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A model and experimental study of phosphate uptake kinetics in algae: Considering surface adsorption and P-stress

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Abstract

Phosphorus is an important limiting nutrient in many ecosystems. Consequently, there is increasing interest on phosphate uptake and algal growth due to the increasing frequency and magnitude of algal blooms induced by eutrophication. The co-existence of surface adsorbed and intracellular phosphorus pools indicate that phosphate uptake by phytoplankton is, to some extent, a two-stage kinetic process. However, almost all previous uptake models considered the internal uptake stage only and ignored the possible impact of surface adsorption. In this article, a two-stage kinetic uptake model considering both surface adsorption and P-stress on phosphate uptake by algae was constructed and compared to conventional one-stage models, based on experimental data on short-term uptake kinetics of a green algae *S. quadricauda*. Results indicated that with suitable parameters, the two-stage uptake model not only fit the experimental data better, but also gave more reasonable and realistic explanations to the phosphate uptake process. The results are meaningful as surface-adsorption of phosphate may affect the uptake process of phosphate and assist in understanding realistic phosphate uptake kinetics in phytoplankton.

Key words: phosphate uptake; surface adsorption; P-starvation; phytoplankton

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Introduction

World-wide water resources such as lakes, rivers, and oceans have suffered severely from eutrophication over the past several decades (Gurkan et al., 2003), and there is increasing interest in developing management schemes to promote growth of beneficial algal forms due to the increasing frequency and magnitude of noxious phytoplankton blooms. Among the factors that influence phytoplankton succession, such as nutrient availability, grazing, parasitism, irradiance, and temperature, nutrient availability is frequently deemed the most important and the easiest to control (Roelke et al., 1999). Phosphorus is an important limiting nutrient in many ecosystems (such as lakes, rivers, and estuaries), and also one of the most likely to limit the rate of phytoplankton production (Falkowski, 1997). In addition, P stress has also been associated with other facets of phytoplankton growth, such as toxin production (John and Flynn, 2000a) and allelochemicals release (Elert, 1997; Granéli and Johansson, 2003; Fistarol et al., 2005), which may threaten the survival of many organisms, including humans, and have the potential to impact ecosystems, services, and economic development at the local and catchment scale (JΦgensen et al., 2002). Many studies have focused, therefore, on phosphate uptake and phytoplankton growth relationships for the past several decades (John and Flynn, 2000b).

Phosphate uptake through algal cell membranes is usually described as Michaelis-Menten kinetics with the parameters of maximum uptake rate (V_{max}) and half-saturation constant (K_s) (Aksnes and Egge, 1991). This approach was originally descriptive, and the parameters V_{max} and K_s were, to some extent, interpreted as estimates of theoretical parameters characterizing the species under study (Harison et al., 1989). However, the common and generally successful use of Michaelis kinetics to describe nutrient uptake tends to obscure the difficulties associated with interpretation of the kinetic parameters derived from the measurements performed. This is especially true for the interpretation of K_s (Droop, 1983), which often indicates competitive ability at low nutrient concentrations and is sometimes denoted as the affinity constant (Button, 1978; Healey, 1980; Harison et al., 1989). Furthermore, the two-parameter Michaelis-Menten model can not encompass the observed phenomenon of phosphate uptake increase due to nutrient deficiency (Syrett, 1956), usually called

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"luxury uptake" or "luxury consumption", which indicate that P-stressed algal cells have an enhanced maximum phosphate uptake rate (Conway et al., 1976; Goldman et al., 1981; Parslow et al., 1984; Harrison et al., 1989). Experimental results have proved that P-starved cells could attain much higher nutrient uptake rates than saturated cells (Goldman and Glibert, 1982; Lehman and Sangren, 1982; Riegman and Mur, 1984), and may uptake phosphate by 8–16 times the minimum cell-quota in phosphate-repletion medium, which were stored as polyphosphate bodies (internal P pool) and could sustain 3–4 generations of growth in phosphate-deplete conditions theoretically (Droop, 1973; Morel, 1987). Therefore, maximum uptake should be determined for both starved and saturated cells, and uptake rate should be related to the size of the internal P pool (Harrison et al., 1989).

Besides the feedback control of internal P pool size, phosphate uptake rate may also be affected by another P pool on algal cell surfaces caused by surface adsorption. Wilhelmy (2004) reported that surface-adsorbed phosphate could attain 60%–90% of total cellular phosphorus in different algal species. The co-existence of surface-adsorbed and intracellular P pools indicates that phosphate uptake by algae is to some extent a two-stage kinetic process: (1) phosphate adsorption on algal surfaces; (2) transportation of surface-adsorbed phosphate into cells through cell membranes. Nearly all previous studies, however, only considered the second step, directly relating uptake rate to substrate phosphate concentration and P-state of algae cells, and phosphate uptake was thus a one-stage kinetic process. Very few studies (e.g., Wilhelmy et al., 2004) have focused on adsorption of phosphate on algal cell surfaces and little is understood so far. It is suggested, however, that surface-adsorption may play an important role in uptake of phosphate as it is an intermediate process taking place at the substrate-cell interface, and a two-way process via equilibrium between adsorption and desorption. Variations in environmental conditions such as P concentration, temperature, or pH in the substrate may change the direction of P flux between the substrate and cell surface. As a result, the amount of surface-adsorbed phosphate and the later transport process could be affected. Furthermore, partitioning total cellular phosphorus into surface-adsorbed and intracellular P pools may affect cell growth, which directly depends on intracellular instead of extracellular phosphorus. Most commonly used growth models at present, however, use total phosphorus (including surface adsorbed and intracellular P). In addition, Redfield ratios are also strongly influenced by partitioning the two pools, which could affect the interpretation of ecosystem models (Wilhelmy, 2004).

We constructed a two-stage kinetic model of phosphate uptake by algae, considering both surface adsorption and P-stress. The model was divided into three main parts: phosphate adsorption/desorption between the substrate and cell surface; transportation of surface-adsorbed phosphate into cells; and cell growth on intracellular phosphorus quota. Additionally, luxury uptake of phosphate under P stress was considered.

1 Materials and methods

1.1 Model description

Phosphate uptake by algae is thought to be a two-stage kinetic process in which substrate phosphate (WP) adsorbs on algal cell surfaces via equilibrium between adsorption and desorption. Subsequently, surface-adsorbed phosphate (AP) is transported into the cells through the cell membrane, which is controlled by the feedback of intracellular P pool size. Additionally, algal cell growth is based on intracellular phosphorus (QP).

