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Phosphorus from wastewater to crops: An alternative path involving microalgae

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A B S T R A C T
Phosphorus (P) is a non-renewable resource, a major plant nutrient that is essential for modern agriculture. Currently, global food and feed production depends on P extracted from finite phosphate rock reserves mainly confined to a small number of countries. P limitation and its potential socio-economic impact may well exceed the potential effects of fossil fuel scarcity.

The efficiency of P usage today barely reaches 20%, with the remaining 80% ending up in wastewater or in surface waters as runoff from fields. When recovered from wastewater, either chemically or biologically, P is often present in a form that does not meet specifications for agricultural use. As an alternative, the potential of microalgae to accumulate large quantities of P can be a way to direct this resource back to crop plants. Algae can acquire and store P through luxury uptake, and the P enriched algal biomass can be used as bio-fertilizer.

Technology of large-scale algae cultivation has made tremendous progress in the last decades, stimulated by perspectives of obtaining third generation biofuels without requiring arable land or fresh water. These new cultivation technologies can be used for solar-driven recycling of P and other nutrients from wastewater into algae-based bio-fertilizers.

In this paper, we review the specifics of P uptake from nutrient-rich waste streams, paying special attention to luxury uptake by microalgal cells and the potential application of P-enriched algal biomass to fertilize crop soils.

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Abbreviations: AISP, acid insoluble polyphosphate; ASP, acid soluble polyphosphate; DW, dry weight; EBPR, enhanced biological phosphate removal; PBR, photobioreactor; P, phosphorus; P i, inorganic phosphate; WSP, wastewater stabilization pond.

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1. Introduction

Phosphorus (P) is a finite, non-renewable resource and a foundation of modern agriculture (Elser, 2012). After nitrogen, P is the second most frequent macronutrient that limits plant growth. It is an essential component of key molecules such as nucleic acids, phospholipids and ATP, and consequently, plants cannot grow without a reliable supply of this nutrient.

Currently, agriculture depends almost exclusively on P extracted from phosphate rock (Cordell et al., 2009, 2011), which is not distributed uniformly over the globe. Morocco, China, Algeria, United States, South Africa, and Jordan possess the majority of the world’s minable P rock, which is expected to be largely exhausted within this century (Jasinski, 2012; Bennett and Elser, 2011; Cordell et al., 2009, 2011; Shilton et al., 2012). The chemical precipitation of P with Al, Fe, or Ca compounds is already widely applied (Mulbry et al., 2005). However, P from chemically precipitated sludge is not readily bioavailable and is typically lost in landfills (de-Bashan and Bashan, 2004) although biological systems can mobilize chemically bound P to a certain extent (Desmidt et al., 2015; Wilfert et al., 2015). Among alternative strategies currently in use, “enhanced biological phosphorus removal” (EBPR) stands out; in this process, P is removed from wastewater through accumulation in heterotrophic sludge bacteria.
(Kulaev et al., 2004; McGrath and Quinn, 2003). EBPR is one of the most investigated biotechnological processes due to its widespread application (García Martín et al., 2006). It is technically more complicated than chemical P recovery, but the sludge is better suited for agricultural application. Often EBPR is combined with chemical P recovery, for example by struvite (magnesium ammonium phosphate) precipitation after anaerobic digestion of EBPR sludge (Desmidt et al., 2015; Wilfert et al., 2015).

Wastewater treatment using photosynthetic organisms also has a long history (Oswald, 1988, 2003; Oswald and Gotaas, 1957). The recent impetus to this application is the dynamic development of microalgal technologies focused towards industrial-scale production of biofuels that could be made economically viable and sustainable when using nutrients from wastewater (Pittman et al., 2011; Wijffels et al., 2010). Due to a number of significant advantages, microalgae-based techniques also gain importance among the possible approaches to manage P in wastewater. Microalgae can take up inorganic phosphate (Pi) at a rate that allows them to successfully compete with bacterioplankton in natural conditions (Currie and Kalff, 1984) and accumulate P up to 2–3% of their cell dry weight (Powell et al., 2009; Wood and Clark, 1988) — a trait that can be applied to remove Pi from treated wastewater (Boelee et al., 2011). The P-enriched microalgal biomass could then be converted, for example, to feed additives and bio-fertilizers, aiding P retention and recycling (Mulbry et al., 2005). There are many added benefits, such as the removal of nitrate, destruction of organic pollutants, suppression of pathogenic microflora, and CO2 sequestration (De Pauw and Van Vaerenbergh, 1984; Pittman et al., 2011).

Phosphorus uptake and turnover in microalgae has been already the subject of intensive research at diverse levels—from cells to ecosystems (Cembella et al., 1984, 1982; Kulaev et al., 2004) and biosphere (Carpenter and Bennett, 2011). Past research focused mostly on environments with low P availability and the mechanisms developed by microorganisms to cope with P limitation. In contrast, reviews dedicated to the biology of microalgae in a P-rich environment are scarce (Brown and Shilton, 2014; Powell et al., 2009). Polyphosphate is the main form of P stored in algal cells, and in spite of its central role in many biological processes, we know little about it (Rasala and Mayfield, 2015).

In this paper, we review the specifics of P uptake from P-rich environments, such as waste streams, as well as strategies for the sequestration and recycling of this element. We pay special attention to luxury uptake of P by microalgal cells, and the potential to convert algae into bio-fertilizers with slow release of P.

2. Phosphorus in the environment and in the algal cell

2.1. Reservoirs and fluxes of phosphorus in geo- and agrosphere

In terms of mass, the P concentration is approximately 1180 ppb in the lithosphere and 70 ppb in the seawater (Fairbridge, 1972), ranking it 10th and 19th, respectively, of the most abundant elements on Earth. The P concentration in the biosphere is estimated to be approximately 250 ppm (Cole and Rapp, 1981), an amount that is also relatively low when one considers the essential biochemical role of the element: no genetic biological information transmission, no biological energy metabolism, no photosynthesis, no cellular respiration (Hofmann and Van Raamsdonk, 2000). By CO2 sequestration, we mean the process in which gaseous CO2 from the atmosphere or other sources is assimilated into algal biomass. One needs to remember, however, that the carbon fixed in the algal fertilizer can be respired back to atmospheric CO2 after application to the soil.

Although the total pool of P in the Earth’s crust is large and its concentration in the biosphere relatively small, biomass and productivity of primary producers are often P limited (Elser et al., 2012; Elser et al., 2007; Smil, 2000). This discrepancy is partially due to: 1) the low content of P in cellulose, hemicellulose, and lignin, which represent a large share of biomass; and 2) the low bioavailability of P in poorly soluble rock apatite, which accounts for 95% of P in the Earth’s crust. The natural P cycle depends on the tectonic uplifting of rocks and exposure to weathering, typically on a timescale of 107–108 years. Fast atmospheric transfers of the element similar to carbon and nitrogen are absent because there are no long-living gaseous P compounds that could facilitate a faster and more uniform P distribution over the globe. Cycling of soluble P compounds is limited by rapid precipitation in aluminum (low pH) or calcium (high pH) containing complexes.

The slow natural P cycle abruptly increased because of the massive P mining for agricultural fertilizer production that facilitated the Green Revolution of the twentieth century and continued to increase thereafter (Cordell et al., 2011). The present massive anthropogenic transfer of P from rock to agriculture is, unfortunately, not a sustainable solution because the minable mineral reservoir will be exhausted in the next several decades as the human population and the demand for quality nutrition continue to increase at an unprecedented pace (Elser et al., 2007; Lima et al., 2003).

