Kinetics of phosphate uptake in the dinoflagellate *Karenia mikimotoi* in response to phosphate stress and temperature

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**A B S T R A C T**

Phosphate (P\(_i\)) and temperature are two important environmental factors affecting algal growth and the occurrence of red tide. In this paper, we conduct experiments on *Karenia mikimotoi* under different P\(_i\) concentrations and temperatures and propose a novel P\(_i\) uptake model by incorporating Arrhenius function and P\(_i\)-stress function into the two-stage model presented in one of our recent paper (Jiang et al. (2019)). In both P\(_i\)-replete and P\(_i\)-deplete, the model parameters are obtained by fitting the experimental data at 24 °C and validated by the experimental data at 20 °C, respectively. *K. mikimotoi* under low P\(_i\) condition entered into the exponential growth phase earlier compared with P\(_i\)-replete groups. Under the P\(_i\)-replete condition, *K. mikimotoi* continually increased, while the P\(_i\)-deplete condition showed a wave trend. The experimental results and the fitting of experimental data both show that *K. mikimotoi* has an obvious response to temperature. In both P\(_i\)-replete and P\(_i\)-deplete, the peak values of the cell quota of intracellular P\(_i\) and surface-adsorbed P\(_i\) at 24 °C were higher than those at 20 °C. Using the luxury coefficient and growth potential, interspecific competition between *K. mikimotoi* and *Prorocentrum donghaiense* is also discussed. These results and conclusions are helpful to understand the P\(_i\) uptake characteristics of algae at different P\(_i\) concentrations and temperatures, and could effectively explain mechanisms of interspecific competition and succession between different algae species under different conditions of P\(_i\) concentration and temperature.

1. Introduction

*Karenia mikimotoi* (formerly named *Gymnodinium mikimotoi* Brand et al., 2012), a widely distributed red tide species, is known to form alternating blooms with some other harmful algae species (e.g., *Prorocentrum donghaiense*, *Heterocapsa circularisquama* and so on) (Chang, 2011; Li et al., 2009; Uchida et al., 1999). Since 1998, the blooms of *K. mikimotoi* have often been seen in the coastal waters of the Zhejiang and Fujian provinces in China and have caused heavy losses in aquaculture and deterioration of the marine ecological environment (Zhao, 2010; Li et al., 2019; Liu et al., 2019). It is found that after diatom blooms, the concentration of phosphate (P\(_i\)) is relatively low in the coastal water of the East China Sea (Li et al., 2009; Zhao, 2010), and the previous studies showed that the P\(_i\) uptake rate in cells with P\(_i\)-stress is higher than that without P\(_i\)-stress (Lehman and Sandgren, 1982; Riegman and Mur, 1984; Lin et al., 2016). On the other hand, the temperature is also a major factor controlling photosynthesis which has a close relationship with the metabolism and growth of algae (Helbling et al., 2011; Shen et al., 2016; Davison, 1991). It can affect the maximum growth rate of algae (Goldman and Carpenter, 1974), thereby changing the P\(_i\) uptake rate of algae cells and affecting the alternations of algal blooms (Karentz and Smayda, 1984). During most *K. mikimotoi* blooms, the sea surface temperature ranges from 18 °C to 26 °C (Zhao, 2010; Chen et al., 2003; Zhou et al., 2017a,b). The highest specific growth rate of *K. mikimotoi* is obtained at 24 °C (Shen et al., 2016), and the average sea surface temperature from 2001 to 2010 was 23.6 °C when blooms of *K. mikimotoi* occurred in the East China Sea (Zhao, 2010). Hence, it is important to understand the kinetics of P\(_i\) uptake by algae under different conditions of P\(_i\) concentration and temperature.