Surface adsorption is a common kinetic step in the uptake of metals (e.g., iron, copper, and mercury) and organic contaminants by algae, and is usually simply described as the Langmuir equation (Knauer et al., 1997; Khoshmanesh et al., 1997; Rhee and Thompson, 2004; Tien et al., 2005; Rezaee, 2006). At present, however, little is known about phosphate adsorption on algal surfaces. Therefore, we simply used the Langmuir equation to describe the adsorption/ desorption processes (Liu et al., 2007), with some assumptions: (1) the adsorption of phosphate on algal cell surfaces was a single-layer adsorption process, reversible through desorption; (2) the adsorption/desorption of phosphate was rapid and the amount relatively small; (3) algal cell surface properties (e.g. cell size and density of sorption sites) were homogeneous and cell-specific differences were ignored; and (4) the impact of cell size changes caused by P-stress was ignored. Thus, the adsorption and desorption processes can be described as:

$$R_a = K_a \times \mathrm{WP} \times (1 - \frac{S_p}{S_{pmax}}) \quad (1)$$

$$R_d = K_d \times \mathrm{AP} \quad (2)$$

where, AP = $S_p \times N$. AP (μmol/mL) is the surface-adsorbed phosphate concentration; S_p (10^{-8}μmol/cell) is the amount of surface-adsorbed phosphate per algal cell; S_{pmax} (10^{-8}μmol/cell) is the maximum of S_p; N (10^8 cells/mL) is the algal cell density; K_a (min^{-1}) and K_d (min^{-1}) are the adsorption and desorption constants, respectively; R_a (μmol/(mL·min)) and R_d (μmol/(mL·min)) are the adsorption and desorption rates, respectively; and WP (μmol/mL) is the phosphate concentration in the substrate.

Although mechanical phosphate transport models, such as the model of Pasciak and Gavis (1974, 1975) and its Michaelis-like approximation (Armstrong, 2008) have been established and seemed reasonable and realistic, they are difficult to use and still require experimental data to be validated due to complexity of parameterization and severe lack of information on cell surface properties (such as density of porters). Therefore, a common Michaelis-Menten equation could be an alternative:

$$T = T_{max} \times \frac{S_p}{S_p + K_t} \quad (3)$$

where, T (10^{-8}μmol/(cell·min)) is the transport rate of surface-adsorbed P into algal cell; T_{max}

(10^{-8} μmol/(cell·min)) is the maximum transport rate; and K_t (10^{-8}μmol/cell) is the half saturation constant, which refers to the amount of surface-adsorbed phosphate at which transport rate may attain 50% of T_{max}.

The value of T_{max} is determined by the maximum assimilation rate of P by algae, and increases with P-stress (Rhee, 1973; Gothan and Rhee, 1981; Rivkin and Swift, 1985). Here, we simply used a coefficient representing the level of P-stress, and thus the maximum transport rate can be defined as:

$$T_{max} = K_p \times Q_{max} \times \mu_{max} \quad (4)$$

where, K_p is a dimensionless coefficient describing P-stress, whose value may equal 1 for cells not stressed by P, or larger values for those under P-stress; Q_{max} (10^{-8}μmol/cell) is the maximum cell quota; and μ_{max} (min^{-1}) is the maximum cell-specific growth rate.

Furthermore, phosphate transport is also controlled by the feedback of internal P pool size, and can be described by a sigmoidal function with a power of 4 (Flynn et al., 1997; John and Flynn, 2000b; Flynn, 2003):

$$F_q = \frac{(1 - Q_c/Q_{max})^4}{(1 - Q_c/Q_{max})^4 + K_q} \quad (5)$$

where, F_q is a feedback function, Q_c (10^{-8}μmol/cell) is the cell quota; and K_q is a dimensionless constant used to control the shape of the feedback function curve.

Thus, the final form of P transport rate per algal cell can be written as kinetic constraints from the external phosphate concentration according to Michaelis-Menten kinetics Eq. (3), plus regulation via transport-inhibition from size of internal P (Eq. (5)):

$$T = T_{max} \times \frac{S_p}{S_p + K_t} \times F_q = K_p \times Q_{max} \times \mu_{max} \times \frac{S_p}{S_p + K_t} \times \frac{(1-Q_c/Q_{max})^4}{(1-Q_c/Q_{max})^4 + K_q} \quad (6)$$

Algal growth depends directly on intracellular instead of extracellular P (Cembella et al., 1984), and the reproductive growth rate correlates better to cell-quota than to ambient phosphate concentrations for many phytoplankton species (Droop, 1973, 1983). Thus the cell specific growth rate based on cell quota can be described as a conventional quota type of control (John and Flynn, 2000b):

$$\mu = \mu_{max} \times \frac{Q_c - Q_{min}}{Q_c - Q_{min} + K_c} \quad (7)$$

where, μ (min^{-1}) is the cell-specific growth rate determined by cell quota; Q_{min} (10^{-8}μmol/cell) is the minimum cell quota for algal existence; and K_c (10^{-8}μmol/cell) is a constant used for cell quota control of growth.

1.2 Model implementation

The two-stage phosphate uptake model described above was implemented by system dynamic software VENSIM® (Vensim DSS32, Version 5.4a) developed by Ventana Systems, Inc. (USA). It is a simulation environment with a Windows®-based user interface used for developing, analyzing, and packaging high quality dynamic feedback models, including dynamic functions, subscripting (arrays), Monte Carlo sensitivity analysis, optimization, data handling, and other features.

The structure of the implemented model is shown in Fig. 1, with main variables and equations listed in Table 1 to give supplemental information to the conceptual model described above.

1.3 Sensitive analysis

A single parameter sensitivity analysis of all model constants was conducted, in which the initial values of the four levels WP, AP, QP, and N were 0.2, 0.0, 0.233, and 0.116 μmol/mL, respectively. The model was run three times and 600 time steps each for the constants, with the control value, double or half of the control value (Table 2), separately. The model response was calculated as maximum of (P_a–P_c) for positive impact or minimum of (P_a–P_c) for negative impact, where P_a and P_c referred to model responses of the altered value (double or half of the control value) and the control value, respectively. The