2.2. Extracellular phosphorus pools

P compounds are often classified based on their reactivity with molybdate, ease of hydrolysis, and particle size. The fractions capable of reaction with acid-molybdate to form a heteropoly blue complex are generally termed “reactive phosphate”. Unfortunately, this classification...
is derived from an arbitrary physical–chemical characterization that is poorly related to the biological significance of the P species (Cembella et al., 1982). With respect to availability to living organisms, P species in the environment are divided into two groups: bioavailable or non-bioavailable (Fig. 2). Algae can take up Pi directly from their environment (Atlas et al., 1976). More complex bioavailable P compounds are decomposed to Pi by extracellular or cell-wall-bound phosphatase enzymes (see Fig. 2). In such a process, for example, dissolved organic P (DOP) is hydrolyzed by alkaline phosphatase, phosphodies- terase, and phytase (Herbes et al., 1975; see also Table 1 in Cembella et al., 1982). As an interesting additional pathway, some cyanobacteria such as *Trichodesmium* can prevail in low P, oligotrophic waters by taking up Pi directly in form of naturally occurring phosphonate (Dyhrman et al., 2006).

Concentrations of individual P species in natural water are highly variable (Paytan and McLaughlin, 2007). The concentration of soluble reactive P in deep, aphotic zones is approximately 1–3 μM. Towards the ocean surface, it is depleted by phytoplankton uptake. Therefore, P often becomes a limiting macronutrient in oligotrophic waters (Thingstad et al., 2005). The concentration of P (and N) in natural waters can be increased by washout from fields and wastewater efflux (Carpenter et al., 1998; Correll, 1998), causingnoxious algal and cyanobacterial blooms (Conley et al., 2009; Schindler, 1977).

Typical municipal wastewater concentrations of P rose in developed countries from 2 to 4 mg L$^{-1}$ in the 1940s to 10–15 mg L$^{-1}$ 20 years later (Litke, 1999; Rybicki, 1997). This led to the massive increase in organic and nutrient loading in ground and surface waters that occurred during the 1950s and 1960s. In the same period, algae blooms accompanied by shifts in phytoplankton community composition occurred in lakes and rivers (Fig. 1). Some of the blooms contained toxic algal species. These phenomena entailed changes in both water turbidity and the abundance and composition of macrophytes, invertebrates, and fish. For surface waters worldwide, correlations were found between chlorophyll concentration, microalgae abundance, and nutrient loading, particularly P (Vollenweider, 1968). Although the debate continues about which nutrient is predominantly responsible for this eutrophication, it is clear that P plays an important if not the central role in anthropogenic stimulation of algal growth (Schindler, 1977; Schindler et al., 2008).

### 2.3. Measures for phosphorus reduction in surface waters

In response to the pivotal role of P in the eutrophication process, three abatement policies developed in order to improve water quality. In Europe, the earliest water legislation (1975) included standards for surface water intended for drinking and quality rules for other water, such as bathing water or groundwater. In 1991, the European Union adopted two important directives to regulate the quality of ground and surface waters of member states: the Nitrate Directive (nitrate pollution from agriculture) and the Urban Waste Water Treatment Directive. Under the latter directive, effluents discharged into sensitive freshwater areas needed to be treated so that either an 80% reduction in P$_t$ was achieved or that the final P$_t$ concentrations would be between 1 and 2 mg L$^{-1}$. Later, new legal measures, such as the Bathing Water Directive (2006) and the Drinking Water Directive (1998), were adopted for sensitive water types. To better integrate and coordinate policies related to water quality management, the EU adopted the Water Framework Directive using river basins as the basis for water quality management (Directive 2000/60/EC of the European Parliament and of the Council establishing a framework for community action in the field of water policy). This framework set guidelines and measures depending on the water type to achieve a desired quality level.

For example, the Dutch great rivers are designated as highly changeable waters, for which a summer average of 0.14 mg L$^{-1}$ P would be required. For sensitive waters, stricter regulations apply. Similarly, the United States Environmental Protection Agency, the Canadian Council of Ministers of the Environment (CCME, 2004, 2007), and the Australian and New Zealand Guidelines for Fresh and Marine Water Quality (ANZECC, 2000) have implemented type-specific water quality regulations.

Two source-oriented measures proved to be highly effective in the 1980s, when approximately 80% of the detergent P load originated from domestic wastewater (Rybicki, 1997). The first was the ban on the use of P in detergents and soaps, causing the per-capita detergent P load to surface water to drop from 0.5–1.1 kg y$^{-1}$ P in the 1970s down to 0.2 in the 1990s’ (De Jong and de Oude, 1988; Rybicki, 1997). The second was increasing the efficiency of P removal in domestic wastewater treatment plants. The contributions of human excreta to domestic effluent have been estimated to be 0.5 kg y$^{-1}$ P per capita, of which two thirds can be attributed to urine and one third to feces (Rybicki, 1997). In a typical treatment plant, only 40–50% of the P can be removed from the liquid phase, whereas more advanced P removal techniques targeting the sludge can lead to a total removal of over 90% of the influent load at a wastewater treatment plant (Desmidt et al., 2015). Phosphorus removal from wastewater is achieved by chemical and biological processes. Chemical phosphorus removal (CPR) is based on the addition of trivalent cations, typically iron (Fe) or aluminum (Al) salts, which form insoluble precipitates with phosphorus (Desmidt et al., 2015; Wilfert et al., 2015). Biological P removal is most often done by EBPR (Mino et al., 1998). To recover the P from digested EBPR sludge, magnesium salts are used for struvite formation (Desmidt et al., 2015; Wilfert et al., 2015). The efforts to reduce P in surface waters were particularly noticeable in rivers such as the Rhine, where a sharp decrease in P levels was observed in the 1980s after the introduction of EBPR and the ban on P in detergents and elimination of several of its major sources (Fig. 3).

### 2.4. Opportunities for and advantages of using algae for phosphorus capture and recovery

The use of algae for P (and nitrogen) removal and recovery has been promoted over the past decades (de-Bashan and Bashan, 2010; Doran and Boyle, 1979; Nesbitt, 1966). Recently, the efforts towards this goal have intensified (e.g. de-Bashan and Bashan, 2004 or Hernández et al., 2013; Sawayama et al., 1998; Shilton et al., 2012; Solovchenko et al., 2013, 2014), stimulated by:

1. the increasing need for lowering the nutrient levels further, particularly for sensitive water types;
2. the economic and ecological arguments against using chemicals, such as iron or aluminum, at wastewater treatment plants;

![Fig. 3. Annual flow-weighted mean concentrations of NO$_3$-N and PO$_4$-P in the Rhine at the German-Dutch border (Lobith). Reproduced from Stålman (2004) with permission.](image-url)
3. the need to decrease the environmental footprint of wastewater treatment facilities (Shilton et al., 2012);
4. the capacity of algae to operate in an aerobic environment, unlike bacteria in EBPR.

Fig. 4 schematically shows that several relevant P-rich waste streams may be accessible for sequestration by algae (dotted arrows). The scheme is consistent with the estimates of Cordell et al. (2009), who suggested the P loss from animal manure amounts to approximately 40% of the annual P mining quota, from human excreta it is up to 10%, and from other industrial uses and products such as detergents, it accounts for almost 10%. These figures, considered in the context of P rock limitation (Cordell and White, 2014), makes recovering P from waste flows an imperative.

