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Biotechnological polyphosphate as an opportunity to contribute to the circularization of the phosphate economy



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Polyphosphates, chains of polymerized phosphate subunits, are used as food additives for various applications such as conservation, water retention, and pH buffering. Currently, the value chain of phosphates is linear, based on mining fossil phosphate rock, which is anticipated to be depleted in a few hundred years. With no replacement available, a transition to a circular phosphate economy, to which biological systems can contribute, is required. Baker's yeast can hyperaccumulate phosphate from various phosphate-rich waste streams and form polyphosphates, which can be used directly or as polyphosphate-rich yeast extract with enhanced properties in the food industry. By maturing the technology to an industrial level and allowing upcycled waste streams for food applications, substantial contributions to a sustainable phosphate economy can be achieved.

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Introduction

Compared with other food additives, phosphates, especially polyphosphates (polyPs), are in a unique position, as other molecules cannot or only partially replace many of the properties. Here, we refer to polyPs as phosphate chains of two phosphate moieties and longer [1]. PolyP is

primarily synthesized chemically through a condensation reaction of phosphate from pure phosphoric acid driven by heat at 800 °C for several hours [2]. Since mainly natural gas is used for energy generation, this is associated with high costs in today's world, including a considerable CO₂ footprint. The resulting polyPs cover 2–40 phosphate subunits on average, with varying physicochemical properties, depending on the chain length [3]. Next to chemical synthesis, organisms can produce polyPs. Here, chain lengths of up to one thousand phosphate subunits have been reported [4].

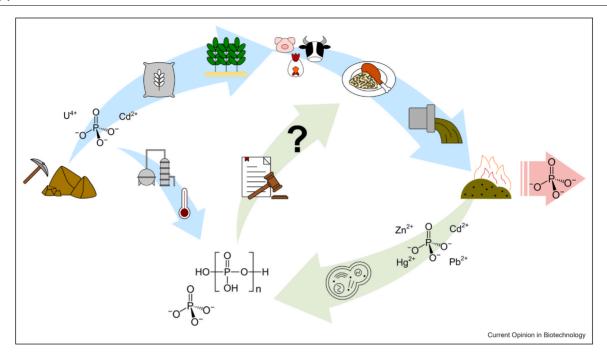
The phosphate economy is primarily linear. Phosphate rock is mined to produce phosphate fertilizer or purified to high-quality phosphoric acid. Phosphate fertilizers are applied to fields and finally lost to the environment [5]. In contrast, phosphoric acid is converted to polyPs and used in food processing with an extensive range of functions, followed by wastewater treatment after consumption, and either lost to the environment or captured in the ash of burned sludge [6]. The leading phosphateusing societies, that is, industrialized countries and eventually the entire world, require a sustainable phosphate value chain, which is difficult to imagine.

Here, we summarize applications of (poly)Ps in the food sector, highlight a new biological technology for polyP production, and suggest how emerging biological technologies can contribute to circularizing the phosphate economy and address changing legislations that challenge the linear phosphate value chain. We discuss how these technologies could improve phosphate reuse immediately and open possibilities emerging for the food industry. Finally, we share our opinions on the need for change, focusing on the end-of-life options of phosphate and the alternatives for polyP synthesis for food applications (Figure 1).

Applications for polyphosphate in food

PolyPs, often referred to as E450 (diphosphate), E451 (triphosphate), and E452 (polyP) in the context of food additives, play a significant role in enhancing the quality and safety of various food products. Notably, in EU biolabeled products, only E451 as CaPolyP is allowed. For completeness, E338 is phosphoric acid, E339 is sodium phosphate, E340 is potassium phosphate, E341 is

Figure 1



Circularization of the linear phosphate economy. The currently linear phosphate economy depends on fossil phosphate rock, which is mined and mainly processed into phosphoric acid or superphosphate. Depending on the contamination grade, the prior is purified for food applications as polyP salts, and the latter is directly used to produce fertilizer for agriculture. Crops are grown as food and as feed for livestock. The phosphate ends up in wastewater and sludge, which is incinerated, and a great portion is lost as only a fraction is recycled due to contamination. Here, biotechnological approaches pose an alternative route for efficient re- and upcycling into polyP, which is purified and could be used purely or, for example, as polyPrich yeast extract in food applications. However, as it originates from waste, legislation must be adjusted to allow recycling into food additives.