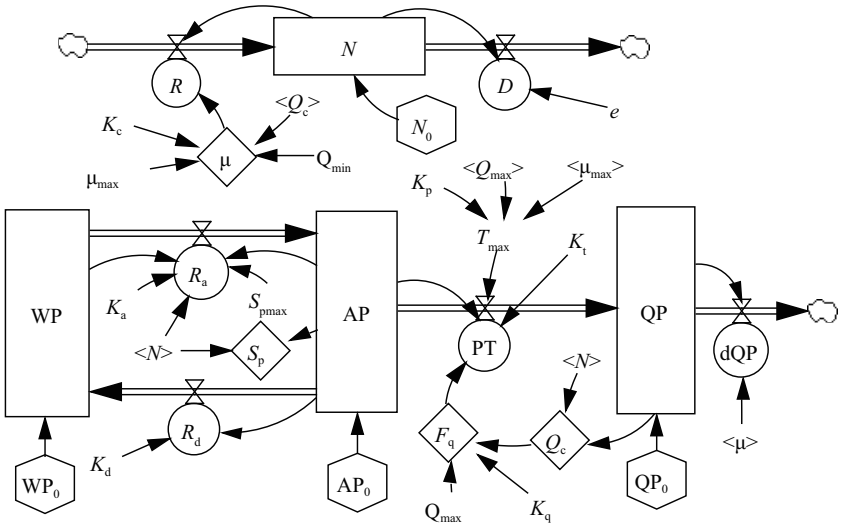

Fig. 1 Structure of the two-stage phosphate uptake model implemented by Vensim. Boxes are levels, circles are rates, hexagons are initial values of levels, diamonds are auxiliaries, square brackets are shadow variables, clouds represent sources or sinks, and the others are constants. e (min^{-1}): death rate of algae cells.

Table 1 Main variables and equations of the implemented two-stage phosphate uptake model

Parameter	Value	Explanation
R (10^8 cells/(mL·min))	$\mu \times N$	Cell growth
D (10^8 cells/(mL·min))	$e \times N$	Cell exertion
N (10^8 cells/mL)	$N_0 + (R - D) \times dt$	Algal cell density
WP (μmol/mL)	$WP_0 + (R_d - R_a) \times dt$	Phosphate concentration in substrate
AP (μmol/mL)	$AP_0 + (R_a - R_d - PT) \times dt$	Surface-adsorbed phosphate concentration
PT (μmol/(mL·min))	$T_{max} \times F_q \times N \times S_P/(S_P + K_t)$	Phosphate transport rate
QP (μmol/mL)	$QP_0 + (PT - dQP) \times dt$	Intracellular phosphate concentration
dQP (μmol/(mL·min))	$\mu \times QP$	Correction of QP with cell growth

Table 2 Results of single parameter sensitivity analysis for constants of the implemented two-stage phosphate uptake model

Parameter	Control value	Test value	Model Response			
			WP	AP	QP	N
μ_{max}	0.0004	0.0008	0.05	0.027	0.077	0.033
		0.0002	0.03	0.003	0.033	0.014
S_{pmax}	0.4	0.8	0.019	0.019	0.0002	0.0001
		0.2	0.016	0.015	0.0004	0.0001
Q_{max}	2.8	5.6	0.346	0.008	0.353	0.018
		1.4	0.176	0.004	0.176	0.0034
Q_{min}	0.28	0.56	0.007	0.001	0.0074	0.0036
		0.14	0.003	0.0006	0.0038	0.0019
e	0.00008	0.00016	0.012	0.0016	0.013	0.0064
		0.00004	0.006	0.0012	0.0068	0.0033
K_a	0.09	0.18	0.018	0.018	0.0002	0.0001
		0.045	0.012	0.012	0.0004	0.0001
K_d	0.06	0.12	0.0076	0.0075	0.0001	0.0001
		0.03	0.0067	0.0067	0.0001	0.0001
K_t	0.001	0.002	0.0006	0.0002	0.0006	0.0001
		0.0005	0.0003	0.0001	0.0004	0.0001
K_c	0.28	0.56	0.004	0.0006	0.001	0.0014
		0.14	0.003	0.0004	0.0006	0.0009
K_p	4	8	0.012	0.0044	0.0132	0.0001
		2	0.0123	0.0027	0.0132	0.0001
K_q	0.004	0.008	0.0085	0.0016	0.0091	0.0001
		0.002	0.0077	0.0017	0.0084	0.0001

results of the sensitivity analyses of all constants are given in Table 2, which suggest that the model was robust with no over-sensitivity control points, and gave reasonable outputs if suitable parameter values were selected.

Phosphate concentration in the substrate (WP) was most affected by Q_{max}, as it determined how much and how fast algal cells took up phosphate from the external environment in the model. It was also largely affected by μ_{max}, e, S_{pmax}, K_a, and K_d because these constants impacted algal cell growth and surface adsorption characteristics. And the factors affecting surface adsorbed phosphate concentration (AP) are similar to those of WP. Intracellular phosphorus concentration (QP) was notably affected by Q_{max}, μ_{max}, e, and K_p as these values controlled internal phosphate demand and phosphate transport rate. Algal cell density (N) was also significantly impacted by Q_{max}, Q_{min}, μ_{max}, and e, but was less sensitive to the other constants.

1.4 Experimental materials and methods

1.4.1 Algae culturing

The *S. quadricauda* algae used was obtained from the Research Center on Lake Environment of Chinese Research Academy of Environmental Sciences. The *S. quadricauda* were grown in axenic M11 culture medium (see culture medium below) in a series of Erlenmeyer flasks, put in a culture incubator at 25°C, with light intensity of 37.5 μmol/m^2 (3000 lx) and a light to dark ratio of 12 hr:12hr. The flasks were shaken 4–6 times a day by hand.

1.4.2 Culture medium

The M11 culture medium contained (mg/L): $NaNO_3$, 100; K_2HPO_4, 10; $MgSO_4 \cdot 7H_2O$, 75; $CaCl_2 \cdot 2H_2O$, 40; Na_2CO_3, 20; Fe·citrate·xH_2O, 6; $Na_2EDTA \cdot 2H_2O$, 1; and buffered to pH 8.0 with 1 mol/L NaOH. Additionally, the starved with P-free culture medium: M11 culture medium was devoid of K_2HPO_4 but contained KCl to keep equivalent concentration of K^+.

1.4.3 Surface wash reagent

Surface-adsorbed phosphate was washed by an oxalic acid reagent following Sanchez et al. (2003) (g/L): $Na_2EDTA \cdot 2H_2O$, 18.6; $C_6H_5Na_3O_7 \cdot 2H_2O$, 14.7; KCl, 0.74; $C_2H_2O_4 \cdot 2H_2O$, 12.6; and buffered to 8.0 with 10 mol/L NaOH. The reagent was initially used to wash out cell surface-bond iron, but also proved very effective for washing out surface-adsorbed phosphate from algal cell surface. It has been suggested that this reagent is easily prepared, has good chemical stability, high wash-out efficiency, and low negative effect on algal cells (Sanchez et al., 2003; Wilhelmy et al., 2004).