In many previous studies, the Michaelis–Menten equation has often been used to describe the nutrient uptake process by algae (Lee et al., 2015). However, the successful use of Michaelis–Menten kinetics model to describe nutrient uptake often masks the difficulties in interpreting the kinetics parameters obtained by measurement (Droop, 1983). In addition, the two-parameter Michaelis–Menten kinetics model cannot...
well describe the observed phenomenon of “luxury uptake” of algae cells under P-stress (Goldman et al., 1981; Harrison et al., 1976, 1989). Therefore, it is not enough to describe the P uptake process of microalgae only by Michaelis-Menten kinetics. Within the adaptive range, algae can maintain the maximum growth rate by adjusting their internal nutrient quota and absorbing nutrients at the maximum short-term nutrient uptake rate according to changes in external nutrient concentration (Morel, 1987). Aksnes and Egge (1991) developed a term nutrient uptake rate according to changes in external nutrient concentration. The area is a type of uptake sites, h is the time required for processing one nutrient ion, n is the mass transfer coefficient, S is the nutrient concentration in the substrate, and confirmed that the Michaelis–Menten equation applied to the uptake process of algae was a special case of the model. In fact, this model can be rearranged as $V = nh^{-1}S/(hAv) + S)$, which can then be transmitted to the classical Michaelis–Menten kinetics model by letting $V_{\text{max}} = nh^{-1}$ and $K_{h} = (hAv)^{-1}$. Harrison et al. (1989) suggested that the maximum uptake rate of cells should be related to the size of the intracellular $P$ pool. Considering the multiple $P$ pools in cells, John and Flynn (2000) established the three-compartment PIM model of microalgal growth (phosphate interaction model, PIM). Recently, Singh et al. (2018) further extended the PIM model by considering the combined effects of light and nutrients on algal growth.

Notice that most studies mentioned above only considered the transportation from substrate into cells. In fact, the intracellular $P$ (QP) pool and surface-adsorbed $P$ (AP) pool have great significance to the investigation of $P$ uptake kinetics (Harrison et al., 1989; Jiang et al., 2019; Yao et al., 2011; Sañudo-Wilhelmy et al., 2004; Jin et al., 2021). The coexistence of QP pool and AP pool demonstrate that the $P$ uptake process of algae should be divided into two stages: substrate $P$ (WP) is first adsorbed on the cell surface and then AP enters the interior of cell to become $P$ through the active transport of membrane. Considering the effects of AP and QP on algal growth, Liu et al. (2007) established a two-stage growth kinetics model of algae. By further considering the regulation via transport-inhibition from size of QP pool, Yao et al. (2011) constructed a two-stage kinetics model of $P$ uptake by algae based on the experimental data of short-term absorption kinetics of a green algae Scenedesmus quadricauda. Based on the phosphate absorption experimental data of Prorocentrum donghaiense under different phosphorus concentrations, in a recent paper (Jiang et al., 2019), we proposed a two-stage $P$ uptake kinetics model with the Droop equation describing the dependence of cell growth on the QP based on the model of Yao et al. (2011). In Refs. Jiang et al. (2019) and Yao et al. (2011), the two-stage model and the traditional one-stage model were compared. From the model fitting results, the biological significance of parameters, and statistical test results et al. the two-stage model provides a more reasonable and realistic explanation for the process of $P$ uptake by algae.

To better understand the effects of different $P$ concentrations and temperatures on the $P$ uptake kinetics of algae, in the present paper, the temperature function (Arrhenius function) and $P$ stress function are further introduced to the model given in Jiang et al. (2019) and obtain a novel two-stage kinetics model. The parameter calibration and validation of the model are carried out based on the experimental data of $P$ uptake by K. mikimotoi under different $P$ concentrations and temperatures. Then, the effects of $P$ concentration and temperature on $P$ uptake characteristics of K. mikimotoi are analyzed by experimental data and parameter fitting results. The impact of different $P$ uptake characteristics of P. donghaiense and K. mikimotoi on interspecific competition between these two species under different $P$ concentrations and temperatures are also discussed.

### 2. Materials and methods

#### 2.1. Model description

The process of $P$ uptake by algae is divided into two steps. In the first step, $P$ is adsorbed on the cell surface and then $P$ adsorbed on the cell surface is transported into the cell (see Jiang et al. (2019) for more details). We improve the model provided in Jiang et al. (2019) from the following two aspects.

Firstly, to clearly consider the influence of temperature on the growth rate of algae, the Arrhenius function is introduced into the model. The early $P$ uptake model proposed by Droop (1973) presents a simple quota model in which the specific growth rate of algae ($\mu$) is assumed to be a function of intracellular $P$ concentration:

$$\mu = \frac{Q_{i} - Q_{\text{min}}}{Q_{i}}, \quad (1)$$

where $Q_{\text{max}}$ is the maximum growth rate of algae, $Q_{i}$ is the cell quota of $P$ (i.e., $Q_{i} = Q_{P}/N$, where $N$ is the cell density of algae) and $Q_{\text{min}}$ is the minimum of $Q_{i}$. The maximum specific growth rate of algae, though independent of limiting nutrient concentrations, is still a function of other environmental variables such as temperature. Using the Arrhenius equation, $Q_{\text{max}}$ can be described as a function of temperature below (Goldman and Carpenter, 1974; Bordel et al., 2009):

