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Review

Wastewater biorefineries: exploring biological phosphorus removal and integrated recovery solutions

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The emphasis on phosphorus removal and recovery from wastewater treatment plants has intensified in recent years due to the urgent need to reduce dependency on nonrenewable phosphorus reserves. Enhanced biological phosphorus removal (EBPR), driven by a diverse community of polyphosphate-accumulating organisms with distinct metabolic capabilities, offers several advantages over chemical precipitation methods. These benefits include reduced chemical use, lower sludge volumes, decreased reliance on costly chemical precipitants, and improved phosphorus recovery quality. Recent advancements in recovery technologies now enable efficient phosphorus extraction from digester supernatant, dewatered digested sewage sludge, and sewage sludge ash, each yielding different recovery efficiencies. Despite these advances, a comprehensive assessment of the phosphorus recovery potential from these target streams in conjunction with EBPR remains crucial and has yet to be fully explored.

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Introduction

Phosphorus is an essential element for agriculture and a broad range of industrial and global food products [1]. It is primarily sourced from finite phosphate rock reserves, and its price has fluctuated considerably in recent decades [2]. Concomitantly, phosphate has been categorised as critical raw material by the European Union, emphasising the need for recovery from waste and wastewater within circular economy initiatives highlighted in the recent revision of the Urban Wastewater Treatment Directive [3,4].

Traditionally, wastewater treatment has focused on nutrient removal, but the field is shifting towards resource recovery and recycling. In wastewaters, phosphorus is predominantly present as orthophosphates, and its removal is achieved through chemical precipitation or enhanced biological phosphorus removal (EBPR), with the latter considered as a more sustainable approach [5]. Despite this, the integration of EBPR with phosphorus recovery remains uncommon in full-scale wastewater treatment plants (WWTPs) globally. Phosphorus recovery from WWTPs holds significant potential to meet a substantial portion of the phosphorus demands for human activities [6,7]. This review explores the biorefinery approach for phosphorus removal and recovery and discusses three key areas: (1) understanding the microbiology that underpins EBPR, (2) highlighting promising methods to identify the physiological traits unique to microorganisms driving EBPR, and (3) evaluating the compatibility of target phosphorus recovery streams in WWTPs with EBPR applications.

Enhanced biological phosphorus removal microbiology

The EBPR process is driven by the activity of polyphosphate-accumulating organisms (PAOs) that synthesise intercellular polyphosphate as a storage polymer. Most of the current understanding of PAOs is derived from studies on the model organism *Ca.* Accumulibacter, including insights into its metabolism, which is well-adapted to the feast-famine conditions typical of WWTPs [8–12]. Phylogenetic classification of *Ca.* Accumulibacter has been based on the polyphosphate kinase (*ppk1*) gene, grouping species into two clades and multiple species, though attempts to link this grouping to distinct functional differences have been unsuccessful [8,13,14]. Recent use of high-quality metagenome-assembled genomes (MAGs) for phylogenomic analysis of *Ca.* Accumulibacter has revealed 19 species, of which eight are known to be present in full-scale WWTPs [15].

In addition to laboratory-scale studies to explore the metabolism of *Ca.* Accumulibacter, integrated multi-omics surveys of full-scale WWTPs provide key insights into the PAO composition and functions that facilitate EBPR in full-scale WWTPs [16]. Such efforts have allowed to confirm PAO species belonging to the genus *Azonexus* (previously identified as *Dechloromonas*) (Figure 1). Notably, *Azonexus*-related PAOs participate in denitrification, with species such as C*a.* Azonexus phosphorivans capable to perform complete denitrification [17]. The genus *Tetrasphaera* has long been regarded as an abundant and important PAO in full-scale plants [18]; however, phylogenetic analysis has reclassified many species to the genera *Ca.* Phosphoribacter and *Ca.* Lutibacillus, with the former being an abundant PAO in many WWTPs [19]. *In situ* studies and metagenomic reconstructions have shown that members of *Ca.* Phosphoribacter lacks the ability to store intracellular storage polymers, such as glycogen or polyhydroxyalkanoates (PHA) but can ferment sugars or amino acids.

