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Review

Polyphosphate accumulation in microalgae and cyanobacteria: recent advances and opportunities for phosphorus upcycling

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Phosphorus (P) must continuously be added to soils as it is lost in the food chain and via leaching. Unfortunately, the mining and import of P to produce fertiliser is unsustainable and costly. Potential solutions to the global issues of P rock depletion and pollution lie in microalgae and cyanobacteria. With an ability to intracellularly store P as polyphosphates, microalgae and cyanobacteria could provide the basis for removing P from water streams, thereby mitigating eutrophication, and even enabling P recovery as P-rich biomass. Metabolic engineering or changes in growing conditions have been demonstrated to improve P removal and recovery by triggering polyphosphates synthesis in the laboratory. This now needs to be replicated at full scale.

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Introduction

Phosphorus (P) is essential for plant growth and, consequently, becomes rapidly depleted from soil during intensive farming, as it is removed with the harvested plant biomass and lost during runoff. Adding P fertiliser is therefore indispensable to sustain land-based food production, and this use accounts for 90% of the global demand for P [1]. Considering the fast depletion of P reserves, the financial cost of extracting P rock, and the environmental issue related to P losses or P discharge [1,2], it is critical to develop solutions to recover P from aquatic bodies [3,4]. P recovery from domestic wastewater could in theory satisfy 15–20% of the world demand for phosphate rock [5].

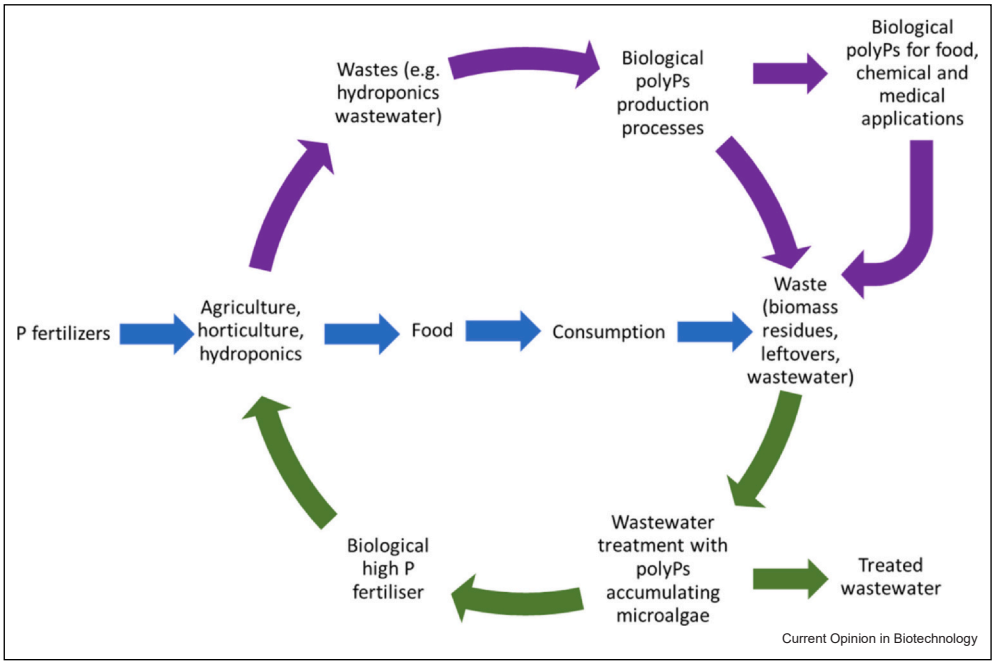
Microalgae and cyanobacteria can accumulate P as polyphosphates (polyPs) under certain conditions [6]. Advances in this field have the potential to improve wastewater treatment and subsequent recovery of P [4,7,8] and may also lead to processes that extract and utilise the biologically produced polyPs [9,10] as summarised in Figure 1.

Microalgal polyP research has considerably increased in the last decade, either to determine the critical functions of polyPs in cells or to develop biotechnologies for P removal and P upcycling as polyPs have industrial applications [4,6,11]. Here, we summarise the advances in the biological mechanisms leading to P accumulation in microalgae and how this knowledge, as well as prior research, is leading the way to the development of processes for P upcycling using microalgae. We discuss P reuse and provide an overview of the future for microalgal P upcycling biotechnology.

