  | 9.87  |

| Group | $R^2$ | $\mu_{\text{max},D}$ | $Q_{\text{min}}$ |
|-------|-------|----------------------|------------------|
| Exp 1 | 0.76  | 2.32                 | 0.033            |
| Exp 2 | 0.50  | 1.35                 | 0.035            |
| Exp 3 | 0.89  | 2.02                 | 0.033            |
| Exp 4 | 0.75  | 2.44                 | 0.033            |

$a$, a constant; $r$ (d$^{-1}$), the intrinsic growth rate; $N_{\text{max}}$ (mg C·L$^{-1}$), the maximal algae density; $R^2$, correlation coefficient; $\mu_{\text{max},D}$ (d$^{-1}$), the maximal specific growth rate; $C_0$ (mg·L$^{-1}$), the residual concentration of nutrients; $K_c$ (mg·L$^{-1}$), the residual concentration of nutrients; $Q_{\text{min}}$ (g N·(g C)$^{-1}$), the minimal cell quota or subsistence quota; Exp 1, first experiment; Exp 2, second experiment; Exp 3, third experiment; Exp 4, fourth experiment. Data were from Benavides et al.’s (2015) study, and parameters were calculated according to Eq. (1), Eq. (4) and Eq. (6).

3.2.2. Application of modified Monod on *D. tertiolecta* growths. Monod function is modified so that it contained more biologically relevant parameters. In Figure 6 and Table 3, modified Monod function combined with Logistic function (equation (4)) is also used to fit relationships between *D. tertiolecta* specific growth rates and nitrate concentrations. The fitting results indicate that the combination can be well applied to describing *D. tertiolecta* growth in Benavides et al.’s study with $R^2=0.24-0.72$ [42].
As shown in Figure 6, *D. tertiolecta* specific growth rates increase with increasing concentrations of nitrate in general. In sum, in the case from Benavides et al.’s study tested [42], the modified Monod function is suitable to describe the growth data of *D. tertiolecta* and is easy to use.

![Figure 6](image)

**Figure 6.** Dots experimental data from Benavides et al.’s study [42] and lines function prediction: modified Monod function combined with Logistic function describing algae growth.

3.2.3. Application of Droop function on *D. tertiolecta* growth. As shown in Figure 7 and Table 3, combination of Logistic and Droop functions (equation (6)) visually give reasonably good fits of *D. tertiolecta* growth, and $R^2$ are 0.76, 0.50, 0.89 and 0.75 for Exp 1, Exp 2, Exp 3 and Exp 4, respectively. Specific growth rates of *D. tertiolecta* increase with the increase of nitrogen quota. As mentioned in the above, nitrogen quota is the comprehensive effect of environmental factors on algal cells themselves. The nitrogen quota determines the synthesis reaction rate directly, and the proliferation of *D. tertiolecta* cells will stop below $Q_{\text{min}}$. $Q_{\text{min}}$ for cellular nitrogen quota from four experiments are close to each other, and they are 0.033-0.035 g N·g C$^{-1}$. And $\mu_{\text{m,D}}$ is 2.32, 1.35, 2.02 and 2.44 d$^{-1}$ for Exp 1, Exp 2, Exp 3 and Exp 4, respectively (Table 3).

In fact, the objective of Benavides et al.’s work is to evaluate the experimental work required to estimate the parameters of the Droop function, and to discuss an experimental case study based on a lab-scale flat-plate photobioreactor [42]. According to the present study, the parameters fitted by the combination of Droop and Logistic functions are similar with the fitted results of Benavides et al. [42]. The Droop function related with intracellular nutrients proves that marine algae also does have the function of storing nutrients, and marine algae also uses the nutrients stored in the cells for growth especially when the nutrients concentrations in the medium is very low.
3.3. Comparison of modified Monod function and Droop function for describing algae growth

As discussed in the above, Monod function relates algae growth rates with available nitrogen and phosphorus dissolved in water, and Droop function relates algae growth with their internal nutrient levels. In freshwater algae experiment in the case of *M. aeruginosa*, equation (6) with $R^2=0.41-0.86$ for describing relationships between algae growth ($\mu$) and intracellular N and P ($Q$) are more suitable than equation (4) with $R^2=0.28-0.93$ for describing relationships between $\mu$ and extracellular N and P ($C$) in general. Whereas, in MHT and MHP group for describing relationships between algae growth and P uptake, modified Monod function combined with Logistic function is better than Droop function, and the reasons are not clear and need to be further studied. In marine algae experiment in the case of *D. tertiolecta* [42], equation (6) is also slightly better for describing the relationships between *D. tertiolecta* specific growth rates and external nitrate concentrations ($R^2=0.50-0.89$) than equation (4) for describing the relationships between specific growth rates and intracellular nutrients concentrations ($R^2=0.24-0.72$).

4. Conclusions

All of HT, HP and ZT fish feed could release nitrogen and phosphorus concentrations in dissolved forms, which will effectively simulate *M. aeruginosa* growth. Results on *M. aeruginosa* growth show that equation (6) could be well applied to fit the relationship between *M. aeruginosa* growth ($\mu$) and TDP, PO$_4^{3-}$-P, TDN and NH$_4^+$-N concentrations, and $R^2$ is 0.64-0.93, 0.76-0.90, 0.28-0.84 and 0.67-0.82 respectively. PO$_4^{3-}$-P and NH$_4^+$-N are important forms of nutrients in determining algae growth kinetics. Meanwhile, combination of Logistic and Droop functions can be better used to fit relationships between *M. aeruginosa* specific growth rates and cellular P and N quota ($R^2=0.41-0.85$ and $R^2=0.84-0.86$ for cellular P and N quota respectively) than the application of Droop function alone ($R^2=0.24-0.33$ and $R^2=0.18-0.44$ for cellular P and N quota respectively). And algae growth need less intracellular P concentrations with $Q_{\text{min}}=7.72-7.43\times10^{-4}$ mg·(10$^4$ cells)$^{-1}$ than intracellular N concentrations with $Q_{\text{min}}=4.76-6.45\times10^{-3}$ mg·(10$^4$ cells)$^{-1}$ obviously.

Data from Benavides et al.’s study on *D. tertiolecta* growth are extracted by Digitizer for Origin 8.6 [42], and the results further indicate that both modified Monod and Droop modelling approaches combined with Logistic function are suitable for describing the relationships between *D. tertiolecta* specific growth rates and external nitrogen concentrations and between specific growth rates and
intracellular nitrogen concentrations, respectively. And the Droop function is slightly better than Monod function to describe *D. tertiolecta* growth.

To sum up, both modified Monod function and Droop function combined with Logistic function can do a good job in predicting the growth of algae. The combination of Logistic and Droop functions is better in general.

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