Biochemical Society Transactions (2018) **46** 483–490
https://doi.org/10.1042/BST20170262

Review Article

Modelling phosphorus uptake in microalgae

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Phosphorus (P) is an essential non-renewable nutrient that frequently limits plant growth. It is the foundation of modern agriculture and, to a large extent, demand for P is met from phosphate rock deposits which are limited and becoming increasingly scarce. Adding an extra stroke to this already desolate picture is the fact that a high percentage of P, through agricultural runoff and waste, makes its way into rivers and oceans leading to eutrophication and collapse of ecosystems. Therefore, there is a critical need to practise P recovery from waste and establish a circular economy applicable to P resources. The potential of microalgae to uptake large quantities of P and use of this P enriched algal biomass as biofertiliser has been regarded as a promising way to redirect P from wastewater to the field. This also makes the study of molecular mechanisms underlying P uptake and storage in microalgae of great interest. In the present paper, we review phosphate models, which express the growth rate as a function of intra- and extracellular phosphorus content for better understanding of phosphate uptake and dynamics of phosphate pools.

Introduction

Phosphorus (P) is a finite non-renewable resource essential for food production. It is required for the synthesis of key biological components such as nucleic acids, phospholipids and ATP, and is the second most frequent nutrient that limits the plant growth after nitrogen. Although, in early years of agriculture, manure, human excreta, and guano were used as phosphorus source, phosphate rock is the only economically viable source in modern era [1]. In the year 2000, it was estimated that ∼80% of total P extracted (19 MT) from P rock was utilised for the production of fertilisers. However, the efficiency of P usage hardly reaches 20% with the rest either ending in waste water or being carried away by runoff from fields to rivers and oceans [2], which not only decreases the water quality but also disturbs ecosystems through eutrophication [3,4]. Moreover, with the increasing global food demand and the utilisation of fertiliser to meet this demand, it is highly likely that the minable P rock will be exhausted in near future and as stated by prominent chemist Isaac Asimov [5]: '*We may be able to substitute nuclear power for coal, and plastics for wood, and yeast for meat, and friendliness for isolation but for phosphorus there is neither substitute nor replacement*'. Hence, it is utmost necessary to develop strategies that allow the recovery of P from waste to be reused in agriculture.

The techniques of P recovery from wastewater can be broadly classified into two groups: chemical phosphorus removal (CPR) and biological phosphorus removal (BPR). CPR involves the precipitation of P, using metals such as Fe and Al, which is then separated through the process of filtration. This process can reduce the effluent total P to <2.0 mg/l which is difficult to achieve through BPR [6]. Therefore, CPR is widely used by industries for wastewater treatment; however, the products are not readily bioavailable and recycling of P is economically expensive [7]. BPR involves absorption of phosphorus by phosphate-accumulating organisms (PAOs), micro-organisms with the ability to store P, which can be further used for biofertilisers or other purposes such as biofuel production [7]. BPR system usually consists of both PAOs and ordinary organisms that are not capable of storing P. Thus, the challenge remains to increase the growth of PAOs in order to enhance the percentage of P

Received: 12 November 2017
Revised: 1 February 2018
Accepted: 16 February 2018

Version of Record published:
17 April 2018

Biochemical Society Transactions (2018) 46 483–490
https://doi.org/10.1042/BST20170262

removal. Enhanced biological phosphorus removal (EBPR) targets the selective enrichment of P-accumulating bacteria through the sequence of anaerobic and aerobic culture conditions. A detailed explanation on EBPR is beyond the scope of this paper, and interested readers are referred to Comeau et al. [8], Smolders et al. [9], Oehmen et al. [10], Wentzel et al. [11], and Nielsen et al. [12].