Molecular pathways

The accumulation of polyPs in microalgae and cyanobacteria can occur when the cells experience a change in the external level of P (i.e. from P depleted to repleted conditions) and/or a lack of another key nutrient (e.g. sulphur) [4]. The responses to P depletion (also often referred to as P starvation) have been well characterised in both prokaryotes and eukaryotes, and while involving different genes, the overall responses are similar. Controlled by transcription factors (the two component SphS-SphR in cyanobacteria and the phosphate starvation response 1 in eukaryotes [11–13]), genes encoding phosphatases, high-affinity P transporters and enzymes involved in the swapping of phospholipids by sulfolipids are upregulated during P depletion [14,15]. In addition, this condition upregulates the genes encoding for the proteins catalysing inorganic polyphosphate synthesis, the vacuolar transport chaperones complex (VTC) in eukaryotes [6,14,15]. In contrast to the prokaryotic polyphosphate kinases (PPKs), the function of VTCs as polyP polymerase was only demonstrated in the last

Figure 1



Current practices in P utilisation (shown in blue) alongside development of wastewater treatment processes to product high P fertiliser (shown in green) and high-value biological polyP production (shown in purple). Shilton et al. [8] provide some details on relative amounts of P that could be recovered.

decade in microalgae. The polyP polymerase activity of the microalgal VTC4 has been demonstrated with bioassays involving repressed mutants and *in silico* [14,16,17]. While the complete structure of the microalgal VTC complex is currently unknown, the study of microalgal VTC4 models showed high conservation between yeast and algae including for the binding of the inositol phosphate InsP6 to the SPX domain of the protein. This suggests that the activation of polyP synthesis is similar in microalgae and yeast [17]. Further work is needed to confirm this finding and to determine if differences in VTC structures (and assembly) could explain the differences in polyP accumulation abilities reported among microalgae species [17]. This knowledge would be critical to identify the 'champion' microalgae to use for biotechnology and would complement bioprospecting from field studies [18,19].

The identification of the P-related genes and specifically the ones involved in P storage has been instrumental for metabolic engineering to improve polyPs accumulation in both microalgae and cyanobacteria. This was done by Wang et al. [11] and Slocombe et al. [13]. For instance, Wang et al. [11] created lines overexpressing the phosphate starvation response 1 (encoding a global transcriptional regulator of P-deficiency responses) and lacking ptc1 (encoding a protein involved in P excretion from the vacuoles where polyPs are stored), leading to the overaccumulation of polyPs in *Chlamydomonas reinhardtii* from 2% in the parental strain to 7% in the mutant. Transformation of Synechococcus expressing ppk genes also led to an increase of polyP accumulation in this cyanobacterium [20]. Alternatively, the down-regulation of the negative regulatory element of the phosphate regulon also led to increasing polyP accumulation in Synechocystis sp. strain PCC6803 by decreasing polyP degradation [21]. While demonstrated in the laboratory, the use of genetically modified organisms (GMOs) for P removal by triggering polyP synthesis has never been demonstrated at relevant scale outdoors. Importantly, certain countries have stringent regulations regarding the use of GMOs and non-native species (e.g. New Zealand). Therefore, for the case of wastewater treatment, simply triggering P accumulation via process design, as further described below, may be the most suitable approach.

Processes for microalgal phosphorus upcycling

Mixed cultures of microalgae from wastewater treatment processes have been shown to contain 0.1–3.85% P [18,22,23]. With an enhanced understanding of P accumulation in microalgae, there is the potential to engineer new P removal process or modify existing ones [24,25].

Table 1

Key variables influencing the P content of microalgal biomass.