$$\mu_{\text{max}} = A \cdot \exp \left( \frac{E}{K_{b} \cdot T} \right), \quad (2)$$

where $T$ stands for the temperature, and the parameters $A$, $E$, $K_{b}$ are respectively the maximum growth rate of algae at a particular temperature, activation energy for algal growth and universal gas constant. By substituting (2) into (1), then the specific growth rate of algae can be written as:

$$\mu = A \cdot \exp \left( \frac{E}{K_{b} \cdot T} \right) \cdot \frac{Q_{i} - Q_{\text{min}}}{Q_{i}}, \quad (3)$$

Secondly, to consider the influence of different $P$ concentrations on the uptake kinetics of algae, the $P$ stress function is introduced into the model. In the model of Jiang et al. (2019), the traditional Michaelis–Menten equation for $P$ transport rate (PT) was modified as:

$$PT = K_{p} \cdot Q_{\text{max}} \cdot \mu_{\text{max}} \cdot \frac{S_{p}}{S_{p} + K_{p} \cdot Q_{\text{max}}} \left( \frac{1 - Q_{i}}{Q_{\text{max}}} \right)^{4} \left( \frac{1 - Q_{i}}{Q_{\text{max}}} \right)^{4} + K_{q}, \quad (4)$$

where $K_{q}$ is a dimensionless coefficient describing $P$ stress, $Q_{\text{max}}$ is the maximum quota of $P$ in the cell, and $K_{p} \cdot Q_{\text{max}} \cdot \mu_{\text{max}}$ represents the maximum transport rate (Yao et al., 2011); $S_{p}$ ($10^{-5}$ mol cell$^{-1}$) is the cell quota of AP (i.e., $S_{p} = AP/N$), $K_{q}$ is the half-saturation constant from algae $P$ uptake; and

$$\left( \frac{1 - Q_{i}}{Q_{\text{max}}} \right)^{4} + K_{q} \quad (5)$$

is a feedback function describing the regulation of the transport rate of $P$ for each algal cell via the inhibition from the size of intracellular $P$ pool, where $K_{q}$ is a coefficient in the feedback control function. Notice that $K_{q}$ describes the change of $P$ uptake rate of algae under $P$ stress, and there are literatures demonstrating that it is inversely proportional to phosphorus concentration in the environment (Dyhrman and Palenik, 1999; Rengelors et al., 2003; Yao et al., 2011). Algal cells can increase alkaline phosphatase activity and the number of high-affinity phosphate transporters under low phosphorus conditions to improve $P$ uptake and utilization efficiency (Ou et al., 2008; Lin et al., 2016). To describe the $P$ uptake characteristics of algae under different $P$ concentrations, we propose the following $P$-stress function:

$$K_{p} = 1 + \frac{1}{a \cdot WP + b} \quad (6)$$
where WP is the $P_i$ concentration in substrate, and the parameters $\alpha$ and $\beta$ are respectively the coefficient of WP affecting transport rate and a dimensionless coefficient in $P_i$-Stress function. Thus the final form of $P_i$ transport rate can be represented as:

$$PT = Q_{max} \cdot \left( 1 + \frac{1}{\alpha \cdot WP + \beta} \right) \cdot A \cdot \exp \left( - \frac{E}{K_T \cdot T} \right) \cdot \frac{S_p}{S_p + K_i}$$

$$= \left( 1 - \frac{Q_{max}}{Q_{max}} \right)^4 \cdot \left( 1 - \frac{Q_{max}}{Q_{max}} \right)^4 + K_i$$

(7)