For example, a tropical cyanobacterium *Phormidium bohneri* was considered as a potential candidate for wastewater treatment due to its high biomass production, along with high rates of ammonium and phosphate removal [13]. Other bacteria have also been exploited for their capability to absorb and accumulate P. For instance, *Staphylococcus auricularis* has been successfully used to remove more than 90% of P [14]. Even the use of photosynthetic organisms, such as unicellular microalgae, for wastewater treatment has a long history. *Chlorella vulgaris* and *Scenedesmus dimorphus* have been reported to be capable of removing up to 55% of the phosphates from dairy industry and pig-farming wastewater [15], while another strain of *Scenedesmus* was able to remove more than 50% of the phosphates from artificial wastewater [16]. The ability of microalgal cells to accumulate large amount of P, which can reach up to 2–3% of cell dry weight [17], makes them a potential candidate for P uptake from wastewater.

Phosphate uptake and storage in microalgae

Algal growth is supported by the transport of soluble inorganic phosphate (SIP)/orthophosphate (ortho-P), consisting of single phosphate residue, across the cell membrane. For many years, mainly SIP was thought to be the only significant biologically available form of phosphate because its transport mechanism was well known. However, utilisation of other organic forms such as cyclic-AMP, phospholipids and pyrophosphate has been demonstrated in microalgae. The uptake of the latter forms is facilitated through enzymatic hydrolysis of exogenous organic phosphate compounds, which has been reviewed in refs [18,19].

The process of algal phosphate uptake is influenced by environmental factors and cell state such as light, temperature, pH, salinity, starvation and growth [17,18,20–24]. P uptake is directly influenced by growth which is known as the metabolic uptake. On the contrary, P-starved algal cells when exposed to P-rich medium are capable of accumulating P in excess to that required for growth. This mechanism is also known as 'overshoot' or starvation uptake. In addition, excess P uptake is observed even in algal cells without P starvation when exposed to P-sufficient environment, known as 'luxury' or storage uptake. In the latter two mechanisms, P uptake can be much faster than what is required for immediate growth, uncoupling the growth rate from P uptake. The excess of P accumulated is stored often as inorganic polyphosphate (Poly-P). Poly-P is a linear, unbranched polymer of three to several hundreds of SIP residues linked by phosphoanhydride bonds. It serves as internal storage for P and can be utilised when needed [25,26]. Poly-P has also been suggested to play a role in regulating the synthesis of phosphorylated compounds including ATP, and in adjusting intracellular pH homeostasis and osmotic pressure [18,27,28]. In addition to SIP and Poly-P pools, Wolfgang [29] categorised intracellular pools based on their function as 'structural' components required to maintain integrity and viability of cells such as P in DNA and membrane lipids, and 'synthetic' or 'functional' components such as P in RNA and other synthetic machinery which directly determine growth rate.

Growth and uptake models

The classical growth model proposed by Jacques Monod dates back to the 1940s [30]. It was based on the assumption that the microbial growth rate is influenced by the external concentration of a limiting nutrient, in a similar fashion as reaction rates are described by Michaelis–Menten kinetics in the following equation:

$$\mu = \mu_m \cdot \frac{S}{(K_s + S)}, \tag{1}$$

where S is the limiting substrate concentration, μ is the specific growth rate, μ_m is the maximum specific growth rate, and K_s is the half saturation constant for growth.

Monod's growth law holds true for substrates that are metabolised immediately after uptake, such as glucose (carbon source), which was also the growth-limiting substrate in Monod's experiment. However, studies on algal growth on other limiting nutrients such as N, P, Si, or Vitamin B_{12} show that the rate of nutrient uptake can exceed that required for growth based on nutrient availability and cell state as also described above. On the other hand, growth can continue even after the depletion of external growth-limiting nutrients by mobilisation

of storage reservoirs [29,31,32]. Thus, Monod's growth model does not fit well in such scenarios. To take the influence of internal nutrient concentration into account, growth rates were better described as a function of cell quota, the amount of limiting nutrient in the cell per total population. This concept allowed describing growth depending on cell quota, regardless of the external nutrient concentration, and zero growth when cell quota was below the threshold.