1.4.4 Preparation of algal cells

A 10-day old culture (with cell density of about 4.0 × 10^6cells/mL) was harvested by centrifugation, washed three times with the oxalic acid reagent, and resuspended in 500 mL fresh P-free medium to give a cell density of about 1.1 × 10^7cells/mL. Two groups of experiments (each group had three parallels) were then carried out: (1) 34 μmol K_2HPO_4 was added immediately to perform short-term uptake experiments; (2) cultured for two days under the same conditions as before, and then 34 μmol $K_2HPO_4$was added to perform uptake experiments. Thus, the first group related to uptake kinetics of algal cells not stressed by P, and the second reflected the impact of certain P-stress on uptake kinetics compared to the first. The short-term phosphate uptake experiments were carried out on a horizontal shaker under the same conditions as algal culturing.

1.4.5 Short-term phosphate uptake experiments

Two groups of comparative phosphate uptake experiments (P-stressed and without P-stress) were conducted as described above. During each experiment, 10 mL of culture was sampled every 5 min within the first 30 minutes, every 10 min during the next 30 minutes, and every 30 min during the remaining time. (1) Each sample was filtered by 0.45 μm filtration membrane immediately after sampling, and phosphate concentration in the filtrate was analyzed with phosphomolybdate-blue spectrophotometry for WP; and (2) the filtration membrane with algal cells

was then carefully washed by 10 mL oxalic acid reagent and filtered again by 0.45 μm filtration membrane, in which the filtrate was analyzed with phosphomolybdate-blue spectrophotometry for AP, and algal cells on the filtered membrane were carefully washed out by 10 mL MQ water, digested by potassium persulfate method, and analyzed with phosphomolybdate-blue spectrophotometry for QP. Algal cell density (N) was determined by microscope (Nikon Eclipse 50i microscope, Japan) count method with an additional 1 mL sample every hour during each experiment.

2 Results

2.1 Experimental results

The results of the short-term phosphate uptake experiments are shown in Fig. 2, in which averaged values of the three parallels of each group were used. Adsorption of phosphate on *S. quadricauda* cell surface was obvious, and the time variations of N, WP, AP, and QP exhibited similar trends: a fast increase of AP and QP led to a decrease of WP within the first 30 minutes after P was added, and AP started to decrease soon after it attained its maximum, with corresponding slower increase of QP and decrease of WP.

However, some notable differences between the two groups were easily found. Compared to the group without P-stress, the P-starved algal cells had lower initial cell quotas, showed a much faster initial uptake rate of phosphate after P was added, and had larger cell quotas in the end. Also, the time variations of QP in the P-stressed group exhibited two obvious different stages: a short-time rapid increase period soon after P was added and a subsequent slow increase period (Fig. 2d), which agreed with previous reports (Parslow, 1984; Litchman and Nguyen, 2008). It is suggested that P-starvation consumed a certain portion of internal stored phosphorus, and stimulated a "luxury uptake" after P was added. As a result, the maximum surface-adsorbed phosphate concentration of the P-stressed group was only about half that of the other group, and much lower by the end (Fig. 2c). Correspondingly, substrate phosphate concentration of the P-stressed group decreased much more sharply and had a much lower final value than the other group (Fig. 2b). In addition, the sharp decrease of WP in the P-stress group was mainly caused by internal uptake, with nearly all added phosphate internalized by algal cells; while in the group without P-stress, surface-adsorbed phosphate took up a large portion during the rapid decrease period, and in the end only about one third of added phosphate was internalized, one third remained on the cell surface, and the last third remained in the substrate (Fig. 2b).

Initial cell density (N) of the P-stressed group and the group without P-stress showed little difference (0.1176×10^{-8} vs. 0.1164×10^{-8} cells/mL) due to cell loss by

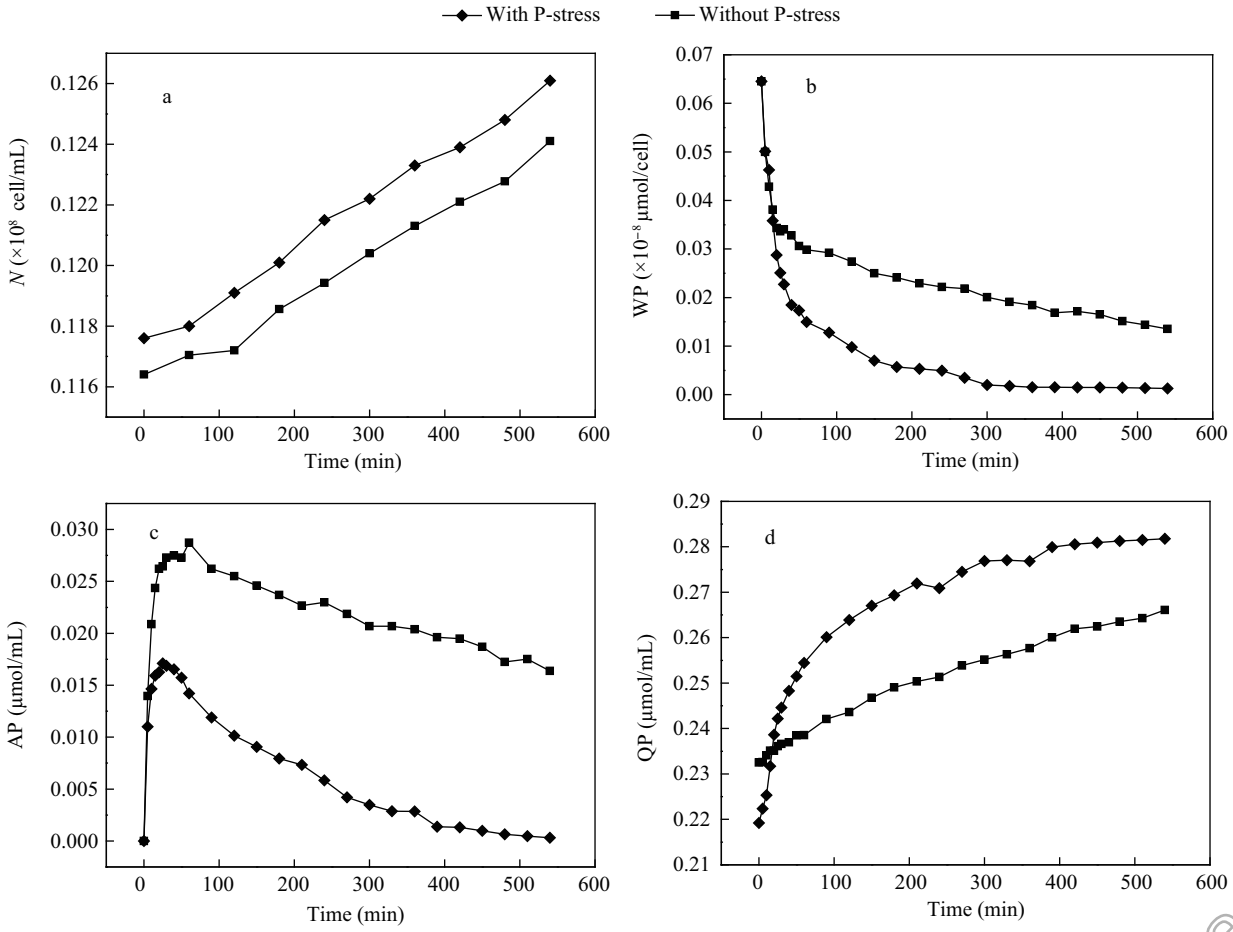

Fig. 2 Results of short-term phosphate uptake experiments of the two comparative groups. (a) algal biomass; (b) substrate phosphate concentration (WP); (c) surface-adsorbed phosphate concentration (AP); (d) total internal phosphorus concentration (QP).

centrifugation in the algal cells preparation process. The P-stressed group took up much more phosphate after P was added, had a larger cell quota, and thus had a greater growth rate than the other group (Fig. 2a).