Based on above, we can write out our novel $P_i$ uptake kinetics model as follows:

$$dN = A \cdot \exp \left( - \frac{E}{K_T \cdot T} \right) \cdot \frac{S_p}{S_p + K_i} \cdot N - e^{-N_{obs}},$$

$$dAP = K_i \cdot WP \left( 1 - S_p \right) - K_i \cdot AP$$

$$= Q_{max} \left( 1 + \frac{1}{\alpha \cdot WP + \beta} \right) \cdot A \exp \left( - \frac{E}{K_T \cdot T} \right) \cdot \frac{S_p}{S_p + K_i}$$

$$\left( \frac{1}{Q_{max}} \cdot \frac{S_p}{S_p + K_i} \right) \cdot \left( \frac{1}{Q_{max}} \cdot \frac{S_p}{S_p + K_i} \right)$$

$$dQP = \frac{Q_{max} \left( 1 + \frac{1}{\alpha \cdot WP + \beta} \right) \cdot A \cdot \exp \left( - \frac{E}{K_T \cdot T} \right) \cdot \frac{S_p}{S_p + K_i} \left( \frac{1}{Q_{max}} \cdot \frac{S_p}{S_p + K_i} \right) \cdot N}{Q_{max} \left( 1 + \frac{1}{\alpha \cdot WP + \beta} \right) \cdot A \exp \left( - \frac{E}{K_T \cdot T} \right) \cdot \frac{S_p}{S_p + K_i} \left( \frac{1}{Q_{max}} \cdot \frac{S_p}{S_p + K_i} \right) + K_i}$$

$$dWP = -K_i \cdot WP \left( 1 - S_p \right) + K_i \cdot AP$$

(8)

where $Q_{max} = QP/N$ and $S_p = AP/N$, other variables and parameters are provided respectively in Tables 1 and 2. Fig. 1 shows the conceptual diagram of model (8). The first equation of $N$ in model (8) describes the cell growth with respect to $QP$ quota. The second and the fourth equations describe respectively the $P_i$ adsorption and desorption between cell surface and substrate. While the third equation describes the intracellular transportation of AP.

### 2.2. Experimental materials and methods

#### 2.2.1. Algal culture conditions

*K. mikimotoi* was provided by Prof. Zhaoli Xu from East China Sea Fisheries Research Institute, Shanghai, China. The algae were precultured at 20 °C in f/2 medium (Guillard, 1975) with a light intensity of 65–70 µmol photons m$^{-2}$ s$^{-1}$ cool-white light (12 : 12 h Light : Dark cycle, the photoperiod is from 6 AM to 6 PM). These cultures were manually shaken twice daily at 7:00 AM and 6:00 PM. These algal cells in the exponential growth phase were used in the following experiments.

#### 2.2.2. Temperature treatment experiments

To compare the growth rules of *K. mikimotoi* at different temperatures, these algal cells were cultured in 50 ml of fresh f/2 media in 100 ml Erlenmeyer flasks at an initial cell density of $0.2 \times 10^8$ cells L$^{-1}$. The temperatures of the flasks were set at 20 °C and 24 °C and the illumination condition as preculture.

#### 2.2.3. Phosphate uptake experiments

The cell density of the algal cultures were about $0.2 \times 10^8$ cells L$^{-1}$, and the experiments were divided into P$_i$-replete group (25.55 µM P$_i$) and P$_i$-deplete group (0.74 µM P$_i$) with three replicates. A 10 mL sample was collected at the interval of 3 days in each group. The determination methods of WP, AP, and QP are based on Yao et al. (2011) and Jiang et al. (2019) with minor modifications. The cell density ($N$) was counted using the method of Jiang et al. (2019) at 0, 3, 6, 9, 12, 15, and 18 day.

#### 2.2.4. Model calibration and validation

In this study, parameter values of model (8) are calibrated based on the experimental data ($N$, WP, $Q_p$, $S_p$) of *K. mikimotoi* at 24 °C under the conditions of P$_i$-replete and P$_i$-deplete by using the least square method, which is implemented by the function of “fmincon” of MATLAB R2016b. The least square estimation method is to find a set of optimal parameter values in parameter space to minimize the objective function. To get the objective function, the following model cost is used in this study (Adhurya et al., 2021):

$$cost_i = \frac{1}{m} \sum_{j=1}^{n} \left( \frac{X_{ij}}{\hat{X}_{ij}} \right)^2$$

where $cost_i$ is the model cost of the $i$th state variable in parameter calibration, $n$ is the number of observed data, $X_{ij}$ is the simulation value of the $i$th day of the $j$th state variable and $\hat{X}_{ij}$ is the corresponding observed value of the $i$th day of the $j$th state variable. Model calibration of multiple variables simultaneously using the average cost of multiple variables as the objective function:

$$f(\Theta, m) = \frac{1}{m} \sum_{i=1}^{m} cost_i,$$

where $\Theta$ is a parameter vector to be calibrated, $m$ is the number of state variables chosen to calibrate simultaneously.