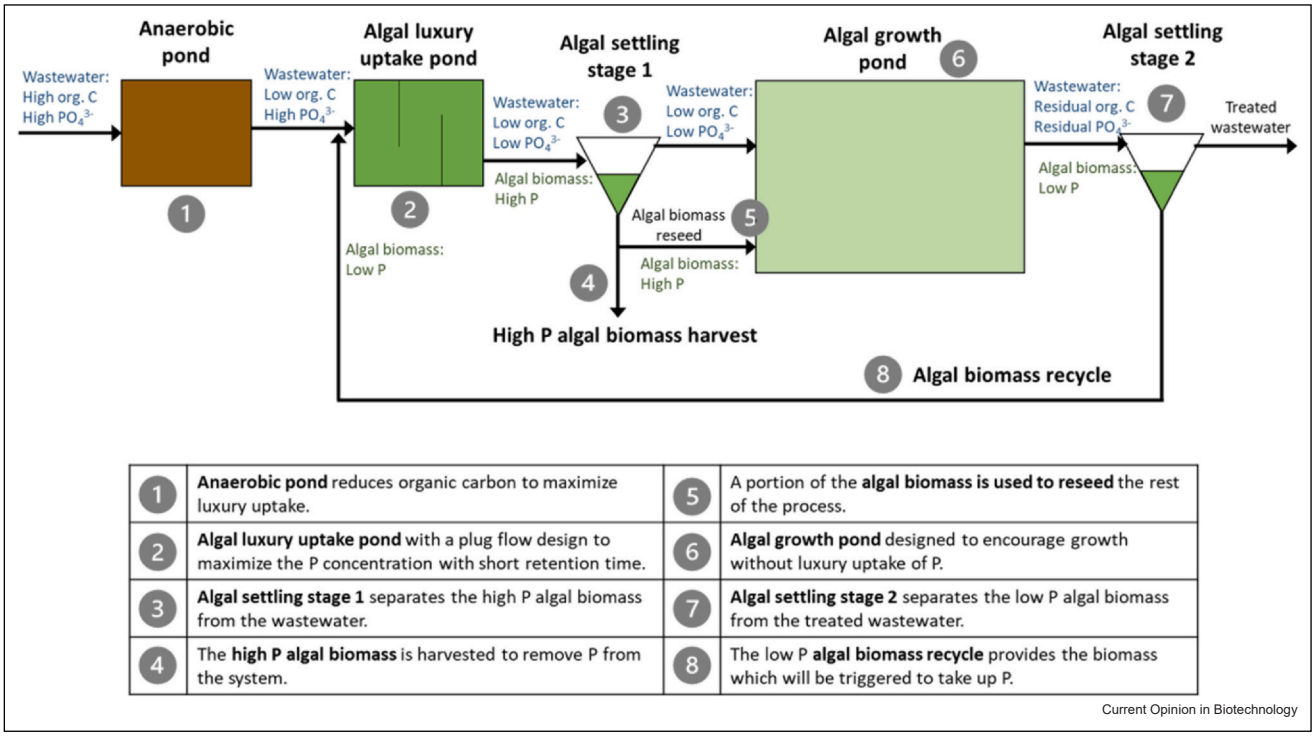
Variable	Summary of effects reported
Phosphate concentration in the wastewater	Increase in phosphate concentration triggers polyP accumulation [18,23,25,27–29].
Temperature	Inconsistencies in the effect of temperature with some reporting a significant effect [22,27,28], while others have found no effect [18,23].
Light intensity	Various effects have been reported, including a negative effect [22], a positive effect [23,28,29], and no effect [18,25,27].
Mixing intensity	No main effect was found by Brown et al. [27], but there were some interactions detected with other variables.
Biomass concentration	No effect, polyP accumulation was not influenced, but there was a positive effect on P uptake [25].
Phosphorus depletion time	Time that the cells were depleted of P has a positive impact on the rate of P uptake [25,26].
Organic carbon in the wastewater	Organic carbon in the wastewater has a negative effect on P luxury uptake[27].

Key conditions needed to maximise the P content of the microalgal biomass are summarised in Table 1. Recent work in this field has begun exploring the differences between microalgal genera and species [26,27], which may lead to further refinement of an engineered process in the future.

This enhanced understanding has led to the proposed luxury P uptake pond, which was first developed by Powell et al. [28] and then further refined by Brown et al. [27] and Plouviez et al. [25]. The term luxury P uptake refers to the uptake and storage of P above levels required for standard growth. An overview of the luxury P uptake process is provided in Figure 2. A key feature of this process is the separation of the growth and luxury uptake phases to optimise the conditions needed for luxury P uptake.