2.5. Inorganic phosphate uptake by algal cells

The negative charge of the P\textsubscript{i} ions prevents its spontaneous diffusion across the lipid bilayer of the algal cell membrane, which is not only hydrophobic but also negatively charged on its internal side (Fig. 2). Similar to fungi (Burns and Beever, 1979) or bacteria (Rosenberg et al., 1977), kinetic and biochemical studies of P\textsubscript{i} uptake in microalgae suggest at least two distinct mechanisms of P\textsubscript{i} transport across the plasmalemma: one is activated when the P\textsubscript{i} concentration in the environment is low and the other when P\textsubscript{i} is abundant. Detailed molecular mechanisms of these uptake processes in microalgae remains scarcely known.

According to the ample evidence reviewed in Cembella et al. (1982, 1984), P\textsubscript{i} uptake is an active process occurring at the expense of ATP hydrolysis and/or membrane potential energy and involving co-transport of cations (most probably H\textsuperscript{+} or Na\textsuperscript{+}). In low-P environments, this energy-intensive process can be facilitated by increasing the bioavailable P concentration in the environment through extracellular alkaline phosphatase (see Fig. 2) or by enzymes excreted by other microorganisms. In P-rich environments, passive diffusion can also take place. For a comprehensive account of the general biology of P uptake and metabolism in microalgae, we refer readers to reviews by Cembella et al. (1982, 1984) and Grossman and Aksoy (2015).

To estimate the suitability of algae for EBPR, one must compare the efficiency of P uptake systems in algae with that of other biotechnologically established microorganisms capable of both inorganic (P\textsubscript{i}) and organic (DOP) P uptake (Blank, 2012). The primary P\textsubscript{i} uptake occurs in the Escherichia coli model by the phosphate-specific transporter (PST) family (Hsieh and Wanner, 2010) that consumes 2 ATP per one P\textsubscript{i} molecule. In a nutrient-rich environment, P is assumed to be transported by low-affinity metal transporters driven by a transmembrane proton gradient and a transport-neutral phosphate complex with divalent ions such as Mg\textsuperscript{2+}, Mn\textsuperscript{2+}, Ca\textsuperscript{2+}, and Co\textsuperscript{2+}.

Unlike in E. coli (Hsieh and Wanner, 2010), fungi, and higher plants (Bucher, 2007; Rausch and Bucher, 2002; Tian et al., 2012), the molecular nature of P\textsubscript{i} in microalgae transporters remains fragmentary, relying mainly on genomic data mining. For example, a sodium-dependent P\textsubscript{i} transporter gene, DvSPT1, was isolated from a cDNA library of Dunaliella viridis (Li et al., 2006) similar to that of TcPHO from Tetraselmis chui. More detailed information on P\textsubscript{i} transporters is available for a model microalgae Chlamydomonas reinhardtii (Grossman and Aksoy, 2015). Similar mechanisms of (active) P\textsubscript{i} transport are assumed to operate in the membranes of cell organelles, for example, in chloroplasts that could resemble those of higher plants (Pfeil et al., 2014).

The P uptake by algae can respond dynamically to changes in nutrient availability. Currently there are two known dynamic mechanisms of P uptake in microalgae in P-rich environments. The first mode, overcompensation or “overshoot” (Cembella et al., 1982), occurs in pre-starved algal cells after exposure to a P-rich environment. The transfer results in a rapid, light-dependent accumulation of acid-soluble polyphosphate (Aitchison and Butt, 1973). The second uptake mode, luxury uptake, was originally identified in studies relating growth to nutrient availability. One of the early seminal works on P storage in algae (Ketchum, 1939) showed that the N:P uptake ratio can change and also that the N:P ratio in biomass varies with the concentration of both nutrients in water. Ketchum concluded that “phosphorus must also be utilized in reactions which do not involve nitrogen,” suggesting P storage mechanisms. Recently, a mathematical model approached the N:P co-limitation (Bougaran et al., 2010), and Saito et al. (2008) discussed the underlying concepts. For example, P\textsubscript{i} availability in the low concentration range affects not only P uptake and storage but also cell quota of nitrogen.

Importantly, no pre-starvation is required for luxury uptake (Eixler et al., 2006), which is a storage of P reserves occurring even when there is a sufficient supply of it in the environment. Luxury P uptake probably evolved as an adaptation of microalgae to unstable P availability. Watanabe et al.’s (1988) observations that the microalgae Heterosigma akashiwo adjusts its P\textsubscript{i} metabolism during its vertical migrations support this hypothesis. At night, this alga migrates to the lower, P\textsubscript{i}-rich water layer and absorbs the nutrient, which is incorporated in polyphosphate, increasing its chain length. During the day, when the alga migrates to the P\textsubscript{i}-depleted surface water, it takes P\textsubscript{i} from its polyphosphate storage for photophosphorylation. De Mazancourt and...
Schwartz (2012) also suggested that luxury uptake was a competitive strategy aiming at starving the competitor.

2.6. Phosphorus pools in algal cells

2.6.1. Integral phosphorus capacity of algal cells

The Pi concentrations inside cells are typically close to 5–10 mM (Brown and Kornberg, 2004; Rasala and Mayfield, 2015), independent of external concentrations that may vary from the low micromolar range in the deep aphotic ocean up to the low millimolar range in wastewater effluent (see above). The Ki for Pi uptake is below 4 μM for most species; this fact is believed to indicate the fundamental similarity of the mechanism(s), presumably active transport, involved in Pi uptake in different algal classes under a wide range of environmental conditions (Cembella et al., 1982). Although the intracellular Pi concentrations are modest, its turnover is immense (Blank, 2012).

The stoichiometric ratio of the essential elements carbon, nitrogen, phosphorus, and sulfur is largely conserved in marine phytoplankton, close to the Redfield ratio (C:N:P) of 106:16:1.7:1 (gravimetric ratio 41:7.2:1.75:1) (Redfield, 1958). In comparison, the relative content of P in land plants is much lower because of the large C-content in the phytomass (Smil, 2000). Using the Redfield ratio, one can estimate a typical gravimetric share of P in dry weight of natural phytoplankton at approximately 0.9%, corresponding to <1% of estimate (Grottelaar, 2004, 2013). However, the dry weight (DW) relative content of P in microalgae fed by swine manure can reach up to 1.8% (Kebede-Westhead et al., 2006; Pizarro et al., 2006), and levels up to 4% DW of P have been reported in Powell et al. (2011a). This confirms that microagal cells can take up much more P than necessary for the next life cycle (Rhee, 1973; Tilman and Kilham, 1976). A rough calculation of the potential of algae non-optimized P uptake capacity leads to a content of 1.8% P in dry weight (Smil, 2000). The required area would be proportionally even smaller (Lívanský, 2014). The exceptions to the widespread occurrence of polyphosphates in phototrophic eukaryotic cells are scarce even though they are the most abundant P reserves in microalgal cells (Cembella et al., 1982; Martin et al., 2014; Powell et al., 2009).

For a better understanding of the role of polyphosphates in algae, one must consider their broader role in nature and biology. Achbergerová and Nahálka (2011) proposed that polyphosphate formed on the prebiotic Earth through dehydration of phosphate rock at high temperature. Polyphosphates were tentatively proposed as the main energy storage molecule in early evolution (Achbergerová and Nahálka, 2011), and if this is true, it is not surprising that polyphosphate became widespread or even ubiquitous in pro- and eukaryote cells (Brown and Kornberg, 2004; Kulaev et al., 2004; Nishikawa et al., 2006; Rasala and Mayfield, 2015). The exceptions to the widespread occurrence of polyphosphates are also interesting. The heterokontophyte Chattonella antiqua as well as Pylaiella sp. are reportedly unable to synthesize polyphosphates (Kimura et al., 1999) although one ought to keep in mind the comment by Raven and Knoll (2010) that “inability to detect polyphosphate in a given cell may be a problem of technique.”