After model calibration, experimental data ($N$, WP, $Q_p$, $S_p$) of *K. mikimotoi* at 20 °C under two P$_i$ concentrations were used for model validation. In addition, the relative error is also calculated as another index to measure model fitness. The relative error between the simulation data and the observation data is calculated by the method.
of Marois and Mitsch (2016),

$$RE_j = \left( \frac{X_{j}^{sim}}{X_{j}^{obs}} - 1 \right) \times 100,$$

where $RE_j$ is the relative error, $X_{j}^{sim}$ and $X_{j}^{obs}$ are the simulation data and the observation data at $j$th day, respectively. The relative error of a state variable is expressed by the average relative error of all experimental data.

3. Results

3.1. Experimental results

3.1.1. Results of short-term phosphate absorption experiments of K. mikimotoi under P$_i$-replete condition

The changes of state variables $N$, WP, $Q_r$ (cell quota of QP), and $S_p$ (cell quota of AP) of $K$. mikimotoi at 20 °C and 24 °C under the condition of P$_i$-replete over time are shown in Fig. 2. With the initial cell density of 0.2×10$^8$ cells L$^{-1}$, $N$ increased slowly during the early 6 days and increased quickly in the latter 12 days. When the initial $P_i$ concentration in the substrate was 25.55 μM, the trends of WP in both 20 °C and 24 °C conditions ultimately reached a plateau after a sharp initial decline. WP at 24 °C decreased more significantly than WP at 20 °C. With the initial $Q_r$ 6.70×10$^{-8}$ μmol cell$^{-1}$, the trends of $Q_r$ at 20 °C and 24 °C were identified as increasing in the early 3 or 6 days then decreasing. However, the maximum value of $Q_r$ (16.33×10$^{-8}$ μmol cell$^{-1}$) at 24 °C was larger than that at 20 °C (11.24×10$^{-8}$ μmol cell$^{-1}$). With the initial $S_p$, 19.68×10$^{-8}$ μmol cell$^{-1}$, $S_p$ at two temperatures increased in the first 3 days and dropped rapidly for the next 9 days then it maintained a more or less stable condition. The peak value of $S_p$ at 24 °C was higher than that at 20 °C. In addition, the decline rates of $S_p$ at the two temperatures were different from day 3 to day 12. At 24 °C, $S_p$ first decreased slowly, then decreased rapidly, and finally decreased slowly, while at 20 °C, $S_p$ declined almost linearly.

3.1.2. Results of short-term phosphate absorption experiments of K. mikimotoi under P$_i$-deplete condition

The changes of $N$, WP, $Q_r$, and $S_p$ of $K$. mikimotoi at 20 °C and 24 °C under the condition of P$_i$-deplete with time are shown in Fig. 3. With the initial cell density 0.2×10$^8$ cells L$^{-1}$, $N$ showed a trend of fluctuation during the experiment at the condition of 20 °C. It increased in the first 9 days, decreased in the next 6 days, and increased in the last 3 days. $N$ at 24 °C decreased in the first 3 days, then increased in the latter 9 days and dropped again in the final 6 days. With the initial $P_i$ concentration in substrate 0.74 μM, WP at 20 °C was almost the same as WP at 24 °C, ultimately reached a plateau after a sharp initial decline. With the initial $Q_r$ 4.57×10$^{-8}$ μmol cell$^{-1}$, $Q_r$ decreased almost linearly in the course of the experiment at two temperatures. In the first 12 days, the trend of decline was relatively fast, and in the last 6 days reached a plateau. With the initial $S_p$, 11.43×10$^{-8}$ μmol cell$^{-1}$, $S_p$ at 24 °C decreased soon after increasing in the first 3 days, while $S_p$ at 20 °C decreased during the first 6 days, finally $S_p$ tended to a plateau at both temperatures.