Quota model

An early phosphate uptake model was proposed by Droop in 1973 [31]. It is a simple quota model that describes a single internal P pool where growth is the function of total internal P concentration (internal nutrient pool),

$$\mu = \mu' \cdot \frac{(q - q_0)}{q} \text{ if } q > q_0 \text{ else } 0, \tag{2}$$

where μ is the specific growth rate, q is the cell quota (concentration of limiting substrate per cell), q_0 is the minimum cell quota required for the growth and μ' is the specific growth rate when q is infinite. The transport of P into the cell is described as a function of the external concentration in Michaelis–Menten fashion irrespective of the internal situation,

$$u = u_{\mathrm{m}} \cdot \frac{S}{(K_{\mathrm{s}} + S)}, \tag{3}$$

where S is the substrate concentration, u is the specific uptake rate, u_{m} is the specific uptake rate when the substrate concentration is very large, and K_{s} is the saturation constant and is numerically equal to the substrate concentration giving half of the maximal uptake rate. Thus, the internal P pool is utilised during cell growth, but is continuously refilled by the uptake of P from the external environment and at steady state the specific P uptake rate is the product of cell P quota and specific growth rate.

Although Droop's equation overcomes Monod's kinetic limitation by taking into account the internal P concentration, it is not free of limitations. For example, it considers a single internal P pool only. However, as discussed earlier, there can be more than one internal P pool in a cell with distinct influence on the growth. In addition, P uptake in Droop's model is a function of external concentration only and is not influenced by the internal pool size, which is biologically unrealistic.

Phosphate interaction model

John and Flynn [33] developed a mechanistic model of ordinary differential equations (ODE) for P uptake and dynamics known as the phosphate interaction model (PIM) (schematic representation in Figure 1). Contrary to a single internal P pool in Droop's model, it contains multiple internal phosphate pools: SIP, Poly-P and structural and other organic P (SOP). The external inorganic phosphate (P_{ex}) pool provides the phosphate source. SIP represents the internal phosphate pool, which can be converted to Poly-P for storage or SOP to support growth.

Phosphate uptake from the external source follows the Michaelis–Menten kinetics and is controlled by the size of the SIP to halt transport when SIP attains a maximum value. Similarly, the relation between internal phosphate pools is simulated using Michaelis–Menten kinetics and the allosteric regulation using a sigmoidal function. The formation of Poly-P is the function of SIP and is restricted by the maximum capacity of a cell to store Poly-P. The process of Poly-P degradation is a function of Poly-P and restricted by the maximum capacity of SIP. SIP is converted into SOP before it can be utilised for growth. The latter process is subject to kinetic constraints from SIP and is limited by the maximum capacity of SOP. The growth rate is a function of SOP only and Poly-P must be degraded to SIP before incorporation into SOP.

Consideration of multiple internal phosphate pools allows accumulation of more phosphate than that required for immediate use. This enables decoupling of transport from assimilation and hence the maintenance of high transport rates for longer time. This behaviour is beneficial to organisms living in a transient nutrient regime and in environments where they need to compete for P with other organisms. PIM, with a moderate

Biochemical Society Transactions (2018) **46** 483–490
https://doi.org/10.1042/BST20170262

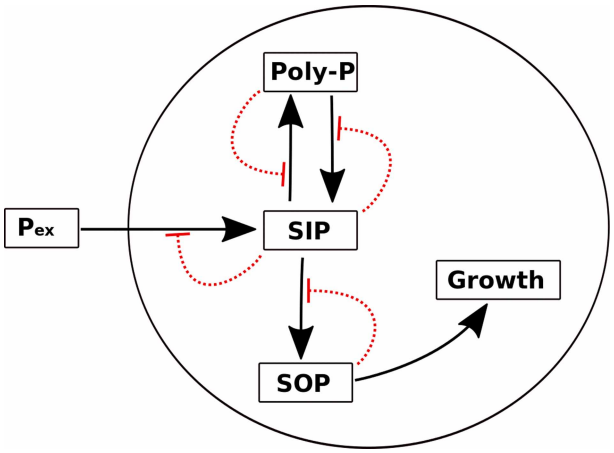

Figure 1. Schematic representation of the PIM.