Additionally, glycogen-accumulating organisms (GAOs), such as *Ca.* Competibacter, *Defluviicoccus*, and *Propionivibrio*, are frequently found to co-exist with PAOs in WWTPs. While GAOs have been assumed to compete with PAOs for carbon substrates, this competition has been mainly observed in laboratory-scale systems where acetate is used in high concentrations [20,21]. In full-scale plants, if intracellular polyphosphate limits the carbon substrates uptake by PAOs, GAOs can utilise the remainder carbon substrates and co-exist with PAOs [22]. Influent wastewater comprises a diverse mix of carbon substrates, which may provide a competitive advantage to PAOs due to their metabolic versatility. When sugars are used in a laboratory-scale EBPR process, fermentative GAOs (fGAOs), like *Micropruina*, have been observed to be dominant in the microbial community. These fGAOs utilise part of the influent glucose for glycogen synthesis and ferment the remaining glucose into lactate, which may subsequently be utilised by some PAOs [23,24].

Many full-scale WWTPs designed for EBPR report coexistence of a diverse PAO community. It is likely that the complex mix of carbon substrates in influent waste streams in EBPR supports the PAO phenotypic diversity and indicates the different ecological niches that various PAO members occupy [16,25]. PAO abundances vary widely across WWTPs, influenced not only by plant design and operation but also by seasonal variations and immigration from sewer systems [26,27]. The efficient and stable phosphorus removal observed in most EBPR plants can be attributed to the high functional resilience offered by the diverse PAO community.

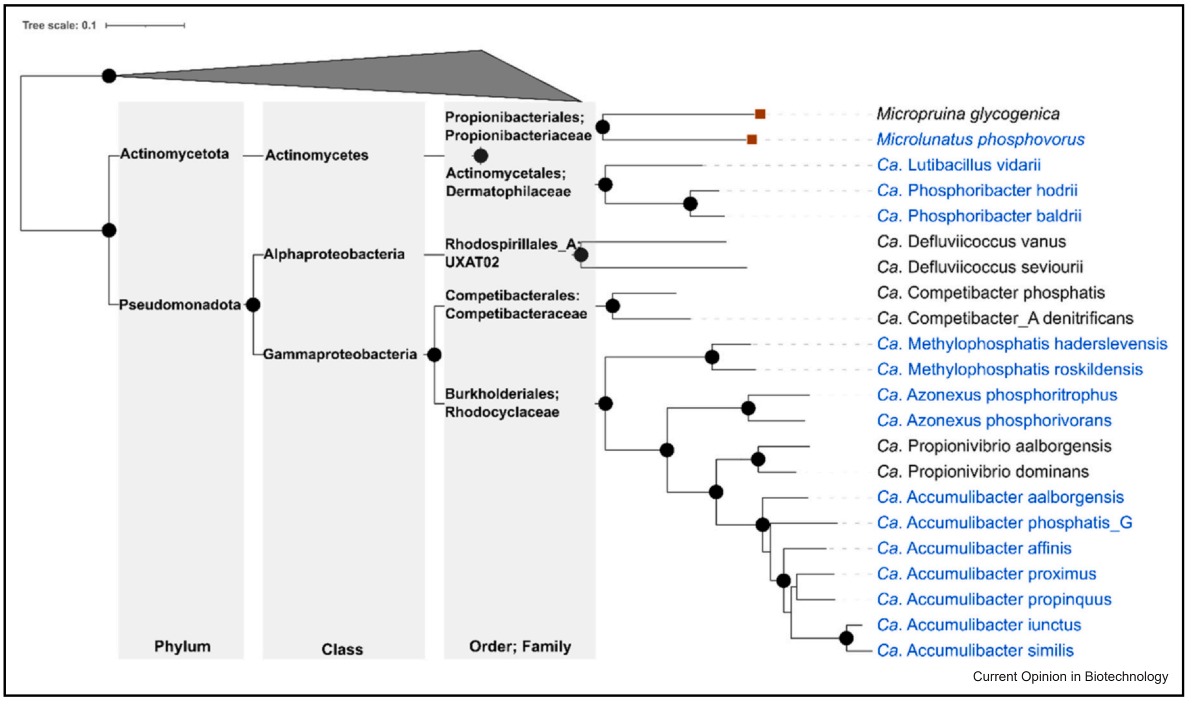
Identifying key physiological traits of polyphosphate-accumulating organisms