3.2. Experimental data fitting

The model calibration and validation results are shown in Figs. 4, 5, 6, and 7. Table 4 shows the model cost and relative error of all state

![Diagram](image-url)
variables in model calibration and validation. The model fitting results of the experimental data of *K. mikimotoi* under Pi-replete condition at 24 °C are shown in Fig. 4. Among the four variables, Qc has the best simulation effect, model cost and relative error both are the smallest, which are 0.63 and −2.79, respectively. The algal cell density N and the substrate Pi concentration WP are simulated moderately well with relative error of 11.21 and −13.83, respectively. For Sp, the simulated curve shows a trend similar to the observed curve and can better simulate the observation data of the first 9 days. However, the model cannot fit the observed data for the last 9 days, and simulation data is lower than the observation data. The fitting results of model (8) under the condition of Pi-deplete at 24 °C are given in Fig. 5. The simulation of Qc performed the best with the model cost of 1.86 and the relative error of 15.34. For N and Sp, the model fitting curve and experimental data have similar trends, but can only fit some data points. For WP, the model can well fit the variation trend of experimental data, but in most cases, the observed value of WP is underestimated. The calibrated parameter values are displayed in Table 3. Figs. 6 and 7 show the model validation results of the experimental data of *K. mikimotoi* under the conditions of Pi-replete and Pi-deplete at 20 °C. It can be seen that the experimental data at 20 °C can be well fitted by using the trained model, here the model parameter values from in Table 3 and only change the temperature value. Combined with the calibration and validation results of model (8), it can be seen that the novel two-stage kinetics model can well describe the Pi uptake characteristics of algae at different Pi concentrations and temperatures.

4. Discussion

In the previous paper (Jiang et al., 2019), we focused on the comparison between the one-stage model and the two-stage model to study the Pi uptake kinetics by algae (*P. donghaiense*). In this study, the effects of different Pi concentrations and temperatures on the Pi uptake characteristics of *K. mikimotoi* are mainly considered. Combined with the experimental data and the fitting results of the model, it can be found that the Pi uptake characteristics of *K. mikimotoi* are different under different Pi concentrations and temperatures. Through these Pi uptake characteristics, the interspecific competition mechanism of two species *P. donghaiense* and *K. mikimotoi* during the red tide can be furthered to understand.

4.1. The role of temperature on Pi uptake

The influence of temperature on the Pi uptake process of algae can be divided into two aspects. On the one hand, temperature affects the activity of rate-limiting enzymes, which could limit the photosynthesis of light saturation and regulate the amount of inorganic phosphorus transported into cells (Von Caemmerer and Farquhar, 1981; Stitt, 1986). When considering the influence of temperature on the rate-limiting enzymes, it is important to consider the independent effect of temperature on the maximum growth rate μmax, which is temperature-dependent and related to Pi uptake directly (Bordel et al., 2009; Goldman and Carpenter, 1974). On the other hand, temperature
could influence other enzymes and physical processes (Raven and Smith, 1978; Raven and Geider, 1988), which may also indirectly influence on phosphorus transport process. Under the same P<sub>i</sub> concentration in the substrate, <i>K. mikimotoi</i> showed different growth characteristics at different temperatures (Figs. 2a and 3a). One possible reason is that temperature can directly regulate algae growth through light fluctuations and nutrient availability. It strongly affects the composition of cells, the absorption rate of nutrients, the fixation of carbon dioxide, and the growth rate of algae (Juneja et al., 2013; Ahmad et al., 2020). Furthermore, the peak values of $Q_c$ and $S_p$ at 24 °C were higher than those at 20 °C in both P<sub>i</sub>-replete and P<sub>i</sub>-deplete (see Figs. 2c, d, 3c, and d).

### 4.2. The role of P<sub>i</sub> concentration on P<sub>i</sub> uptake

Phosphorus is one of the main elements in phytoplankton growth, which is directly engaged in many processes of photosynthesis, such as the Calvin cycle and adjustment of some enzymes activity (Wang et al., 2004; Shen and Li, 2016). In the present study, <i>K. mikimotoi</i> obviously grows fast under P<sub>i</sub>-replete than those under P<sub>i</sub>-deplete (Figs. 2a and 3a). One possible reason for this phenomenon is that the growth rate of algae is proportional to the P<sub>i</sub> concentration in a range of low and medium P<sub>i</sub> concentrations (Long and Du, 2005; Shen and Li, 2016). About the condition of low P<sub>i</sub> concentration, <i>K. mikimotoi</i> ended the exponential growth phase earlier and entered the decline phase (Figs. 2a and 3a). This may be due to the luxury uptake of P<sub>i</sub> by algal cells under low phosphorus conditions, which increases the growth rate of cells in a short time, thus rapidly entering the exponential growth period to reach the peak, and then entering the decrease stage due to the depletion of phosphorus.