It consist of an external phosphate pool (P_{ex}) and three internal phosphate pools: SIP, Poly-P and SOP. The process of phosphate uptake and the conversion between different phosphate pools follows Michaelis–Menten kinetics and is regulated by the maximum capacity of the pools, as shown by the red lines with flat head. Growth is a function of SOP only.

level of complexity, seems to be appropriate for studying the dynamics of phosphate uptake and intracellular phosphate pools when compared with Droop's model.

Extended phosphate interaction model

In addition to nutrients, light is a major limiting factor for growth of phototrophic organisms. The local light intensity available to cells varies along the radius/depth of the culture vessel due to various factors such as scattering, reflection and absorption by cells that contribute to light attenuation. As the current focus is to use microalgae for P uptake and light is a vital source of energy for phototrophic growth, we have further extended PIM (schematic representation in Figure 2), referred as 'Extended Phosphate Interaction Model' (ExPIM), to

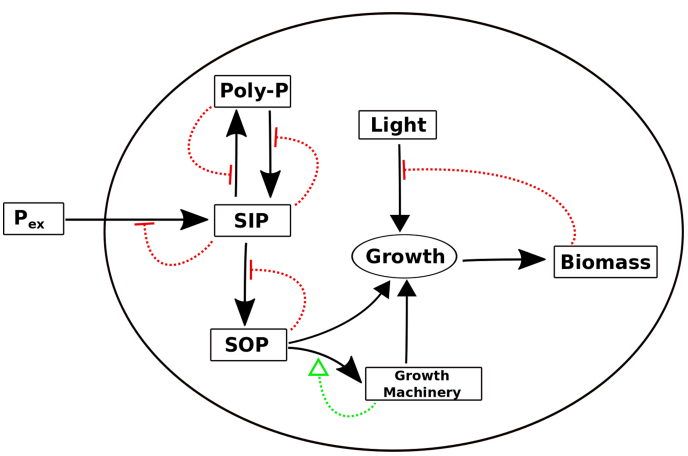

Figure 2. Schematic representation of the ExPIM.

The extended phosphate interaction model (ExPIM) consists of an external phosphate pool (P_{ex}) and three internal P pools: SIP, Poly-P and SOP. Alike PIM, the process of phosphate uptake and the conversion between different phosphate pools follow Michaelis–Menten kinetics and are regulated by the maximum capacity of the pools, as shown by the red lines with flat head. Growth machinery is a function of SOP and is positively regulated by itself, represented by the green line with arrow head. Biomass is determined by the growth rate (represented by the term 'Growth' in oval box), which is a function of SOP, growth machinery and light intensity available after attenuation.

account for the influence of light on growth and P uptake mechanism. ExPIM also takes into account the effect of starvation period on cell growth. Proteins, RNA and other cell components, required for cell growth/division, are degraded during the starvation period and need to be re-established to support growth. In the model, these growth components, which we will call 'growth machinery', are modelled as a function of SOP and need to build up to support growth.

ExPIM consists of four phosphate pools alike PIM: the external inorganic phosphate pool (P_{ex}) and three internal algal phosphate pools: SIP, Poly-P and SOP. The model takes into account light attenuation due to increasing biomass concentration and scattering and ultimately the influence of available light on algal growth. Light attenuation is assumed to obey Beer-Lambert law:

$$I_z = I_{ex} \cdot e^{-a \cdot z}, \tag{4}$$

where I_z is the light intensity at distance z from the light source, a is the extinction coefficient which accounts for light absorption by algae and scattering and I_{ex} is the external light intensity. The influence of light on algal growth rate is modelled after Dauta et al. [34],

$$\mu = \mu_m \cdot 2 \cdot (1 + \beta) \cdot \frac{I_r}{(I_r^2 + 2 \cdot \beta \cdot I_r + 1)}, \tag{5}$$

where $I_r = I/I_{opt}$, I is the light intensity experienced by a cell, I_{opt} is the optimal light intensity, β is the attenuation coefficient, μ is the specific growth rate and μ_m is the maximum specific growth rate. The synthesis of growth machinery is modelled as Michaelis–Menten kinetics which is limited by its maximum capacity and promoted by itself. Ultimately, growth rate is a function of not only SOP, as in PIM, but also of available light intensity and growth machinery, as shown in Figure 2.