Inorganic polyphosphate is ubiquitously stored in cells where it serves as a source of energy and phosphate [28,29]. In bacteria, polyphosphate storage has diverse roles in metabolic activities, such as energy source in cells, phosphorylation of carbon substrate, anaerobic substrate uptake, balance regulatory functions by protein phosphorylation, quorum-sensing, stress response, and virulence [29–32]. In PAOs, the primary function of inorganic polyphosphate is for energy generation to support organic carbon substrate uptake in the absence of electron acceptors. Under anaerobic conditions, *Ca.* Accumulibacter utilises polyphosphate to generate energy for the uptake and storage of organic carbon substrates as PHA. During aerobic conditions, stored PHA is metabolised for growth, while orthophosphate in the mixed liquor replenishes the intracellular polyphosphate reserves. The cyclic feature of polyphosphate accumulation and utilisation in PAOs is attributed to the presence of polyphosphate kinases (encoded by the genes *ppk1* and *ppk2*), high-affinity phosphate transporter (*pst*), and inorganic phosphate transporter (*pit*) [33]. It is important to note that these genes are widely conserved within the bacterial domain [32,34,35]. While PAO metabolism is well understood, the fundamental distinction between PAOs and other heterotrophic organisms in terms of polyphosphate acquisition and regulation is still unknown.

Integrated multi-omics techniques measuring gene expression and metabolite profiling have provided valuable insights into the metabolic regulation in *Ca.* Accumulibacter under dynamic environmental conditions [37]. Previous studies on phosphate uptake regulation have shown inconsistent transcriptomic regulation of the key two-component regulatory system in *Ca.* Accumulibacter, *Dechloromonas*, *Microlunatus phosphovorus*, and *Tetrasphaera elongata* [14,33,35,36]. This inconsistency results in excess orthophosphate transported into the cell whereas the elevated transcription levels of polyphosphate kinases in *Ca.* Accumulibacter suggest efficient orthophosphate condensation to polyphosphate [38,39]. Assessing polyphosphate accumulation and utilisation traits in known PAOs can help distinguish their physiology from that of heterotrophic organisms and support the identification of potential novel PAOs in WWTPs.

Our understanding of the PAO metabolism arises from enrichment cultures, while the inability to cultivate representative PAO members limits physiological insights. Combining the rapidly growing capability of assembling high-quality MAGs with metabolic

Figure 1



Phylogenetic tree showing the diversity and phylogenetic classification of the most abundant genera of PAOs and GAOs. The species representative high-quality MAGs are shown for every genus. The PAO species are in blue. For the *Azonexus* genus, only confirmed PAOs are shown. For *Ca.* Accumulibacter, only species found in WWTPs are shown. The tree is based on the alignment of 120 concatenated proteins created by GTDB-Tk v2.4.0 [36]. Bootstraps are shown with the value of ≥95%. Isolates are identified with dark orange squares. Taxonomic ranks (phylum, class, order, and family) are identified by grey areas. High-quality MAGs from Chloroflexota phylum were chosen as an outgroup.

modelling reveals resource allocation strategies that allow PAOs to thrive in dynamic environmental conditions [12,40,41]. This approach aided in identifying an Nicotinamide adenine dinucleotide-preferring acetoacetyl-CoA reductase in PAOs, suggesting PHA accumulation primarily as a fermentation product rather than a stress response function under anaerobic conditions [42].

Integrating PAO metabolic insights has guided effective process design for stable EBPR in full-scale WWTPs (Table 1). For instance, understanding orthophosphate release during organic carbon uptake without electron acceptors has informed plant designs such as Phoredox and modified University of Cape Town, as well as sidestream applications such as Phostrip and BCFS [43,44]. Furthermore, in the now widely applied aerobic

Table 1

Critical process design parameters that afford stable EBPR in full-scale WWTPs.