2.2 Fit to the experimental data

The two-stage phosphate uptake model was calibrated and compared with the experimental data, with values of model constants listed in Table 3. As algal cell densities of the two groups showed a little difference, AP and QP were changed into units of 10^{-8}μmol/cell divided by cell density (expressed as S_p and Q_c, respectively). It can be seen that with suitable parameter values, the model could give a good fit to the experimental data (Fig. 3). Also, it is suggested that the model fitted WP better than S_P and Q_c, as the determination of AP and QP by cell surface wash may have induced additional errors compared to that of WP.

It also can be seen from Table 3 that the main difference between the two groups of parameter values was the value of K_p, where the P-stressed group had a K_p value six times that of the group without P-stress. This indicated that algal cells under two days P-starvation had a larger initial phosphate uptake rate than those without P-starvation. It appeared that P-stressed algal cells had a larger value of K_p than those without P-stress, which seems reasonable and in accordance with the experimental data.

Table 3 Comparison of constant values of the two-stage and one-stage phosphate uptake model

Parameter	Two-stage model		One-stage model	
	With P-stress	Without P-stress	With P-stress	Without P-stress
μ_{max} (min^{-1})	0.0002	0.0002	0.0002	0.0002
S_{Pmax} (10^{-8} μmol/cell)	0.6	0.6	–	–
Q_{max} (10^{-8} μmol/cell)	2.52	2.52	2.45	2.4
Q_{min} (10^{-8} μmol/cell)	0.25	0.25	0.25	0.25
e (min^{-1})	0.00005	0.00005	0.00005	0.00005
K_a (min^{-1})	0.06	0.06	–	–
K_d (min^{-1})	0.038	0.038	–	–
K_t (10^{-8} μmol/cell)	0.002	0.002	–	–
K_m (μmol/mL)	–	–	0.015	0.01
K_c (10^{-8} μmol/cell)	0.25	0.25	0.25	0.25
K_P (dmnl)	6	1	10	30
K_q (dmnl)	0.005	0.005	0.0005	0.001

K_m is half-saturation constant for the substrate concentration at that P transport rate attains half of its maximum.

Modeled time variations of the phosphate transport rate of the two groups are plotted in Fig. 4, which shows the P-stressed group exhibited an obvious two-stage phosphate uptake kinetics process, the initial rapid uptake stage and subsequent lower uptake stage, while the group without

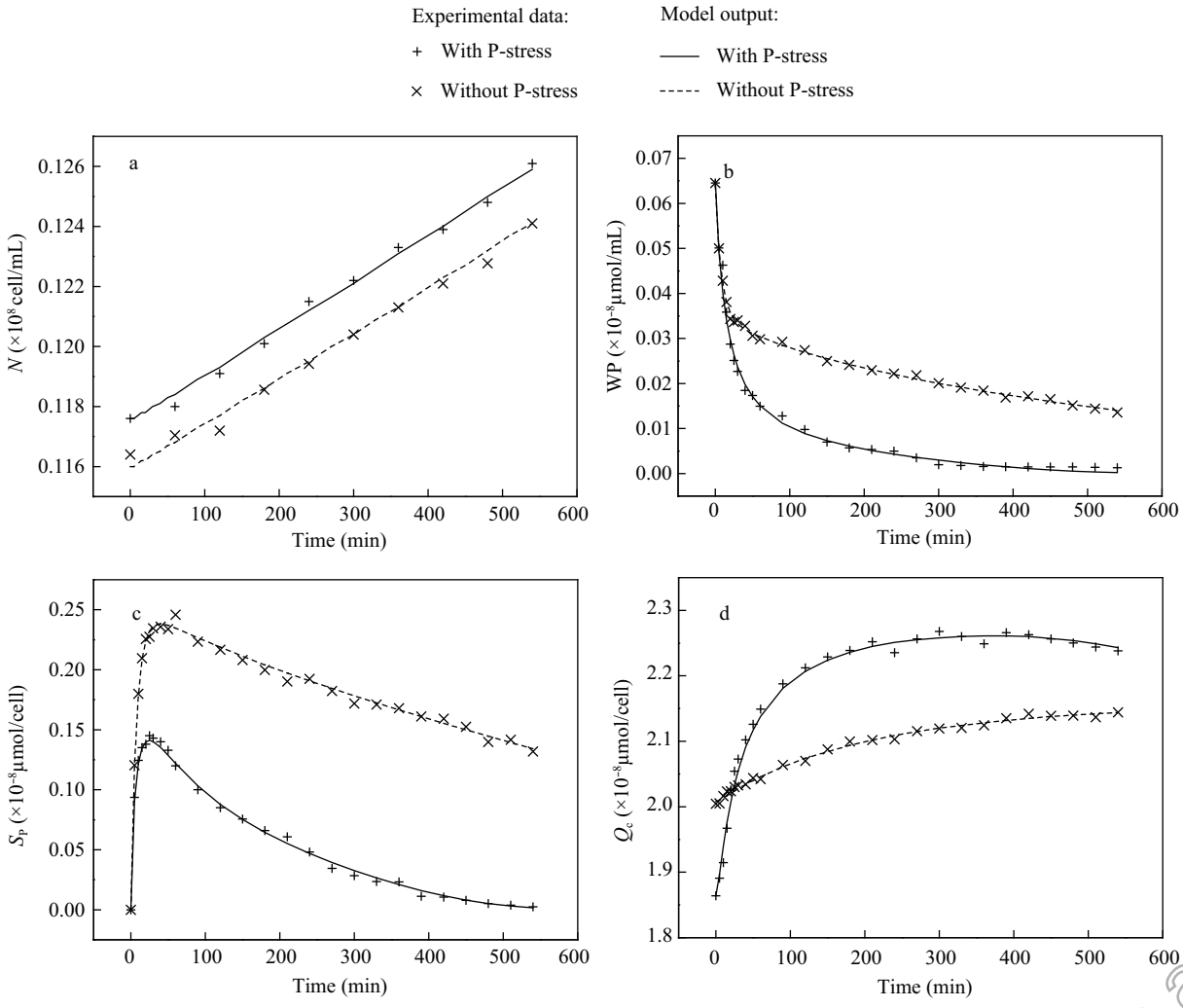

Fig. 3 Comparison of the experimental data and model outputs. (a) N; (b) WP; (c) amount of AP per algal cell S_p; (d) amount of QP per cell (Q_c).