Miyachi and colleagues (Miyachi et al., 1964; Miyachi and Miyachi, 1961; Miyachi and Tamiya, 1961a, 1961b) differentiated at least four fractions of polyphosphate in algal cell. The different polyphosphate pools are linked as P donors to specific metabolic pools depending on illumination conditions and cell cycle phase. During the life cycle of microalgae in synchronous cultures, the newly absorbed P was first discovered in polyphosphate C, then in polyphosphate A, nucleotidic phosphate, and in DNA (Miyachi and Tamiya, 1961a). In Fig. 2, A and C represent the more readily available acid-soluble polyphosphate (ASP) fractions that are generated when light and P are abundant and that can transfer P to DNA and protein. Fraction A was found in volutin granules, named after the bacterium Spirillum volutans, in which polyphosphate was discovered as metachromatic granules in the cytoplasmin which polyphosphate was discovered as metachromatic granules in the cytoplasm (reviewed by Achbergerová and Nahálka, 2011). Fraction A’s accumulation depended largely on photosynthesis, probably because it is derived from fraction C, which is presumably localized either in chloroplasts or their vicinity. Fraction C donates P for the biosynthesis of chloroplast DNA, whereas fraction A is involved in the synthesis of nuclear DNA (Miyachi and Miyachi, 1961; Miyachi and Tamiya, 1961a).

Phosphorus also plays an indispensable role in energy storage and transduction. The short-term energy storage and effective “energy currency” are the triphosphate bonds of ATP, which is a ubiquitous energy storage form. ATP plays a central role in algae because it is the primary product of photosynthesis. A considerable part of intracellular P is also associated with different P metabolites, primarily sugar phosphates formed with direct or indirect participation of ATP and other energy-rich phosphorylated compounds.

The typical mid- and long-term energy storage compounds are carbohydrates or lipids, but significant free energy is also often contained in polyphosphate, which contains tens to hundreds of bonds similar to ATP. Because of its crucial role in P metabolism of algal cells, we discuss polyphosphate in more detail below.

2.6.2. Storage polyphosphates

Through luxury uptake, microalgal cells can take up much more P than is necessary for their immediate growth. The intracellular (cytosolic) P may be stored in polyphosphate pools deposited in vesicles or vacuoles (storage in Fig. 2). Polyphosphates serve as an internal P depot that cells can use when needed (Kuhl, 1974; Nishikawa et al., 2006). Enzymologists and biochemists have studied polyphosphate synthesis in prokaryotic and eukaryotic heterotrophic organisms (for example, in yeast) relatively well (Wood and Clark, 1988). By contrast, the data on the mechanisms of the biosynthesis and degradation of polyphosphates in phototrophic eukaryotic cells are scarce even though they are the most abundant P reserves in microalgal cells (Cembella et al., 1982; Martin et al., 2014; Powell et al., 2009).

Interestingly, the species with higher P uptake rates (in P-starved cultures) are encountered more often among dinoflagellates than among chlorophytes; see Cembella et al., 1982.
endogenous P_i reservoirs that are used in the absence of external P_i. The prominent role of polyphosphate B is in the synthesis of RNA in the dark and in low P_i conditions (Fig. 2).

2.6.3. Subcellular distribution of polyphosphates

Polyphosphates are in different compartments of microalgal cells. The presence of polyphosphate in the volutin granules of *Auxenochlorella pyrenoidosa* (earlier *Chlorella pyrenoidosa*) and *Scenedesmus fuscus* (earlier *Chlorella fusca*) was demonstrated using electron microscopy (Adamec et al., 1979; Atkinson et al., 1974). With a more advanced electron microscopy technique known as X-ray microanalysis, researchers were able to locate granules containing P in the cytoplasm, vacuoles, and chloroplasts of *Scenedesmus quadricauda* (Voříšek and Zachleder, 1984). The cells of microalgae, such as *C. reinhardtii* (Ruiz et al., 2001), possess special polyphosphate and Ca\(^{2+}\) storage organelles known as acidocalcisomes, which have some traits in common with vacuoles, for example, possessing H\(^+\)-pumping pyrophosphatase in their membranes (Kulaev et al., 2004).

Electron microscopy in combination with the results of \(^{31}\)P NMR spectroscopy of living cells can provide a realistic picture of the P distribution within microalgal cells. The aforementioned technique provides information (though not always comprehensive) on P speciation, compartmentalization, its chemical surrounding, P chain length, formation of cation complexes, and P exchange (Ginzburg et al., 1988; Martin et al., 1982; Mitsumori and Ito, 1984). Wood and Clark (1988) compiled a comprehensive account of polyphosphate study methods.

2.6.4. Physiological roles of polyphosphates

The mechanisms of polyphosphate’s biosynthesis and catabolism and its physiological significance for photosynthetic microorganisms remain largely elusive. Polyphosphate is undoubtedly a P reserve but may also play a number of other essential roles that remain largely unexplored (Rao et al., 2009). Polyphosphates are secondary products of photophosphorylation, meaning that they are formed after organic phosphates (including ATP) and that their turnover rate is slow relative to that of other P species in the cell, even under conditions of P starvation. Thus, polyphosphate may serve as an energy reservoir for ATP formation through the polyphosphate kinase enzyme(s), similar to analogous enzymes identified in bacteria and yeast (Wood and Clark, 1988).

Another suggested function for polyphosphate is regulatory; it is involved in regulation of the synthesis of ATP and other phosphorylated compounds, acting as a metabolic “buffer” between different cell compartments (Cembella et al., 1982; Kuhl, 1974). The intracellular concentrations of ATP and P_i as well as the P_i level in the culture medium, regulate the biosynthesis and degradation of polyphosphate in microalgae (Cembella et al., 1982).

Purified granules contained polyphosphate complexes with calcium and magnesium as the most common inorganic components (see Kulaev et al. (2004) and references therein). X-ray microanalysis of the electron-dense vacuoles or polyphosphate bodies of *C. reinhardtii* showed large amounts of P, magnesium, calcium, and zinc. Immuno-fluorescence microscopy revealed a vacuolar-type proton pyrophosphatase (H\(^+\)-Pase) in this compartment. Purification of the electron-dense vacuoles using iodixanol density gradients showed preferential localization of H\(^+\)-pyrophosphatase and H\(^+\)-ATPase activities in addition to high concentrations of pyrophosphatase and polyphosphate (Ruiz et al., 2001). There are also reports implying that polyphosphate could be involved in the sequestration of heavy metals in microalgal cells (see Nishikawa et al. (2006) and references therein). Studies carried out with *C. reinhardtii* suggested that distinct mechanisms regulate cell-wall-associated and intracellular polyphosphate, and that the former might protect the cells against toxic compounds or pathogens during cytokinesis, which is when they are more vulnerable (Werner et al., 2007). The \(^{31}\)P NMR studies carried out in vivo by Pick et al. (1990) showed that in the unicellular alga *Dunaliella salina* long-chain polyphosphates are hydrolyzed, generating protons and/or energy which could facilitate the maintenance of pH homeostasis under condition of alkaline stress.

*Phaeodactylum tricornutum* responds to hyperosmotic stress by a sizeable elongation of polyphosphate chains and a decrease in the total amount of polyphosphate, whereas exposure to hypoosmotic stress resulted in a higher content of shorter polyphosphate chains and increased total polyphosphate content. These changes might reflect the buffer role of polyphosphate and its involvement in adjusting intracellular osmotic pressure and storing elements in an osmotically inactive form (Raven and Knoll, 2010; Werner et al., 2007).