Operational parameters	Physiological relevance	Criteria	Reference study
Oxidation reduction potential (ORP)	Reduced conditions characterised by low ORP lead to anaerobic substrate uptake by PAOs	Below −250 mV in anaerobic tank	[44,45]
Solid retention time (SRT)	PAOs are slow-growing organisms, and phosphorus removal performance is strongly linked to operational SRT and temperature.	6–10 days For integration with A-stage processes, a minimum SRT of 4 days is recommended for an operational temperature range of 16–25ºC.	[44,46–48]
Proportion of return activated sludge diverted through sidestream	Impact of low-influent VFA or readily biodegradable COD concentrations can be overcome by hydrolysis/fermentation activity promoted by sidestream processes.	5–20%, with residence time of 15–40 hours Only applied when there is low VFA in the influent	[45,46][43,49]

VFA: volatile fatty acids, COD: chemical oxygen demand granular sludge technology, PAOs are observed to be key to the EBPR process and critical to the structural integrity of the granules. With increasingly stringent effluent discharge criteria, a deeper understanding of PAO physiology can guide technical strategies in full-scale EBPR applications to achieve effluent orthophosphate concentrations as low as 0.5 mg P/l [43].

Efficient phosphorus recovery strategies coupled to biological phosphorus removal processes

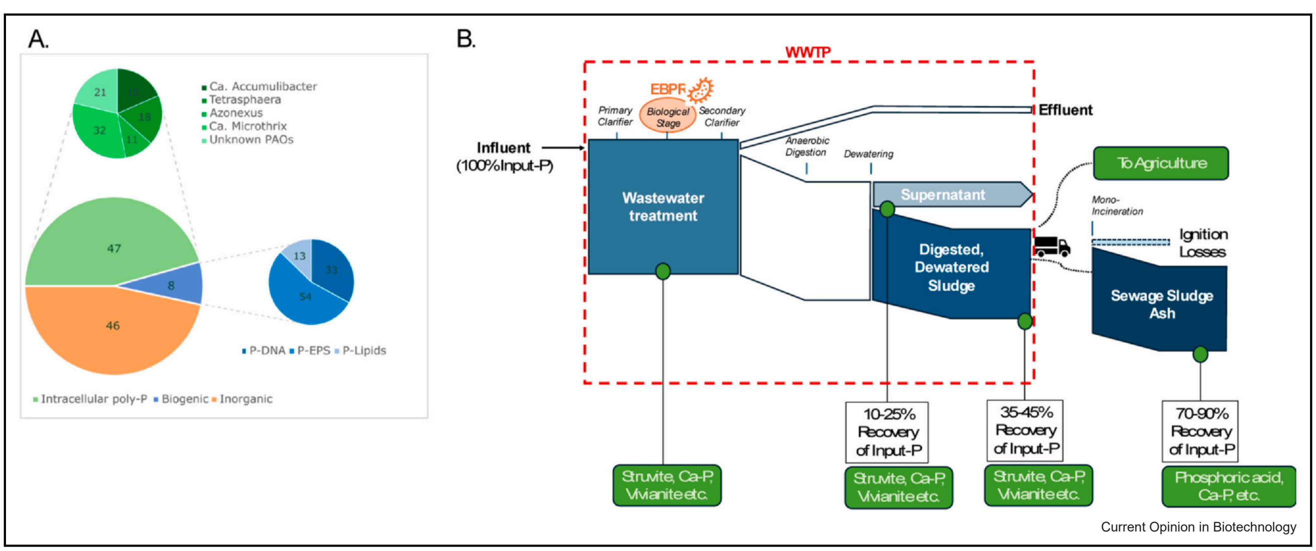
EBPR can be implemented in several ways in municipal WWTPs, with a general distinction between systems targeting the entire wastewater flow ('mainstream') and processes that treat a fraction thereof ('sidestream'). Compared to chemical precipitation methods for phosphorus removal, EBPR minimises chemical use, results in lower sludge volumes, lowers dependence and cost accrued for chemical precipitant, and significantly improves the quality of phosphorus recovered [50]. Furthermore, WWTPs often face significant challenges from pipe clogging caused by the unwanted precipitation of phosphorus-bound salts, which can greatly increase operation and maintenance costs. Following a recent iron-precipitant shortage observed in Germany, the establishment of EBPR in WWTPs was recommended as a strategic response to ensure adequate phosphorus removal and enhance water quality [51].