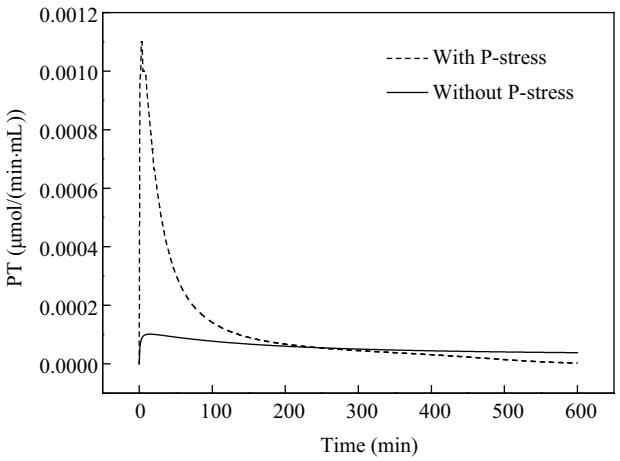

Fig. 4 Modeled phosphate transport rate (PT) of the two comparative groups.

P-stress maintained a relatively stable low uptake rate during the whole experimental period. This phenomenon agreed with the experimental data and previous studies (Parslow, 1984; Litchman and Nguyen, 2008).

3 Discussion

Conventional phosphate uptake models commonly used in algae only consider the relationship between external (substrate) phosphate concentration and total cell quota, ignoring the adsorption of phosphate on algal surfaces. Phosphate uptake is thus often considered a one-stage kinetics process. For the convenience of comparison, an uptake model based on Michaelis-Menten equation and feedback control of cell quota similar to Eq. (6) was used, in which phosphate transport could be described as:

$$T = K_{\mathrm{p}} \times Q_{\max} \times \mu_{\max} \times \frac{\mathrm{WP}}{\mathrm{WP} + K_{\mathrm{m}}} \times \frac{(1 - Q_t/Q_{\max})^4}{(1 - Q_t/Q_{\max})^4 + K_{\mathrm{q}}} \tag{8}$$

where, K_{m} (μmol/mL) is half-saturation constant for the substrate concentration at that P transport rate attains half of its maximum; Q_t (10^{-8}μmol/cell) is the total cell quota including surface-adsorbed phosphate and internal phosphorus content.

The one-stage phosphate uptake model was also implemented with VESIM, used to fit the experimental data (model constants are listed in Table 3), and compared with the two-stage model given by this paper (Fig. 5). Results suggested that the conventional one-stage phosphate uptake model without consideration of cell surface-adsorbed phosphate also had a good fit to the experimental data, although it could not describe some details as good as the two-stage model does.

With careful comparison of the constant values of the two models (Table 3), however, the two-stage phosphate uptake model was better able to explain the real phosphate uptake process by algal cells both stressed and not stressed by P, and its parameters also had more reasonable meaning. The parameter K_{p} represented the impact of P-starvation on phosphate uptake rate, with a larger value denoting heavier P-stress. In the two-stage uptake model, the K_{p} value of the P-stressed group was six times that of the group without P-stress, which was in accordance with the experimental data (Table 3 and Fig. 3) and previous reports (Parslow, 1984; Litchman and Nguyen, 2008). In the one-stage uptake model, however, K_{p} did not appear to represent P-stress, as the group not stressed by P had a much larger K_{p} value than the group stressed by P (Table 3). Here, the larger value of K_{p} was mainly used to fit the rapid decrease of phosphate concentration in the substrate soon after P was added, because algal cells not stressed by P had a relatively larger initial cell quota and lower initial uptake rate (a feedback function F_{q} is used), and as a result a larger value of K_{p} should be used to give a larger P transport rate (Fig. 5e and f). According to the experimental data, however, this was unrealistic as the rapid decrease of substrate concentration was mainly caused by surface adsorption instead of internal uptake, and nearly one third of phosphate added still remained adsorbed on algal cell surfaces in the group not stressed by P. A larger initial P transport rate of the algal cells not stressed by P is unreasonable and does not agree with previous reports (Goldman and Glibert, 1982; Lehman and Sangren, 1982; Riegman and Mur, 1984; Parslow, 1984; Litchman and Nguyen, 2008).

It should be noted that, unlike previous studies, the two groups of algal cells were both washed by the oxalic acid reagent before the P-uptake experiment was performed, which may explain why the one-stage model could not give reasonable explanation to the uptake process. However, we did not pull down the one-stage model (or similar model) which is common and successful in many ecological models, as we can see that the two models did not have notable differences in fitting the experimental data (WP and Q_t) mathematically. Instead, we emphasized the importance of surface-adsorption in the phosphate uptake process, and partitioning of the surface-adsorbed and intracellular P pools was theoretically and practically reasonable. The combination of the two pools may have lead to an over-estimation of uptake capacity of phosphate in algae, especially those with large surface-adsorption capacities, as well as misinterpretation of some ecological observations (Wilhelmy, 2004).

4 Conclusions

Phosphate uptake by algae is a process determined by both environmental conditions and cell state with complex reactions and feedbacks, and its modeling is often paradoxical and controversial. Although large numbers of related studies have been done before, our knowledge on phosphate uptake in phytoplankton is still limited. The commonly used one-stage kinetic uptake models satisfied most of our requirements. Detecting cell surface-adsorbed phosphate in some algal species may be meaningful, however, and indicates that phosphate uptake by algae may be a two-stage uptake kinetics process that includes surface adsorption and internal uptake. Surface adsorption of phosphate may play an important role in the phosphate