The decrease of $Q_c$ of <i>K. mikimotoi</i> from day 6 to day 18 at 20 °C and 24 °C under the P<sub>i</sub>-replete condition (Fig. 2c), which may be due to the dilution of intracellular P<sub>i</sub> concentration caused by the increase of cell density ($N$), and the decrease of P<sub>i</sub> concentration in the substrate (WP) resulted in the decrease of the amount of P<sub>i</sub> transported into cells. The quantity $Q_c$ decreased in <i>K. mikimotoi</i> during the whole treatment time at 20 °C and 24 °C under the condition of low P<sub>i</sub> concentration (Fig. 3c). It may be because the P<sub>i</sub> concentration of substrate was lower.
so that *K. mikimotoi* cannot grow and survive. Therefore, as cell density grew, previous cells had to allocate part of their own $Q_c$ to newborn cells.

$S_p$ reached its maximum value faster and entered into stable phase earlier under $P_i$-deplete than that at $P_i$-replete (Figs. 2d and 3d). The reason for this phenomenon may be that algal cells at the condition of low $P_i$ concentration took little time to make $P_i$ of surface-adsorbed transfer into intracellular than that at high $P_i$ concentration. Furthermore, this can be explained as dinoflagellate cells can accelerate $P_i$ uptake by increasing the number of high affinity phosphate transporters when the deterioration of the external environment is in the state of phosphorus stress (Lin et al., 2016). In model (8), a decreasing function $K_p$ of external phosphorus concentration is used to describe this phenomenon. When $P_i$ is deficient, $K_p$ has a large value, and the uptake rate of $P_i$ by algal cells increased at this time. In addition, the parameter values of $Q_{\text{max}}$, $Q_{\text{init}}$, and $S_{\text{max}}$ at $P_i$-replete group were higher than those at $P_i$-deplete group (Table 3). This may be because when phosphorus is sufficient, the survival strategy of algae cell has changed, increasing its internal and external $P_i$ pools and storing a large amount of $P_i$ to cope with sudden deterioration of the external environment (Kwon et al., 2013). The value of $e$ at $P_i$-replete is lower than that at $P_i$-deplete, which may be increased cell mortality due to the lack of nutrition in the environment.

4.3. The role of $P_i$ uptake on the competition between *K. mikimotoi* and *P. donghaiense*

Jiang et al. (2019) studied the $P_i$ uptake characteristics of *P. donghaiense* by using the two-stage kinetics model under different $P_i$ concentrations, compared it with the one-stage kinetics model, and found that the two-stage kinetics process was better than one stage kinetics process. Here the mechanism of competition between *P. donghaiense* and *K. mikimotoi* under various $P_i$ concentrations at 24 °C will be discussed.
Fig. 5. Comparison of model fitted curve and experimental data of *K. mikimotoi* at 24 °C under the condition of *P*<sub>i</sub>-deplete. (a) *N*; (b) WP; (c) the cell quota of QP (*Q*<sub>c</sub>); (d) the cell quota of AP (*S*<sub>p</sub>). The parameters of model (8) can be estimated by fitting the four state variables at the same time. The parameter values are displayed in Table 3.

5. Conclusion

Based on the two-stage model in our previous work (Jiang et al., 2019), a novel two-stage kinetics model is developed by combining the Arrhenius temperature function and *P*<sub>i</sub> stress function. This model will help predict *P*<sub>i</sub> uptake kinetics of algae at different phosphorus and temperatures concentrations. The model parameters are calibrated by the experimental data of *K. mikimotoi* at 24 °C under the conditions of *P*<sub>i</sub>-replete and *P*<sub>i</sub>-deplete, and then the model is validated by the experimental data of two *P*<sub>i</sub> concentrations at 20 °C. Experimental data and fitting results suggest that the *P*<sub>i</sub> uptake characteristics of the *K. mikimotoi* vary under different cultural conditions of *P*<sub>i</sub> concentration and temperature. Furthermore, by using the results of experiments and parameter values, the interspecific competition between two species *K. mikimotoi* and *P. donghaiense* under different *P*<sub>i</sub> concentrations are discussed. In order to predict the occurrence of algal blooms in oceans using observational information from marine and experimental results, some factors need to be explored further, for example, *pH* value, solar irradiance, water velocity, environmental toxins and the direct or indirect interactions of algal species (Yang and Yuan, 2021; Xu et al., 2021). Constraints of solar irradiance and phosphate on algae or algae-*Daphnia* interactions were modeled in Wang et al. (2008), Li and Wang (2012), Peace et al. (2014), Peace and Wang (2019), Yuan et al. (2020), Shen et al. (2014). Similarly, the large values of *R* and *T<sub>p</sub>* in *K. mikimotoi* (48.75, 16.83) than those in *P. donghaiense* (26.84, 8.45) under *P*<sub>i</sub>-deplete condition at 24 °C imply that the viability of *K. mikimotoi* is better than *P. donghaiense* in bi-algae substrate at higher temperature under *P*<sub>i</sub>-deplete condition (24 °C and 28 °C) (Shen et al., 2014). Similarly, the large values of *R* and *T<sub>p</sub>* in *K. mikimotoi* (48.75, 16.83) than those in *P. donghaiense* (26.84, 8.45) under *P*<sub>i</sub>-deplete condition at 24 °C imply that the viability of *K. mikimotoi* is also better than *P. donghaiense* in bi-algal culture medium under *P*<sub>i</sub>-deplete condition at 24 °C.