Finally, accumulation of P reserves in the form of polyphosphate rather than P_i also increases the relative cell density and affects the vertical migration of algae between the aphotic, P-rich, deep-water layers and photic but P-poor, surface waters (Raven and Knoll, 2010). A high polyphosphate content, which functions as ballast, may impede the upward migration.

2.7. Strategies of P_i sequestration in algal cultivation systems

Exponential growth of algae is observed when a culture is limited by neither nutrients nor light (e.g. due to self-shading). Sustained exponential growth can be maintained in turbidostat or chemostat regimes, leading to a constant uptake of P that is proportional to the stoichiometric concentration of the element in the biomass (Saito et al., 2008). The growth and P uptake rate are linearly proportional to the biomass and its P content, respectively. In this scenario, the rate of P uptake is constrained by cultivation technology and algal biology. On the technological side, the rate of P uptake per unit volume increases with the number of cells in the culture volume until the energy-requiring uptake becomes limited by self-shading or by CO\(_2\) and O\(_2\) mass transfer. For example, a high rate of P uptake per culture volume can be expected in a thin layer photobioreactor where algae are saturated by light and CO\(_2\) under high cell densities, provided they still attain a high growth rate (Doucha and Livansky, 2014). The higher the culture density that still allows exponential growth, the higher the P uptake rate attained with the given cultivation system and algal species is. On the biological side, the faster the specific growth rate of the alga and the higher the quota (stoichiometric fraction) of P in the algal cells, the higher the P uptake rate is.

The turbidostat or chemostat cultivation systems discussed above lead to a stable P cell content. However, the cell P stoichiometry can largely change in dynamic conditions, such as during the overshoot phenomenon when starved cells are exposed to a high P environment (Herbert, 1961). Eppley and Strickland (1968) concluded that the growth rate under dynamic environmental conditions is more closely related to cellular content than to the actual external concentration of nutrients. It was suggested that the kinetics of P uptake depend on both the nutritional status (“nutritional history,” cell P quota) and the growth rate of the alga (Cembella et al., 1982; Droop, 1983; Grobbelaar, 2013).

3. Phosphorus uptake under different cultivation conditions

3.1. Orthophosphate concentration

In continuous cultures, such as turbidostats or chemostats, the P_i concentration in the medium is constant, allowing the identification of different uptake regimes (Brown and Shilton, 2014) and allowing rigorous P control to discriminate between the uptake of different ionic species (Cembella et al., 1982). Water’s typical pH range (which encompasses the optimum pH range for many microalgal species) is 5–9, and the dominant free P_i species are HPO\(_4\)^{2-} and H\(_2\)PO\(_4\). Ullrich-Eberius (1973) postulated that the cell only takes up H\(_2\)PO\(_4\), which is predominant at a lower pH. Nevertheless, the optimum pH for P_i uptake covers a wide range, suggesting that HPO\(_4\)^{2-} is also transported. The higher ionic
charge of HPO₄²⁻ must be compensated for with a higher ratio of cation co-transfer to facilitate passage through membranes.

In this context, one should take into account that the pH in wastewater stabilization ponds (WSPs) is highly variable. Thus, vigorous uptake of nitrate and dissolved inorganic carbon by microalgal cells might shift the pH to alkaline values, displacing the P species equilibrium and thereby affecting the bioavailability of the inorganic P dissolved in wastewater.

With respect to concentration, Powell et al. (2008, 2009) demonstrated that in the range of 5–30 mg L⁻¹ P, the luxury uptake by a microalgal consortium was independent of the P concentration in the medium. In the lower part of this range, between 5 and 15 mg L⁻¹ P in the medium, the algae stored polyphosphate primarily in the form of acid-soluble polyphosphate, whereas storage of acid-insoluble polyphosphate depot occurred above 15 mg P L⁻¹. This observation can be tentatively generalized as a threshold below which the luxury uptake is directed towards acid-soluble polyphosphate (C and A in Fig. 2), whereas above this threshold concentration, storage goes also to acid-insoluble polyphosphate (D and B in Fig. 2).

3.2. Photosynthesis, irradiance, and temperature

Phosphorus uptake and storage are energy-requiring processes that, under autotrophic conditions, require sufficient light to drive photosynthesis. According to the ample evidence reviewed by Cembella et al. (1982), photophosphorylation, with a significant contribution from the ATP generated during cyclic electron flow around Photosystem I, provides a major source of energy for active Pi uptake. The role of cyclic photophosphorylation in the uptake of Pi was supported by the investigation of simultaneous uptake of Pi and nitrate in Ankistrodesmus braunii (Ullrich-Eberius, 1973).

Interestingly, increasing light intensity may have no significant effect on acid-insoluble polyphosphate and may even induce a decline in the acid-soluble polyphosphate (Brown and Shilton, 2014). Probably, the cells that divide more rapidly in high irradiance start to assimilate acid-soluble polyphosphate for the biosynthesis of cellular constituents at a higher rate than the polyphosphate replenishment (Powell et al., 2008).

Kulaev et al. (2004) suggested that photosynthesis is required to provide the necessary free energy for polyphosphate synthesis, but the size of the polyphosphate pools is a complex function of polyphosphate synthesis and hydrolysis, as well of P-dependent metabolic pathways requiring phosphorylation (e.g. biosynthesis of phospholipids, sugar phosphates, and nucleotides). Polyphosphate C is synthesized only in the light, but polyphosphate B can be formed using energy reserves regardless of illumination (Fig. 2). Consequently, the P₁ required for RNA synthesis could be obtained directly from external P₁ in the light or indirectly from polyphosphate B in the dark. Polyphosphate D is mobilized in the light for RNA synthesis only in the absence of external P₁ (Miyachi and Miyachi, 1961; Miyachi and Tamiya, 1961a, 1961b). The complexity of the interactions between photosynthesis, Pi uptake, and polyphosphate pools was further revealed by findings in the chlorophytes A. pyrenoidosa (Senft, 1978) and Pediasstrum duplex (Lehman, 1976), the rate of photosynthesis at saturating irradiance depended upon the cell P quota, suggesting a relationship between P₁ uptake and photosynthesis. The relationship may be circular: increased photosynthetic capacity probably elevates P demand for anabolic reactions, and the anabolic reactions further stimulate P₁ uptake.

The cultivation of algae at a low CO₂ level and plentiful irradiance slowed cell growth and division but increased polyphosphate content per cell weight or volume, suggesting that the luxury P₁ uptake continues under carbon limitation (Ullrich, 1972). This observation further supports the significance of cyclic and pseudocyclic electron flow for luxury P₁ uptake in microalgae (Allen, 2003; Raven and Glidewell, 1975). With sufficient P₁ supply, the energy that cells cannot use for linear photosynthetic energy transfer is probably directed to luxury P uptake (Cembella et al., 1984).

From a biotechnological perspective, this means that the microalgal culture performing the sequestration of P ought to be optimally illuminated. With high cell density systems (>1 g dry cell weight per L), high irradiance is provided only to cells that pass near the surface (<10 mm), whereas the photon flux densities in deeper and thus shaded suspension layers are not sufficient to drive photosynthesis (Richmond, 2004). On the other extreme, the light ought not to be strong enough to cause photo-inhibition (Richmond, 2004; Zarmi et al., 2013). One needs to maximize the surface-to-volume ratio of the cultivation system and optimize the mixing of the suspension. This is the foundation on which general principles for photobioreactor design have been formulated (Lee et al., 2014; Zarmi et al., 2013; Zittelli et al., 2013). These general principles should be taken into account when developing solar irradiance-driven microalgal-based systems for P sequestration. Irradiance may also have different effects depending on the suspension temperature. Powell et al. (2009) suggested that increased P uptake at optimal temperatures is due to an elevated rate of acid-insoluble polyphosphate formation.