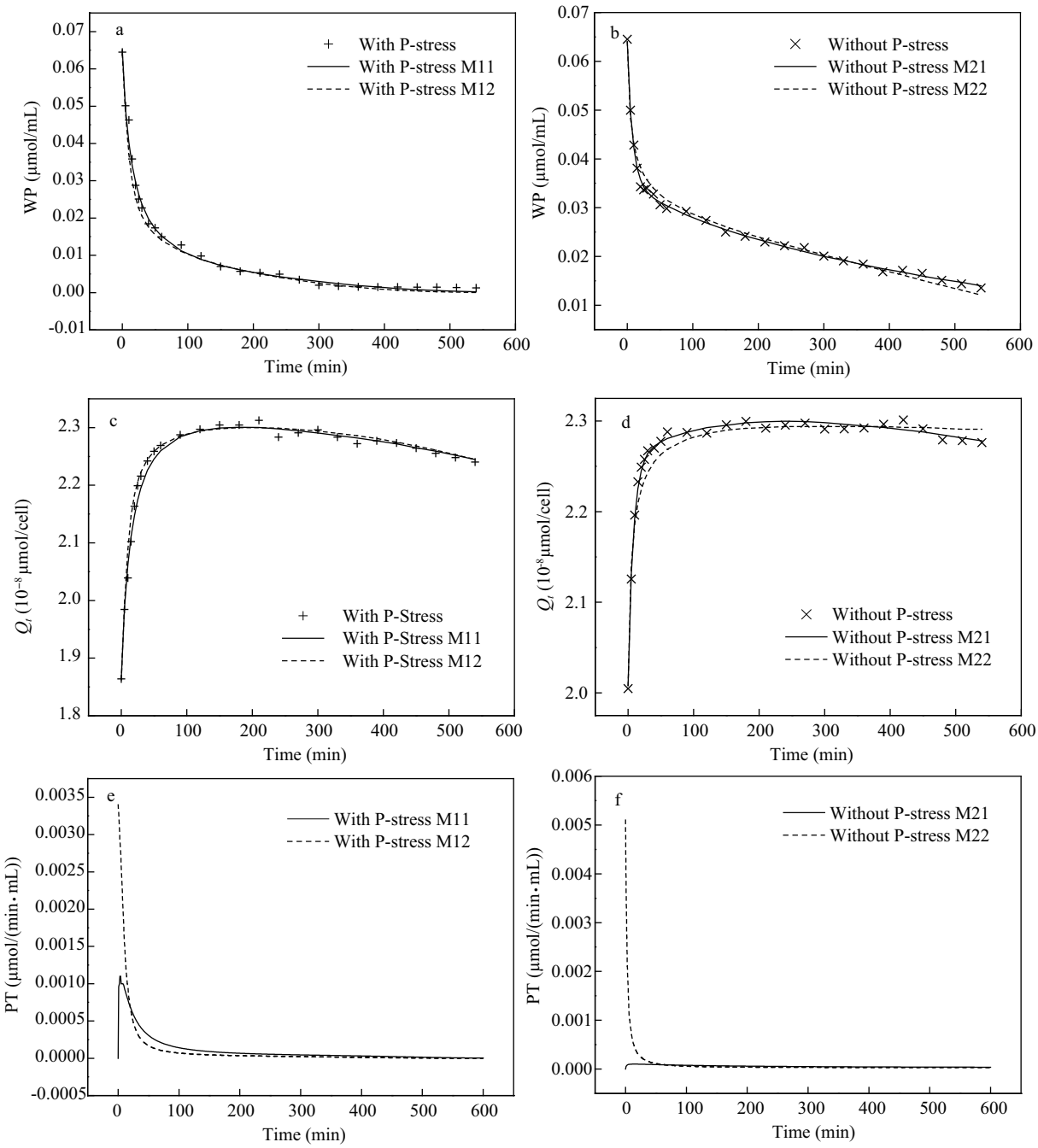

Fig. 5 Comparison of the two-stage uptake model to the conventional model without consideration of surface adsorption. WP concentration of the P-stressed group (a) and without P-stress group (b); Q_t of the P-stressed group (c) and without P-stress group (d); P transport rate (PT) of the P-stressed group (e) and without P-stress group (f). The scattered points (expressed by plus or multiply signs) represent experimental results, the real lines represent the two-stage uptake model outputs (M11 and M21), and the dotted lines refer to the conventional model outputs (M12 and M22). The total cell quota (Q_t) equals the surface-adsorbed phosphate (S_p) plus the internal phosphorus content (Q_c).

uptake process as it is reversible via equilibrium between substrate and surface adsorbed phosphate. Therefore, variations in substrate concentration and/or environmental conditions may shift the equilibrium direction and affect subsequent internal uptake. Directly relating external phosphate concentration to total cell quota and ignoring the role of surface adsorption may lead to misunderstanding of observed phenomenon, especially for algal species which have large surface adsorption capacities.

This article constructed a two-stage uptake model considering surface adsorption and P-stress, based on two groups of short-term phosphate uptake experiments, and compared it to a conventional one-stage uptake model. Results suggested that the two-stage model may better explain the experimental data than the one-stage model, both mathematically and realistically. With suitable parameters, the two-stage model fit the surface adsorption and internal uptake process well, and gave a realistic and reasonable explanation to P-stress. However, good fitness does not mean a perfect model, as we make efforts to construct models based on experimental data to explain physical and/or physiological phenomenon observed, instead of playing mathematical games. As little is known about phosphate adsorption on algal cell surfaces, the

reasonability and reality of the two-stage uptake model still require further validation, such as how environmental conditions (e.g. temperature, light intensity, pH) and cell surface properties (e.g. cell size, density of sorption sites) affect surface adsorption and desorption processes. A mechanism model could be established if enough detailed data were available and a multi-compartment cell quota model could be developed to better interpret the phosphate uptake process.

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References

Aksnes D L, Egge J K, 1991. A theoretical model for nutrient uptake in phytoplankton. *Marine Ecology Progress Series*, 70: 65–72.

Armstrong R A, 2008. Nutrient uptake rate as a function of cell size and surface transporter density: A Michaelis-like approximation to the model of Pasciak and Gavis. *Deep-sea Research Part I: Oceanographic Research Papers*, 55: 1311–1317.

Button D K, 1978. On the theory of control of microbial growth kinetics by limiting nutrient concentration. *Deep Sea Research*, 25: 1163–1177.

Cembella A D, Antia N J, Harrison P J, 1984. The utilization of inorganic and organic phosphorus compounds as nutrients by eukaryotic microalgae: A multidisciplinary perspective. Part 2. *Critical Reviews in Microbiology*, 11: 13–81.

Conway H L, Harrison P J, Davis C O, 1976. Marine diatoms grown in chemostats under silicate or ammonium limitation. II. Transient response of *Skeletonema costatum* to a single addition of the limiting nutrient. *Marine Biology*, 35: 187–199.

Droop M R, 1973. Some thoughts on nutrient limitation in algae. *Journal of Phycology*, 9: 264–272.

Droop M R, 1983. 25 years of algal growth kinetics: Personal view. *Botanica Marina*, 26: 99–112.