ExPIM has been simulated for conditions where P-starved *C. vulgaris* was resuspended into phosphate-rich medium. Figure 3 shows the simulated growth rate and phosphate uptake, together with experimental data, for P-starved cells grown in P-rich medium for 96 h. The model is able to simulate growth rate including the characteristic lag phase at the beginning of algal growth. The uptake of external phosphate into algal cells is predicted by the model to be initially also delayed, which contradicts experimental data that indicate the well-known rapid 'overshoot' uptake by the starved cells described above. In this, the ExPIM model must be further improved to reflect not only the luxury uptake but also the overshoot uptake by the starved cells. The luxury uptake is responsible for the decline of P concentration in the medium shown in the right panel of Figure 3. Difference between the simulation and experimental data for growth is visible also during the stationary phase. To address this, future experiments must provide data directly on algal biomass rather than use optical density (OD) as optical proxy. As shown in Figure 3, cells enter stationary phase with time even though

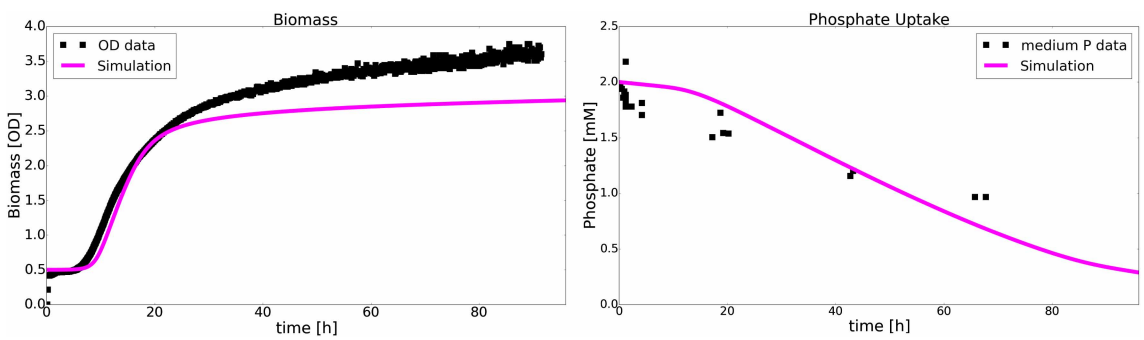

Figure 3. Simulations and experimental data for growth and P uptake of *Chlorella vulgaris*.

Shown are experimental data and simulation results for *C. vulgaris* biomass approximated by OD (left) and for phosphate concentration in the medium (right). At the time origin $t = 0$, P-starved *C. vulgaris* was resuspended in phosphate-rich medium. Experimental measurements are shown by dots, while simulation results are shown by solid line. In the experimental and the model simulation, cells enter the stationary phase, despite the availability of P in the medium, due to light limitation.

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https://doi.org/10.1042/BST20170262

phosphate is available in the medium. This is attributed to light limitation due to increased cell density. The model further suggests storage of phosphate as Poly-P during the non-growth phase (lag phase) which is later utilised for growth. The strength of the model is its ability to make predictions, e.g. about the levels of the different internal P pools, which are difficult to access experimentally. Model formulation, a simulation script and the experimental data, presented here, are available from https://gitlab.com/singhdi/ExPIM_BST2018

Conclusions

The phosphate uptake mechanism, consisting of many reactions and feedback loops, is a complex process influenced by environmental conditions and cell status. Although a large number of studies exist, knowledge on this topic is rather sparse. In such a scenario, the use of mathematical models allows to shed light on the process, test existing hypotheses, make predictions and develop new hypotheses in a cost and time effective manner.