calcium phosphate, E342 is ammonium phosphate, and E343 is magnesium phosphate. To meet the diverse needs of the food industry, polyPs of different molecular masses (length, P-content) and different countercations combine multiple favorable properties, making polyPs attractive food additives [7,8]. They exhibit good pH buffering capacity, high water retention, antimicrobial effects, a low-toxicity profile, and chelation activity of multivalent metal cations. The acidifying function is prominent in baking powder, where sodium diphosphate can be used [3], although phosphate-free alternatives exist using tartaric acid instead [9]. Applied to meat and fish products, polyPs lower the shear force of muscle fibers, thereby increasing tenderness and improving water-holding capacity during storage, freeze-thawing, and cooking [10]. Cut portions are kept in form due to lubrication effects, and the product's color is retained by applying polyP [11]. In dairy applications, polyPs act as emulsifying salts in processed cheese by binding calcium, thereby solubilizing casein, an instrumental feature of these products [12,13]. In the European Union, the 'Kebab' debate was prominently decided at the end of 2017 in favor of polyP use, as no suitable replacement for protecting the raw meat while retaining the liquid has been available [14].

Beyond these applications, polyPs have been explored as anticaking agents [15] and iron carriers in iron-fortified food products. This is particularly important to address nutritional deficiencies in developing countries [16]. An additional property of polyPs is their antimicrobial activity. They effectively control various foodborne pathogens [17–19], including *Bacillus cereus*, *Staphylococcus* aureus, Clostridia, and Listeria. This antimicrobial activity improves the safety and shelf-life of food products, thereby diminishing the likelihood of foodborne illnesses. The probable mechanism involves the sequestration of polyvalent cations to reduce the availability of essential nutrients and direct interference with the cell membrane, although little direct evidence exists.

Notably, after decades of discussion on polyPs in food applications, no replacements covering all functions of (poly)Ps have yet emerged [20–22]. The suggestion of polyP as a cause for hyperactivity in children and osteoporosis in adults could not be confirmed [8]. Still, a recommended phosphate dose of 40 mg/kg body weight and, therefore, maximum permitted levels ranging in food from 500 to 20 000 mg/kg expressed as P₂O₅ (depending on the phosphate and the type of food) exist [8], however, mainly motivated by the challenge of phosphate excess for

kidneys [23]. Thus, although detrimental if consumed in high quantities, applying polyP in food is only partly replaceable, and hence, food-grade polyP production is required.

The dilemma of a linear phosphate economy

Food-grade polyP is produced using pure phosphoric acid derived from mined phosphate rock. Reserves are limited, unequally distributed, and exploited at a high rate [24], guaranteeing sufficient supply for food production for a few hundred years only. Deteriorating, most ores are contaminated with heavy metals, that is, uranium and cadmium, requiring intensive refinement before application as food additives [25]. New legislation by the European Union introducing lower thresholds for contamination of phosphoric acid with heavy metals even for its use in fertilizers (i.e. 60 mg/kg of cadmium in phosphate fertilizers today, aiming for a reduction to 20 mg/kg in little over one decade) [26] is challenging as this limit cannot be met by most of the large phosphate rock reserves. However, it is necessary to avoid health and environmental hazards [27]. Owing to a growing world population, food production and the requirement for purified phosphate will increase in the following years [28], not only in the direct application as fertilizer but also in the form of processed polyP in the food industry. Further, the push toward a circular bioeconomy could increase the demand for fertilizer to produce renewable carbon sources, in turn intensifying the phosphate shortage as described by Anlauf [29]. If humanity aims to prevent a food crisis and succeed in establishing circular bioeconomies for other resources, the linear phosphate economy needs to be circularized, allowing the reuse and recycling of phosphate. New technologies are required to refine spent phosphates and sustainably produce polyP [30-32].