Elert E V, 1997. Phosphorus limitation and not light controls the extracellular release allelopathic compounds by *Trichormus dolioulum* (Cyanobacteria). *Limnology and Oceanography*, 42: 1796–1802.

Falkowski P J, 1997. Evolution of the nitrogen cycle and its influence on the biological sequestration of CO_2 in the ocean. *Nature*, 387: 272–275.

Fistarol G O, Legrand C, Granéli E, 2005. Allelopathic effect on a nutrient-limited phytoplankton species. *Aquatic Microbial Ecology*, 41: 153–161.

Flynn K J, Fasham M J R, Hipkin C R, 1997. Modelling the interactions between ammonium and nitrate uptake in marine phytoplankton. *Philosophical Transactions of the Royal Society of London Series B – Biological Sciences*, 352: 1625–1645.

Flynn K J, 2003. Modelling multi-nutrient interactions in phytoplankton; balancing simplicity and realism. *Progress in Oceanography*, 56: 249–279.

Goldman J C, Taylor C D, Gilbert P M, 1981. Nonlinear time-course uptake of carbon and ammonium by marine phytoplankton. *Marine Ecology Progress Series*, 6: 137–148.

Goldman J C, Glibert P M, 1982. Comparative rapid ammonium uptake by four species of marine phytoplankton. *Limnology and Oceanography*, 27: 814–827.

Gotham I J, Rhee G Y, 1981. Comparative kinetic studies of phosphate-limited growth and phosphate uptake in phytoplankton in continuous culture. *Journal of Phycology*, 17: 257–265.

Granéli E, Johansson N, 2003. Increase in the production of allelopathic substances by *Prymnesium parvum* cells grown under N- or P-deficient conditions. *Harmful Algae*, 2: 135–145.

Gurkan Z, Zhang J J, JΦrgensen S E, 2006. Development of a structurally dynamic model for forecasting the effects of restoration of Lake Fure, Denmark. *Ecological Modelling*, 197: 89–102.

Harrison P J, Parslow J S, Conway H L, 1989. Determination of nutrient uptake kinetic parameters: a comparison of methods. *Marine Ecology Progress Series*, 52: 301–312.

Healey F P, 1980. Slope of Monod equation as an indicator of advantage in nutrient competition. *Microbial Ecology*, 5: 281–286.

John E H, Flynn K J, 2000a. Growth dynamics and toxicity of *Alexandrium fundyense* (Dinophyceae): The effect of changing N:P supply ratios on internal toxin and nutrient levels. *European Journal of Phycology*, 35: 11–23.

John E H, Flynn K J, 2000b. Modeling phosphate transport and assimilation in microalgae: How much complexity is warranted? *Ecological Modelling*, 125: 145–157.

JΦrgensen S E, Ray S, Berec L, 2002. Improved calibration of an eutrophication model by use of the size variation due to succession. *Ecological Modelling*, 153: 269–277.

Khoshmanesh A, Lawson F, Pricne I G, 1997. Cell surface area as a major parameter in the uptake of cadmium by unicellular green microalgae. *Chemical Engineering Journal and the Biochemical Engineering Journal*, 65: 13–19.

Knauer K, Behra R, Sigg L, 1997. Adsorption and uptake of copper by the green alga *Scenedesmus subspicatus* (Chlorophyta). *Journal of Phycology*, 33: 596–601.

Lehman J T, Sandgren C D, 1982. Phosphorus dynamics of the procaryotic nanoplankton in a Michigan lake. *Limnology and Oceanography*, 27: 828–838.

Litchman E, Nguyen B L V, 2008. Alkaline phosphatase activity as a function of internal phosphorus concentration in freshwater phytoplankton. *Journal of Phycology*, 44: 1379–1383.

Liu X A, Ran Y, Luo Y F, 2007. Multi-layer model for algal growth under different behaviors of nutritious salt. *Environmental Science*, 28: 2163–2168.

Morel F M M, 1987. Kinetics of nutrient uptake and growth in phytoplankton. *Journal of Phycology*, 23: 137–150.

Parslow J S, Harrison P J, Thompson P A, 1984. Use of a self-cleaning in-line filter to continuously monitor phytoplankton nutrient uptake rates. *Canadian Journal of Fisheries and Aquatic Sciences*, 41: 540–544.

Pasciak W J, Gavis J, 1974. Transport limitation of nutrient uptake in phytoplankton. *Limnology and Oceanography*, 19: 881–888.

Pasciak W J, Gavis J, 1975. Transport limited nutrient uptake rates in Ditylum brightwellii. *Limnology and Oceanography*, 20: 605–617.

Rezaee A, Ramavandi B, Ganati F, 2006. Equilibrium and spectroscopic studies on biosorption of mercury by algae biomass. *Pakistan Journal of Biological Sciences*, 9: 777–782.

Rhee G Y, 1973. A continuous culture study of phosphate uptake, growth rate and polyphosphate in *Scenedesmus* sp. *Journal of Phycology*, 9: 495–506.

Rhee G Y, Thompson P A, 2004. Sorption of hydrophobic organic contaminants and trace metals on phytoplankton and implications for toxicity assessment. *Journal of Aquatic Ecosystem Stress and Recovery*, 1: 1386–1980.

Riegman R, Mur L R, 1984. Regulation of phosphate uptake kinetics in *Oscillatoria agardhii*. *Archives of Microbiology*, 139: 28–32.

Rivkin R B, Swift E, 1985. Phosphorus metabolism of oceanic dinoflagellates: Phosphate uptake, chemical composition and growth of *Pyrocystis noctiluca*. *Marine Biology*, 88: 18–198.

Roelke D L, Eldridge P M, Cifuentes L A, 1999. A model of phytoplankton competition for limiting and nonlimiting nutrients: implications for development of estuarine and nearshore management schemes. *Estuaries*, 18: 92–104.

Sanchez A T, Wilhelmy S A S, Vargas M G, Weaver R S, Popels L C, Hutchins D A, 2003. A trace metal clean reagent to remove surface-bound iron from marine phytoplankton. *Marine Chemistry*, 82: 91–99.

Syrett P J, 1956. The assimilation of ammonia and nitrate by nitrogen-starved cells of *Chlorella vulgaris*. II. The assimilation of large quantities of nitrogen. *Physiologia Plantarum*, 9: 19–27.

Tien C J, Sigee D C, White K N, 2005. Copper adsorption kinetics of cultured algal cells and freshwater phytoplankton with emphasis on cell surface characteristics. *Journal of Applied Phycology*, 17: 379–389.

Wilhelmy S A S, Sanchez A T, Fu F X, Capone D G, Carpenter E J, Hutchins D A, 2004. The impact of surface-adsorbed phosphorus on phytoplankton Redfield stoichiometry. *Nature*, 432: 897–901.