Energy for P₁ uptake and polyphosphate synthesis can also be obtained by oxidative phosphorylation, e.g. in the dark or under heterotrophic growth conditions. Under P limitation, this contribution can outweigh the amount of energy coming from photophosphorylation in certain organisms such as Euglena or dinoflagellates, but not in chlorophytes studied (Ch. pyrenoidosa, Scenedesmus sp., Hydrodictyon africanum; see Cembella et al., 1982, 1984). In summary, the capability of polyphosphate formation in darkness might be beneficial for the sequestration of P from wastewater enriched with organic pollutants.

4. The potential of microalgal biotechnology for phosphorus recycling

4.1. Real-world phosphorus sequestration

An overwhelming majority of studies on P uptake by microalgae have been carried out under controlled conditions in the laboratory and using artificial growth media or synthetic wastewater. Although such studies are necessary to obtain a detailed insight into the process of luxury P uptake, it is the field studies with industrial-scale P sequestration facilities, such as high-rate algal ponds or waste stabilization ponds (WSPs), that can lead to practical applications. It was shown recently that the development of P uptake by microalgae does indeed regularly occur in full-scale WSPs where the typical P content is in the range 15–30 mg L⁻¹, resulting in 0.21% to 3.85% per volatile suspended solids dry weight (Brown and Shilton, 2014; Powell et al., 2011b). These figures are consistent with laboratory results supporting the feasibility of scaling up from laboratory to industry scale (see Brown and Shilton (2014) and Fig. 5).

For effective P-sequestration on an industrial scale, the P-load rate in the facility must correspond to the P uptake capacity of the microalgal culture. In such optimal conditions, Mulbry et al. (2008) reported a microalgal P removal efficiency of 70% to 90% at a P supply rate of 0.15 g m⁻² day⁻¹. An increase in P supply above the P uptake capacity of the algal cells will not result in an increase of P sequestration.

4.2. Monoculture or association?

Associations of several microorganisms, including microalgae, cyanobacteria, and heterotrophic bacteria, have distinct advantages for environmental applications, including better stability and improved P
sequestration by increasing bioavailability of P in wastewater (de-Bashan and Bashan, 2004; Posadas et al., 2014). In many cases, wastewater contains organic P species that are scarcely available for uptake by eukaryotic microalgae. At the same time, their associated bacteria often possess enzymes to mineralize the organic P (Lim et al., 2007). Nevertheless, microalgae such as Chlorella are able to hydrolyze and take up organic P in the form of phytates (Cembella et al., 1982), which are abundant in waste streams generated by the agricultural, raw-material processing industry. The extracellular phosphatase responsible for this is likely to enhance the release of P, from colloidal P as well (see Fig. 2). Dissolved alkaline phosphatases are reportedly capable of directly degrading particulate matter, resulting in rapid P release (Jansson et al., 1988). S. quadricauda, which is incapable of utilizing phosphomonoesters or pyrophosphate, appeared to overcome this limitation by associating with bacteria that released P from the substrates, which was not directly available to the microalga (Kuenzler, 1965).

The most radical combination of microalgae and bacteria suggested so far is plant growth-promoting (PGPR) bacteria (e.g., Azospirilla), used in agriculture to enhance the growth and nutrient removal capacity of microalgae from wastewater (de-Bashan and Bashan, 2004). Furthermore, these artificial associations (thus far not found in nature) significantly increased the release of ammonium and soluble P ions compared to the use of microalgae alone (de-Bashan et al., 2002, 2004).

Another benefit of the association of microalgae with heterotrophic bacteria is a more sustained supply with inorganic carbon (CO2). The atmospheric concentration of CO2 limits microalgal growth, so sparging the cultures with CO2-enriched gas mixtures generally increases microalgal growth rate but the cost might be significant (Brennan and Shilton, 2014). This cost can be mitigated, for example, by the use of point sources of CO2, such as flue gas from heat and power plants, but this requires additional infrastructure that may not be available. In the case of wastewater treatment, respiration by heterotrophic bacteria normally present in the culture generates additional CO2.

### 4.3. Providing algal strains for luxury P uptake

It is preferable to select strains capable of rapid growth and possessing large cell P quota. In a P-eutrophic environment, cells with increased abilities for P uptake and incorporation in the cell structures should be selected. This approach seems to be feasible due to high clonal variability within and between microalgal subpopulations (Cembella et al., 1982). Accordingly, wastewater treatment plants and stabilization ponds deserve a closer look as sources of promising microalgal isolates because in these P-rich systems natural selection of the strains with high potential of taking up and storing large quantities of P in the cell may take place. Another area worth further exploration is the selective pressure of cycles of P enrichment and starvation, similar to EBPR (Mino et al., 1998), creating a “feast and famine” regime that may effectively select for polyphosphate-accumulating algae.

An alternative to using or selecting native organisms is to engineer strains with higher P uptake and storage capacities. However, according to current legislation, the use of engineered algal strains is permitted only in closed cultivation systems such as PBRs (Shilton and Blank, 2012). Whereas P uptake and regulation systems are relatively well studied in prokaryotes (Keasling et al., 2000) and in higher plants (Raghothama, 2000; Rausch and Bucher, 2002), our knowledge about the genes responsible for P uptake and their expression, regulation, and conservation in algae is scarce, limiting genetic manipulation towards this goal. Nevertheless, the rapid progress in microalgal genomics (Blaby et al., 2014; Grossman, 2005; Grossman and Aksoy, 2015), metabolomics, and phosphoproteomics opens new opportunities for significant breakthroughs in genetic manipulations towards enhanced microalgal (luxury) P uptake in the near future.

Possible directions for engineering microalgae for enhanced P uptake include: 1) increasing the rate of P transport through membranes; 2) boosting cell storage capacity by promoting the biosynthesis of polyphosphate; and 3) knocking out the regulation mechanisms preventing P uptake when the cell is saturated with P. The targets for manipulation may include genes coordinating the P-starvation response in microalgae. A nuclear gene-regulator, P starvation response 1 (PSR1) of the chlorophyte C. reinhardtii can serve as an example (Shimogawara et al., 1999; Grossman and Aksoy, 2015). Relevant information can also be obtained from homologous structures and processes in intensely studied prokaryotes (Keasling et al., 2000) and higher plants (Raghothama, 2000; Rausch and Bucher, 2002). A detailed account of the molecular mechanisms and functional genomics of the P uptake in higher plants and mycorrhiza can be found in excellent reviews by Bucher (2007); Schachtman et al. (1998), and Walder et al. (2015).

### 4.4. Choosing the cultivation mode for microalgal phosphorus sequestration

#### 4.4.1. Open ponds

Waste stabilization ponds (WSPs) are shallow man-made basins accumulating wastewater, which is treated over a long time with a combination of aerobic and anaerobic bacteria as well as microalgae (Mara, 1996; Von Sperling, 2007; Shilton and Walmesley, 2008). WSPs are well established and widespread for the treatment of domestic and
industrial wastewater in regions with ample land and sunshine (Mara, 2004; Von Sperling, 2007). Phosphorus removal in WSP is mainly accomplished by adsorption to sediment and uptake by heterotrophic and autotrophic microorganisms such as microalgae and cyanobacteria (Bitton, 1999). WSPs are further divided into anaerobic, facultative, and maturation/oxidation ponds that are often set up in a series to achieve a reasonable efficiency of wastewater treatment (Mara, 2004). Microalgae exert a sizeable contribution to P removal in the upper (photic) layer of facultative ponds which can be primary or secondary (Bitton, 1999; Von Sperling, 2007). In addition, the elevated pH caused by photosynthesis and nitrogen fixation by the microalgae facilitates phosphorus precipitation in form of phosphates (Bitton, 1999).