Droop first proposed the concept of luxury coefficient *R* (*Q*<sub>max</sub>/*Q*<sub>min</sub>) and used it to express the nutritional storage capacity of algal cells. A larger *R* gives a stronger *P*<sub>i</sub> storage capacity of algal cells (Droop, 1974; Li, 2007). The utility value of the nutrient storage in algal cells for cell growth depends also on the maximum growth rate of the algae. For this reason, Pedersen and Borum (1996) proposed the concept of growth potential *T<sub>G</sub>* (ln*R*/*μ*<sub>max</sub>) to evaluate the utility of nutrients stored in algal cells for cell growth and reproduction. Table 5 shows the calculation results of luxury coefficient *R* and growth potential *T<sub>G</sub>* of *K. mikimotoi* and *P. donghaiense* at two *P*<sub>i</sub> concentrations of 24 °C. The values of *R* of *P. donghaiense* and *K. mikimotoi* under *P*<sub>i</sub>-replete condition at 24 °C are 26.87 (Jiang et al., 2019) and 41.68, respectively. *T<sub>G</sub>* values of *P. donghaiense* and *K. mikimotoi* under *P*<sub>i</sub>-replete condition at 24 °C are 8.76 (Jiang et al., 2019) and 16.45, respectively. The higher values of *R* and *T<sub>G</sub>* of *K. mikimotoi* than those of *P. donghaiense* under the condition of *P*<sub>i</sub>-replete concentration at 24 °C imply that the viability of *K. mikimotoi* is better than *P. donghaiense* in the bi-algae substrate at higher temperature under *P*<sub>i</sub>-replete condition (24 °C and 28 °C) (Shen et al., 2014). Similarly, the large values of *R* and *T<sub>p</sub>* in *K. mikimotoi* (48.75, 16.83) than those in *P. donghaiense* (26.84, 8.45) under *P*<sub>i</sub>-deplete condition at 24 °C imply that the viability of *K. mikimotoi* is also better than *P. donghaiense* in bi-algal culture medium under *P*<sub>i</sub>-deplete condition at 24 °C.
Fig. 6. Validation of model (8) with the data of four state variables of *K. mikimotoi* under the condition of $P_i$-replete at 20 °C. Here $T = 293.15$ and the rest parameter values are from in Table 3.

Davies and Wang (2021) and Zhao et al. (2020) via stoichiometric theory. The integration of stoichiometric models with the multi-stage algal nutrient uptake process would provide a fundamental modeling framework of nutrient cycling and energy flow in trophic cascades. This would be a promising cutting-edge area to explore in the future.

CRediT authorship contribution statement

Shufei Gao: Conducted and analysis data, Writing – original draft. Anglu Shen: Conducted the experimental work, Conducted and analysis data, Writing – original draft. Jie Jiang: Conducted and analysis data, Writing – original draft. Hao Wang: Designed this study, Commented and amended on the manuscript. Sanling Yuan: Designed this study, Commented and amended on the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data accessibility

All data used in this study can be found in the manuscript and its supplementary materials.

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Appendix A. Supplementary data

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.ecolmodel.2022.109909.
Fig. 7. Validation of model (8) with the data of four state variables of *K. mikimotoi* under the condition of \( P_i \)-deplete at 20 °C. Here \( T = 293 \) and the rest parameter values are from in Table 3.

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