Biotechnological production of polyphosphate using baker's yeast

While industrial-scale production of food-grade polyP is currently exclusively based on chemical processes, alternative biological technologies have recently emerged. A well-known polyP-producing organism is Saccharomyces cerevisiae, classified as Generally Recognized as Safe. The production of yeast (extract) is well-established in the food industry [33]. Biotechnological polyP synthesis was reported using S. cerevisiae by incubating them in a phosphate-free medium, causing a starved phenotype. Subsequently, phosphate-starved S. cerevisiae is transferred to a phosphate-rich medium, causing S. cerevisiae to rapidly take up phosphate polymerized to polyP intercellularly. Phosphate starvation and feeding lead to polyP hyperaccumulation, resulting in high intracellular concentrations of up to 28% (w/w) polyP in S. cerevisiae cell dry weight [34] (Figure 2). PolyP hyperaccumulation is nontoxic to S. cerevisiae cells, as they accumulate polyP mainly in the vacuole [35]. PolyP-rich S. cerevisiae can be used to manufacture either polyP-rich yeast extract [34] or pure biopolyP [36]. This approach has not been transferred to industry yet but fulfills several prerequisites for a successful transition.

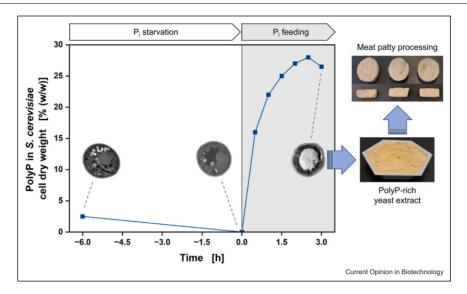
Closing the phosphate value chain in the food and feed sectors

The production of polyP requires a phosphate source to feed starved S. cerevisiae. While baker's yeast can accept various phosphate sources [37,38], applying polyP in requires a food-grade phosphate source. The sources of biophosphate are oil press cakes, for example, from canola or soybean oil mills [39] or brans from wheat and other cereals [40]. Press cakes are frequently used as feed for livestock and contain 1-3% phosphate bound to the sugar inositol, called phytate [41]. Enzymes, namely phytases, have been used in the animal feeding industry for the last decades to mobilize the phosphates from phytate. While usually not all six phosphates are released, Hermann et al. [38] presented a phytase mixture that effectively released most phosphates from phytate. Subsequently, the free phosphate in the hydrolysate was used to feed phosphate-starved S. cerevisiae, resulting in polyP hyperaccumulation. The polyP-rich yeasts were used to produce polyP-rich yeast extract via the established industrial route of yeast extract manufacturing. The polyP-rich yeast extract was tested in meat patty production, acting simultaneously as a texture stabilizer and taste enhancer (Figure 2). This demonstrates the successful utilization of nonfossil, food-grade phosphate sources for polyP production and their application for food production [38]. Thereby, the described technology can contribute to transforming the linear phosphate value chain, exhibiting the severe challenges and dilemma mentioned above, into a circular one, in which spent phosphate is made accessible for recycling and reuse while developing a value-added product for food processing.

Accessing new phosphate sources for foodgrade polyphosphate production

Although pretreating and extracting press cakes could be suitable for acquiring food-grade phosphate, no such process exists in the industry. Instead, the press cake used as feed is mixed with the phytase, making the stored phosphate accessible to livestock. However, most spent phosphate is present in sludge, which is incinerated and partly recovered from the resulting ash and slag (Table 1). New legislation demands higher recovery efficiencies (e.g. the Sewage Sludge Ordinance of Germany, ≥ 50% by 2028 or 2032, depending on plant capacity [42]). The recovered phosphate cannot be used directly to produce food but only indirectly to produce phosphoric acid and phosphate fertilizers for agriculture, as communal wastewater in many cities is contaminated

Figure 2



Hyperaccumulation of polyP and meat patty processing with polyP-rich yeast extract. *S. cerevisiae* is phosphate-starved for six hours, causing the depletion of intracellular phosphate reserves (P_i starvation). Subsequently, when subjected to phosphate in excess (P_i feeding), the cells rapidly take up phosphate and polymerize it to produce polyP. The fed cells can be used to produce polyP-rich yeast extract, which can be applied as a stabilizer and taste enhancer in meat patty processing. Alternatively, polyP can be isolated and purified.

Table 1
Main phosphorus streams in Europe and Germany as potential
phosphate sources for biotechnological recycling.