High-rate algal ponds (HRAPs, Nurdogan and Oswald, 1995) were the first systems for intensive algal cultivation adapted for wastewater treatment and involving mixing and CO₂ addition. HRAPs further evolved into the concept of “luxury uptake ponds,” facilitating uptake of nutrients such as P by microalgal cells from wastewater. P uptake rates from wastewater could be increased at least three-fold by triggering luxury P uptake mechanisms in microalgae (Powell et al., 2009). The characteristic features of high-rate algal pond and luxury uptake ponds include: 1) relatively high P load rates; 2) low pond depth (typically <30 cm) and hence high light intensity; and 3) aeration and/or mixing of the suspension in the pond. Importantly, sustained P uptake in a high-rate algal pond or a waste stabilization pond requires a periodic removal of the microalgal biomass, otherwise the P would simply cycle between the liquid and the sludge layer resulting in minimal net P removal (Powell et al., 2011b).

As discussed by Powell et al. (2009), the luxury uptake pond should be vertically mixed to ensure uniform illumination of microalgal cells. After the P-rich biomass is harvested, microalgae leaving the “luxury uptake pond” would then be harvested and the remaining liquid would be further treated in the main pond system.

Generally, for efficient recovery of P the treatment process in the luxury uptake pond should focus on concentrating the maximal amount of P in a minimum amount of biomass over the shortest possible retention time (i.e., a high P supply rate). Nevertheless, the complexity of P transformations in the pond environment and fluctuating factors make the development of a luxury uptake process challenging.

### 4.4.2. Photobioreactors for suspension cultures

The dominant technology for microalgal P sequestration is based on the open-pond systems because they are inexpensive and simple to construct. Closed photobioreactors (PBRs) can be considered for special situations in which, for example, a wastewater effluent is used as an inexpensive nutrient source in emerging large-scale applications, such as third generation biofuels or CO₂ sequestration. Also, PBRs may be considered when high costs of harvesting microalgae from diluted suspensions, water evaporation, dependence on climatic conditions, large footprint, and unstable yield and biomass quality (Abeliovich, 2004) in open-pond systems outweigh their lower cost.

Zittelli et al. (2013) recently reviewed the design of low-cost, energy-efficient PBRs. Vertical, horizontal, tubular, circular, and flat-panel reactors are the most widespread PBR types. The key factors of successful use of microalgae for sequestration of P from wastewater are: 1) the choice of rapidly-growing strains that are tolerant to eutrophic conditions and a particular wastewater composition; 2) an energy-efficient PBR design supporting intensive cultivation of microalgae; and 3) fine-tuning of the illumination intensity, mixing rate, and temperature based on a deep understanding of the physiology of luxury P uptake by microalgal cells. For detailed account of specific advantages and disadvantages of different PBR designs, we refer the reader to recent reviews (Lee et al., 2014; Zittelli et al., 2013). Microalgae grown in PBRs possess a high potential for P removal (>90%; Shilton et al., 2012; Posadas et al., 2014) and PBR allows, as a closed system, the use of engineered algal strains, so we believe that the application of the closed cultivation systems holds promise for P recovery from wastewater, especially in regions with temperate and cold climates or high land cost.

### 4.4.3. Immobilized microalgae

An interesting alternative to suspension cultivation is the use of immobilized microalgal cells (de-Bashan and Bashan, 2010). Immobilized-algae PBR designs include fluidized- and packed-bed, parallel plane, hollow fiber constructions; for efficiency comparisons, see Mallick (2002). The advantages of immobilized algae cells include steady growth, lack of mixing requirement, high water-purification efficiency, simplified biomass harvesting, and increased cell retention (reduced wash-out) (Boelee et al., 2011; Mallick, 2002). Cells are usually immobilized in alginate gels (Mallick, 2002) or form biofilms themselves (Boelee et al., 2011). The cells of Chlorella vulgaris immobilized in alginate granules remained up to 70% of P from wastewater (Zhang et al., 1992). Thus, algal-turf scrubbers (ATS) used for farm waste treatment support the load rate of 2700 kg of N and 400 kg of P per 1 ha per year, yielding 27,000 kg ha⁻¹ of dry microalgal biomass (Pizarro et al., 2006). A significant advantage of this technique is that the turf with P-enriched algal biomass immobilized in it will be an almost ready-made P bio-fertilizer. Nevertheless, the main problems with this method are nutrient limitation by diffusion, photoinhibition, a suitable immobilization method, and biomass harvesting. Immobilized algae also offer the possibility to regenerate the P uptake capacity of the immobilized microalgae through P starvation (Kaya and Picard, 1996).

### 4.4.4. Thin-layer cultivation systems

As argued above, the systems with a short light path across the algal suspension represent a significant advantage because they can support much higher cell densities taking into account that the light absorption is governed by light path (layer thickness) and culture density. The same areal density in a 5-mm, thin-layer system as compared to a 30-cm-deep pond means about the same light capture efficiency but with a volumetric density that is 60 times higher in the thin layer. A higher volumetric density means proportionally lower harvesting costs and lower costs of pumping or transporting. Because the surface-to-volume ratio of such a thin-layer cultivation system is 60 times higher, the mass transfer of CO₂ and O₂ is proportionally better in the thin layer. Also assisting the mass transfer, the vertical flow gradient in the thin-layer system is high, thus promoting efficient mixing of the suspension close to the liquid surface. The high surface-to-volume ratio, effective mixing, short light pathway, and high culture density in the late exponential phase are typical for thin-layer systems (Douc and Livansky, 2014). At Forschungszentrum Jülich’s AUFWIND facility, O. Pulz of IGV GmbH has recently constructed an interesting modification of this approach, in which the algal suspension is sprayed in droplets over a system of multiple stacked horizontal nets (manuscript in preparation).

### 4.5. Production and use of the phosphorus rich microalgal biomass

#### 4.5.1. Production of phosphorus rich algal biomass using waste streams

Microalgae can be grown in wastewater or other liquid waste streams that are rich in nutrients, including phosphorus (Park et al., 2011; Cabanelas et al., 2013; Ray et al., 2013). Åkerström et al. (2014) described how residual P and N in sludge liquor resulting from a solid–liquid separation of digestate could be a suitable medium for Chlorella spp. biomass production. The production of algae biomass to recycle and recover nutrients from manure, as well as the treatment of manure effluents for algae biomass production has been demonstrated successfully (Mulbry et al., 2005, 2008; Wilkie and Mulbry, 2002).
Anaga and Abu (1996) reported the efficient removal of P and N from the effluent of a fertilizer company using Chlorella and Spirulina, demonstrating both effluent purification and biomass production for further applications (Cho et al., 2013). In this way, algae biomass production can become an integral part of treating and re-cycling the wastewater streams (Cabanelas et al., 2013).

4.5.2. High-value P-rich products

The P-rich biomass of microalgae has a number of different uses. Kulaev et al. (2004) reviewed present applications of polyphosphate. In particular, P-rich microalgal biomass is considered to be a potential source of polyphosphate. Polyphosphates are in high demand in different areas of medicine as well as in the biomaterials and food industry. Medical usage of polyphosphate is defined by its anitseptic, cytotoxic, and antiviral activities. At a concentration of 0.1% or higher, polyphosphate exerts a bactericidal and bacteriostatic effect. Interestingly, polyphosphate with more than four Pi residues inhibited human immunodeficiency virus type 1 (HIV-1) infection of cells in vitro, presumably by inhibiting adsorption of the virus on cell surface (Lorenz et al., 1997). Polyphosphates are used in the synthesis of new biocompatible materials such as Ca-polyposphate bioceramics and scaffolds for tissue regeneration (Waldman et al., 2002). The food industry is also an important consumer of polyphosphates, which are used as multifunctional ingredients (E-451 and E-452) in food, such as ham, bacon, meat poultry, fish, and shellfish due to their high buffering capacity as well as sequestering and antibacterial effects. At the same time, cultivation of microalgae in wastewater may preclude its use for high-value-added products such as food additives or medical applications. In this context, one should also consider that the high-value products are typically niche market products. The algal biomass produced on waste streams is typically much larger than the demands from these markets and media costs are rarely an issue there.