While much of this work has originated from open pond systems, other cultivation methods have been proposed, including photobioreactors [30], immobilised microalgae, thin layer cultivation methods [3] and revolving algal

Figure 2



Proposed microalgal luxury uptake process (not to scale). The process consists of an anaerobic process (shown in brown) to reduce the organic carbon, wastewater processes with concentrated P accumulating microalgae (shown in dark green) to reduce the phosphate, and processes with low P microalgae (shown in green) where the microalgal biomass is grown.

biofilm systems [19]. The EcoRecover system described by Molitor et al. [30] has been shown to provide efficient tertiary P removal via luxury uptake for a small community (~568 $m^3 \cdot day^{-1}$).

An emerging area of research combines the polyphosphate accumulating organisms (PAOs), typically found in Enhanced Biological Phosphorus Removal (EBPR), with algal biomass [31–34]. This process is referred to as Photo-Enhanced Biological Phosphorus Removal (PEBPR). In contrast to EBPR, PEBPR relies on light/dark cycles to create aerobic/anaerobic conditions that are needed for efficient removal of P via polyp accumulation by both PAOs and microalgae. The microalgae provide the oxygen for the process that reduces the energy requirements for aeration, an advantage considering that aeration is one of the main costs in wastewater treatment plants [31].

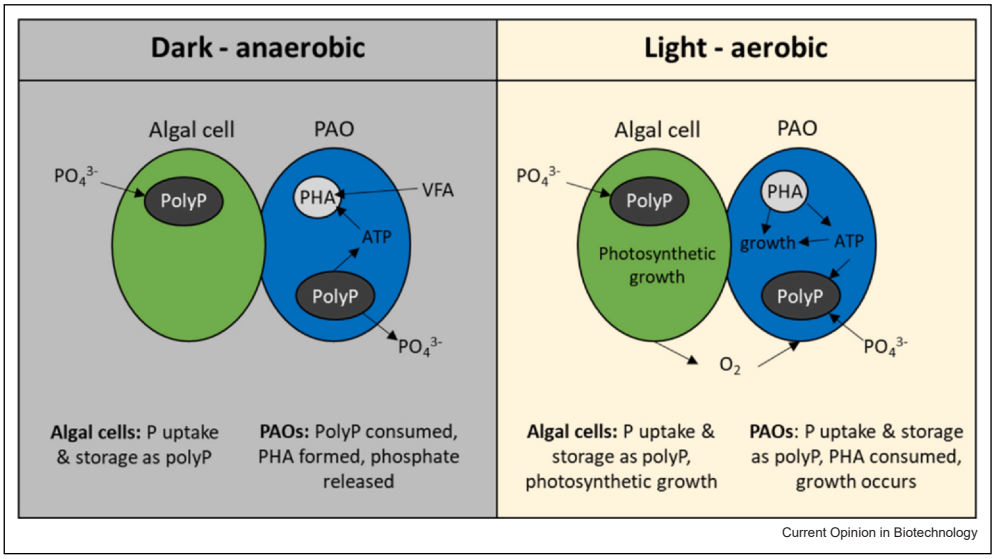
The balance in populations of the microalgae and PAOs can be manipulated by several variables, including light intensity [31,33,35], substrate composition [31,33,36], sludge retention time [31,37] and length of light/dark cycles [31]. Knowledge of P transformations in PAOs and microalgae can be used to propose potential mechanisms occurring within the PEBPR process (Figure 3). In the dark (i.e. anaerobic conditions), PAOs release phosphate, and in the light (i.e. aerobic conditions), they accumulate P. Because the rate of P uptake in the light phase is higher than the P released in the dark phase [31], and with microalgae also accumulating P in both the light and dark phases [25,30], there is net P removal. Consequently, the microalgal-bacterial consortium can accumulate high levels of P. Importantly, while PAOs release their polyP in anaerobic conditions, microalgae retain their polyPs, which would result in a more stable sludge for further processing and utilisation, but this deserves further investigation.

Reuse and potential of phosphorus-rich microalgal biomass

PolyPs have applications in the food, chemical and medicinal industries [10,38,39], but polyPs with different structures and chain lengths have different industrial applications. While the central paradigm in microalgae is that the polyP granules are formed from linear orthophosphoric acid units linked by phosphoanhydride bonds surrounded by counter-ions, microalgae can accumulate P with organics compounds [15] or under different forms such as inositol phosphate (phytate) as recently demonstrated [40]. Therefore, the in-depth characterisation of microalgal polyPs structure is still needed. While studies have shown the kinetics and yield of P accumulation and polyP synthesis vary with the species studied and the conditions experienced [17,24], it is unclear if the type of polyPs produced (e.g. organic vs inorganic, short vs long chain length) also varies. Inorganic polyPs with chain lengths of 10 s of monomers up to 500 monomers have been reported in microalgae [41,42]. Chain length determination is especially important to determine the cellular function and potential industrial value of the various types of polyPs in microalgae.