P stream	Amount in Europe [kt P/a] ^a	Amount in Germany [kt P/a] ^b
Manure	1810	120–260
Municipal wastewater	416	55–70
into sewage sludge	374*	43-51
into sewage sludge ash	262*	24-49
Slaughterhouse waste	312	120-150
Plant meal from oil	n.a.	83°
production (soy, rapeseed)		

- a [51].
- ^b [52].
- * calculated with rates of 90% P from wastewater into sewage sludge and 70% P from sewage sludge into sewage sludge ash (according to [52]).

with pharmaceuticals and heavy metals [43,44]. Only if the recovered phosphate can be purified, an application in food production can be considered. Biotechnology may play a vital role in this regard [45,46]. Microbes can selectively take up phosphate, while heavy metals are left behind, as shown by Dott et al. [47]. A microbial cascade was used, first producing sulfuric acid for phosphate leaching and subsequently phosphate hyperaccumulation for phosphate recovery. This phosphate purification is also easily integrated into the existing polyP production process based on baker's yeast as there is no requirement to adjust the technology; only the

phosphate source is exchanged [38]. As S. cerevisiae is robust toward various phosphate sources and heavy metals [48], the technology could solve multiple challenges in phosphate recovery from sludge, purifying the phosphates to reach food grade, and producing an actual food additive with beneficial properties. However, a maturation of the technology to industrial application, a highly selective phosphate uptake, interlacing of currently separate industries, and adjusted legislation to reuse waste streams to produce food additives would be required [49,50]. In part, these can potentially be addressed by engineering yeast or bacteria to enhance phosphate uptake and polyP accumulation. However, with current legislation regarding the direct application of genetically modified organisms in food, purified polyP instead of polyP-rich (yeast) extract would be the targeted product, requiring extensive progress and financial effort in downstream processing.

Outlook

When will the first bio-polyP be available? The price of polyP is an order of magnitude higher than phosphate fertilizer, however, at sub-10 €/kg, it is still cheap for a microbial intracellular product. The challenge is, hence, to identify a sweet spot in the food industry that goes beyond privileged users in organic food manufacturing. Indeed, the latter high-price market segment could be an entry point for a product whose synthesis is not optimized in every aspect. As suggested, such a product might be polyP-rich yeast extract [34,38].

Although researchers have warned for over a decade [5], phosphate scarcity and the resulting phosphate crisis are not very prominent in the public discussion. The possibilities to either replace phosphates in food applications or use bio-polyP, when available, sketch a future in which food manufacturing contributes to a reduced rate of phosphate use. Again, the primary use of phosphate is fertilizers for efficient plant growth. The envisaged bioeconomy might even increase the need for phosphate fertilizers, and indeed, the discussion on an 'extractive bioeconomy' is in full swing [29]. At the same time, it is reported that the usable land for agriculture stopped increasing but has been decreasing in some regions due to soil loss. Hence, the main driver of increased fertilizer use might not be the envisaged bioeconomy but rather the intensification in areas where fertilizer use was minimal. Anyway, phosphate fertilizer use, despite an ever-growing world population, must not increase. Intense agriculture in greenhouses improves phosphate efficiency enormously. Establishing these for more crops is, however, material- and energy-demanding. A meatreduced diet in the Western world would directly contribute to lowering the phosphate demand. In any case, we should start to reduce the use of this precious element and research possibilities of a sustainable phosphate future. A sustainable phosphate value chain seems feasible [54], requiring enhanced phosphate recycling, a potential opportunity for microbial biotechnology as outlined above.

CRediT authorship contribution statement

Philipp Demling: Conceptualization, Writing - original draft, Writing - review & editing, Visualization, Funding acquisition. Makarius Baier: Conceptualization, Writing - original draft, Writing - review & editing. Alexander Deitert: Conceptualization, Writing - original draft, review & editing. Iana Conceptualization, Writing - original draft, Writing - review & editing. Lars M. Blank: Conceptualization, Writing - original draft, Writing - review & editing. Funding acquisition.

Data Availability

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

Declaration of Competing Interest

The authors declare no competing financial interest. LMB filed two patent applications: "Zusammensetzung, enthaltend getrocknetes Polyphosphat und Verfahren zur Gewinnung von Polyphosphat aus polyphosphat-haltigen Hefezellen dazu" (DE 10 2019 131 561.1) and "Polyphosphatreiche Hefeextrakte und Herstellverfahren dazu" (DE 10 2018 130 081.6, PCT/EP2019/082709).

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