Therefore, microalgae grown on waste streams are typically considered for low-cost, high volume biofuel production (Park et al., 2011). In this context, one can foresee the P-rich residue that remains after the biofuel lipid extraction being used as a bio-fertilizer or as a source of polyphosphate. In another large volume approach, algal biomass was used as a co-ferment, increasing the yield of methane (Schwede et al., 2013). The integration of microalgae production with anaerobic fermentation for biogas generation and the further processing of the resulting digestates for bio-fertilizers opens research avenues for producing added-value products and closing nutrient loops (Uggetti et al., 2014). The use of pure digestates as bio-fertilizers and for soil conditioning purposes comprises numerous benefits for plant production (Möller and Müller, 2012).

4.5.3. Algal bio-fertilizer

The use of algal biomass grown on P-rich waste streams for bio-fertilizer production occurs on a scale where supply and demand can be balanced (Fig. 4). As reviewed by Metting (1996), some rice growers are currently using cyanobacteria rather than eukaryotic algae as a bio-fertilizer. Cyanobacteria’s ability to fix nitrogen from the atmosphere comprises a significant added value. Cyanobacteria applied as living cells and pretreatment of the plant seeds with cyanobacterial inocula led to enhanced germination rates of numerous species. This effect is likely due to phytohormonal and enzymatic activities that together with nutrients promoted plant performance (Adam, 1999). Application of living algal suspensions may, however, be hampered by difficult biomass conservation, implying that more manageable forms of algal biomass processing, logistics, and application must be found.

To assess the competitiveness of P-rich microalgal biomass versus traditional synthetic P fertilizers as well as their compatibility, one must critically compare the biomass with direct application of digestate or raw manure to soils (Laboski and Lamb, 2003). Mulbry et al. (2005) published one of the few studies evaluating the fertilizer value of dried algal biomass grown on anaerobically digested dairy manure. They found that for two soil types, levels of Mehlich-3 extractable P (available to plants) rose with increasing levels of algal amendment. However, the P levels in the soil before the application also influenced the positive effect. As described in the study by Mulbry and fellow researchers, plant growth experiments showed that 20-day-old cucumber and corn seedlings grown in a potting mix containing algae assimilated 38% to 60% of the P applied with the microalgal biomass. The plants grown in algae-amended potting mixes were equivalent to those grown with comparable levels of fertilizer-amended potting mixes with respect to seedling dry weight and nutrient content. An added benefit of algal biomass is that it does not need to be tilled into soil, which is necessary for mineral P fertilizers. The algal biomass could be side-dressed into growing crops, thereby saving much labor and energy. According to the estimations by Mulbry et al. (2005), the amount of algal biomass grown on the manure from a 100-cow dairy farm could support 4 ha of production, and at these application rates (6.5–10 t biomass ha$^{-1}$), loading of heavy metals from the algal biomass would be well below the permitted level (Mulbry et al., 2005). Also, Powell et al. (2011b) showed that sludge from waste stabilization ponds used for wastewater treatment with microorganism consortia including microalgae has potential as a bio-fertilizer. The presence of pollutants such as pathogens and micropollutants may be a point of concern. However, the risks associated with the use of bio-fertilizers in agriculture are typically low (Mulbry et al., 2005; Willett et al., 2015). Even when a material is technically safe, legal barriers may prohibit such a product from entering the market. For example, the European Fertilizer Regulation (EC2003/2003) contains a list of materials that are allowed as fertilizer, mainly single (mineral) compounds. Currently, a revision is being made towards a more functional classification, including specifications on minimal nutrient levels and maximum levels of undesired compounds. Finally, and probably most importantly, the public perception on safety of bio-fertilizers may hinder market introduction. This hindrance can only be eliminated by the careful monitoring of the possible sources of concern and by presenting this information in a transparent manner.

The microalgae that settle into waste-stabilization pond sludge contain significant quantities of polyphosphate (up to 71% of the total P in the sludge). At 25 °C, the sludge releases P from the polyphosphate at a rate of 4.3–12.4 mg P d$^{-1}$ per gram of total suspended solids. The rate of P released from fresh microalgal sludge grown under laboratory conditions was even higher, comprising 160 mg P d$^{-1}$ per gram of total suspended solids. It is noteworthy that after the initial release phase, P was re-assimilated in this system and some polyphosphate was re-suspended solids. It is noteworthy that after the initial release phase, P was re-assimilated in this system and some polyphosphate was re-

5. Conclusions and outlook

The reliance of humanity on the rapidly depleting, non-renewable phosphate rock is a grave, though currently underestimated threat to food security. Continued wasteful use of phosphorus from phosphate rock also leads to a permanent excess of P in the environment, which
Microalgae have a significant potential for P recycling, particularly via algae-based bio-fertilizers produced on P-rich waste streams. Algae can perform sustained luxury P uptake driven by sunlight, grow fast, use nutrients available in wastewater, and form biomass suitable for bio-fertilizer production. Positive synergistic effects can be achieved by coupling wastewater treatment with bio-fertilizer and biofuel production. Algae assimilate CO2, which is among the most abundant and noxious greenhouse gases. To turn this potential into a widely used cycle going from waste streams to crop plants, further research is needed on P uptake mechanisms, their regulation, and their integration in metabolic networks of algae. The research on P transport and transformation pathways may involve advanced “omics” techniques to identify target genes and enzymes. The new knowledge generated may enable the engineering of strains with enhanced capability of luxury P uptake. Simultaneously, this will be supported with bioprospecting native microalgae strains and associations that are capable of rapid sustained uptake of P and withstand the fluctuating background of other pollutants in real-world wastewaters. Further optimization of the current cultivation system will occur in parallel with efforts towards the development of new low-footprint platforms for sequestration of P from wastewater. The cultivation conditions leading to the most effective accumulation of P in the algal biomass must be identified, as well as optimal harvesting procedures. A further objective is to identify the form of algal biomass that is best suited for application as a fertilizer in view of logistics, stability, application approach, and delay of the phosphorus release. This will, for example, include investigation of P availability and potential adverse effects of surface-applied algae suspensions, such as clogging the soil surface or soil aggregate formation, potential decline in surface water absorption or nutrient access for the crops. A better understanding is required about the interaction of algal biomass with the soil microbial community, particularly with bacteria and mycorrhiza (Malik et al., 2012), including detailed mapping of the phosphorus flux from algal biomass to soil and plant roots. The pathways of phosphorus release from algal cell walls (Sanudo-Wilhelmy et al., 2004; Xu et al., 2014) versus intracellular phosphorus pools require further differentiation. Direct effects of phytohormones such as auxins, cytokinins, abscisic acid, and ethylene contained in algae on plants (Riahi et al., 2013) also need further attention. Of separate concern are allelochemicals and/or cyanotoxins which effects are likely plant-dependent and need further investigation (Berry et al., 2008). Comprehensive studies of the effects of algae-based bio-fertilizers on various crops and arable soils remain essential.

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References