Here, we have discussed many phosphate models: the conventional phosphate uptake model, which considers a single internal pool, to more complex phosphate models, that consider multiple internal phosphate pools. Though these models vary in complexity, they are all designed based on certain assumptions to represent the behaviour of a system. The choice of the complexity level depends on the issue under investigation and the efficacy of the model to test/challenge experimental data. For instance, the quota model may be an appropriate choice if the intension is to investigate the relation between total P and growth. The validation of this model would require a rather simple experimental procedure to measure total cell P. On the contrary, to understand the dynamics of internal phosphate pools, it is necessary to study a complex model such as PIM. The evaluation of this model requires measuring different phosphate pools, which might be experimentally challenging, however necessary, if we intend to validate the model and further challenge it to make novel predictions. PIM, described in Figure 1, assumes a single Poly-P pool. However, there exist at least four known Poly-P pools, termed A, B, C and D [35]. Acid-soluble polyphosphates (A and C) are readily available for DNA and protein synthesis, while acid-insoluble polyphosphates (B and D) serve as storage. Taking this into consideration, four distinct Poly-P pools could be considered in the model where acid-soluble pools would directly contribute to SOP, while acid-insoluble pools must be converted to SIP before incorporation to SOP, as was described in John and Flynn [33]. Furthermore, the SOP pool could also be split into structural and an energetic pool to further represent the complexity of P pools. However, regardless of single or multiple Poly-P/SOP pool, PIM fails to explain the influence of light and/or starvation phases on the system. Here, we have further extended PIM to account for the light limitation and the effect of starvation on growth components. ExPIM with a moderate level of complexity can be regarded appropriate to simulate phototropic growth and starvation uptake.

Models discussed in this review, so far, are mechanistic models, which focus on P uptake, growth rate and dynamics of internal storage. Recently, Vijay and Yuan [36] derived an empirical model of P removal via assimilation from lagoons by observing data over 1 year. This model shows a good fit to the experimental data and has the potential to be developed into a predictive tool. However, because it relies on purely heuristic equations, it cannot provide any information about regulatory mechanism or the dynamics of internal P pools. All the models discussed here, based on different assumptions and of varying complexity, have contributed to understand the phosphate uptake mechanism. Nevertheless, throughout the long history of applying mathematical models to understand biological systems, selecting the appropriate level of complexity has always been a critical and heavily debated problem. In conclusion, the simplifications entering the model assumptions, which thus determine the complexity of the model, should be carefully chosen such that available data can be optimally exploited to address the scientific questions which motivated model development in the first place.

Abbreviations

BPR, biological phosphorus removal; CPR, chemical phosphorus removal; EBPR, enhanced biological phosphorus removal; ExPIM, extended phosphate interaction model; P, phosphorus; PAOs, phosphate-accumulating organisms; PIM, phosphate interaction model; Poly-P, polyphosphate; SIP, soluble inorganic phosphate; SOP, structural and other organic phosphate; μ, specific growth rates; μ_m, maximum specific growth rates; S, substrate concentration; K_s, half saturation constant; q_0, minimum cell quota required for the growth; μ', specific growth rate when q is infinite; u, specific uptake rate; u_m, specific uptake rate when the substrate concentration is very large; I_{ex}, external light intensity; I, light intensity experienced by a cell; a, extinction coefficient; z, path length travelled by light; β, attenuation coefficient; I_{opt}, optimal light intensity; OD, optical density.

Acknowledgement

The authors acknowledge financial support through the BioSC AlgalFertilizer project funded by the Ministry of Innovation, Science and Research of the German State of North Rhine-Westphalia.

Competing Interests

The Authors declare that there are no competing interests associated with the manuscript.

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