PolyPs produced by microalgae and cyanobacteria are currently not commercially produced for the food,

Figure 3



Summary of possible P transformations in the PEBPR process during the dark and light phases. Algal cells can assimilate P under both light and dark conditions. PAOs take up volatile fatty acids (VFA) during the dark phase and accumulate P during the light phase.

chemical, and medical industries. However, it was shown that polyPs produced by Synechococcus sp. can be assimilated by human cells and reduce inflammation and oxidative damage in human and mouse cell cultures [6,20]. As microalgae are already produced for food [43], polyP-rich biomass could be of interest as polyPs are widely used as additives [10], as done in yeast [44].

Of particular interest, microalgal biomass shows promise as a biofertiliser with polyP shown to improve plant growth performance [11,21]. The benefit of microalgae production is that it can be integrated with modern agricultural practices, such as vertical farming and aquaponics [43]. Therefore, microalgae-based water treatment could be used to recycle water and generate polyP biofertilisers [3,4], improving the sustainability of the agricultural system. While biomass rich in polyPs is of good quality as a fertiliser, some consideration is needed to prevent issues of nutrient imbalance. Specifically, in the case of wastewater treatment, organic micropollutants, pathogenicity and accumulation of heavy metals would also need to be carefully considered.

Conclusions and future prospects

P rock reserves are found in just five countries, meaning supply chain disruptions, political changes and other unforeseen events can already profoundly impact the cost and availability of P rock imports and consequently P products. With the P rock reserves depleting fast, it is expected to get worse soon, threatening food security. Policies are being put in place by some governments to recover P (e.g. Germany) [9,10,45].

Significant research and development are ongoing with yeast and microalgae to develop bioprocesses for P recovery and P upcycling. The P removal processes discussed in this paper are still being developed and are future opportunities rather than well-established solutions being used today. The advances in the understanding of the biomolecular pathway related to P metabolism and polyPs synthesis achieved in the past two decades revealed the importance of polyPs in the cells and how polyPs synthesis could be triggered either via a change in the growing conditions or metabolic engineering. Processes are now being scaled up and integrated within the food sector (e.g. yeast). However, polyP molecules are diverse and could be developed for other industrial applications. This is an area of further investigation.

Commercial microalgae farming has globally increased, and its food and high-value chemical industries are currently worth ~US$3.0 billion [46]. Considering that the global biofertiliser market is expected to exceed US$3.0 billion by 2024 [47,48], the development of microalgae-based biofertilisers rich in polyPs could contribute to the expansion of the microalgae farming sector. This expansion could also occur from other markets such as the food industry as microalgae are already sold as a food supplement and there is a need for polyPs as food preservatives.

Despite a future increase in price due to increasing costs of extraction and purification of lower quality remaining P stocks, polyPs produced chemically are still relatively cheap; it is therefore unlikely that microalgal polyPs could be competitive. This is why the integration of microalgae-based biotechnology within the agri-food sector is promising. The increased use of more sustainable agricultural practices via integration will therefore support transition to economies that are more sustainable and self-reliant. Collaborative efforts between policy makers, industries and scholars will make this transition a reality. Critically, the potential of microalgae bioprocesses for P upcycling should be demonstrated at scale. While most of the focus has been on microalgae growing on domestic wastewater, microalgae can grow on different types of wastes [49–51], which requires further investigation. In addition, the interest in microalgal heterotrophic growth at scale is increasing [52]. This means that there are possibilities to optimise P accumulation and synthesis of polyPs via microalgae utilising different types of waste and/or during heterotrophic growth. Broadening the application of microalgae-based biotechnology provides several opportunities to develop sustainable solutions for P upcycling.

CRediT authorship contribution statement

Maxence Plouviez: Conceptualization, Writing – original draft, Writing – review & editing, **Nicola Brown:** Conceptualization, Writing – original draft, Writing – review & editing.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

The authors declare no conflict of interest.

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- •• of outstanding interest.

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