Phosphorus in activated sludge is available as inorganic phosphates (in the form of salts), biogenic phosphorus (incorporated in nucleic acids and phospholipids), and polyphosphate stored in cells (Figure 2a) [52]. Most recovery technologies require redissolution of the inorganic- and organic-bound phosphorus. The different stages of treatment in WWTPs offer opportunities to recover these different phosphorus fractions. After biological treatment, excess activated sludge undergoes anaerobic digestion and subsequent dewatering to produce a supernatant and digested dewatered sludge. The latter can be incinerated, resulting in phosphorus-rich sewage sludge ash (SSA; Figure 2b), although in some countries, it can also be directly applied to farmland as fertiliser. Thus, the three key streams for phosphorus recovery are the digester supernatant, digested dewatered sewage sludge (SS), and SSA from mono-incineration. Numerous technologies and concepts have been proposed and reviewed extensively [53]. This section focuses on identifying key products that can be recovered from each target stream and exploring how integrating EBPR can improve phosphorus recovery from these target streams [53].

Digester supernatant

During anaerobic digestion, the bacterial phosphorus fraction stored as polyphosphate is released into the liquid phase under the prevalent anaerobic conditions and commonly recovered from digester supernatant by precipitating struvite ($MgNH_4PO_4{\cdot}6H_2O$) and can directly be applied as a slow-release fertiliser in agriculture. Although some heavy metals may be incorporated during crystallisation, the pollutant concentrations are

Figure 2



Overview of phosphorus presence and fate in wastewater treatment. **(a)** Forms of phosphorus, including inorganically bound phosphorus, phosphorus stored in bacteria as polyphosphate, and biogenic phosphorus stored in lipids, nucleic acids, and extracellular polymeric substances. **(b)** Key streams for phosphorus recovery: digester supernatant, digested dewatered SS, and SSA from mono-incineration. The potential recovery products from these key streams such as struvite, vivianite, phosphoric acid, slow-release fertilisers, etc. have been identified.

low enough for safe fertiliser use [54,55]. EBPR implementation increases phosphorus recovery rates from digester supernatant, as more biologically bound P redissolves from the sludge to the liquid phase during anaerobic digestion, resulting in higher orthophosphate concentrations in the supernatant. However, challenges include low recovery efficiency and limited market potential of struvite, partly due to lack of large-scale applications [53,56]. One factor for the low struvite recovery is the presence of iron-phosphate mineral in the form of vivianite ($Fe(II)_3[PO_4]_2{\cdot}8H_2O$) [57]. Dosing iron in the digestor can lead to 60–80% recovery of total phosphate in the influent [5,57–60]. An investigation of vivianite recovery with EBPR implementation requires future attention.

Dewatered digested sewage sludge

Phosphorus recovery from dewatered digested sludge is achieved by dissolving the bacterial and inorganic phosphorus fractions by acidification [61]. The recovered products after chemical precipitation are an amalgamated mixture of struvite, calcium phosphate, magnesium phosphate, brucite, etc, that severely lower recovered phosphorus purity. The extensive use of chemicals and maximum recovery rates reaching 40% of total phosphorus in influent, further limit the viability of this method. Some countries, such as Denmark, France, and Australia, promote the safe use of dewatered sludge as a slow-release fertiliser in agriculture. To alleviate concerns of the presence of emerging contaminants in this recovery method, thermal processing of the SS fraction to destroy organic contaminants may be a viable option [62]. The implementation of EBPR in WWTPs will typically increase intracellular polyphosphates and biogenic phosphorus in the SS, which is expected to enhance the phosphorus recovery from the SS stream.

Sewage sludge ash

When dewatered SS is treated in mono-incineration plants, phosphorus is concentrated in the ash as organic compounds combust. SSA allows highest phosphorus recovery rates by wet-chemical extraction of P from the ash matrix by acidic or alkaline leaching [53]. This method removes organic and microbial contaminants but requires significant chemical use for leaching and subsequent purification of the leachate (e.g. Fe, Al, and other heavy metals). The recovered product, often phosphoric acid, is a well-accepted chemical compound for use in the fertiliser and chemical industry. Industrial-scale phosphorus recovery plants from SSA are currently planned and installed more frequently in Switzerland and Germany due to the respective phosphorus recovery obligation and the associated requirements for the recovered products [63–65]. However, phosphorus recovered from SSA is significantly costlier than direct phosphorus recycling, so in countries where it is allowed, direct land application of SS improves the economic prospects for phosphorus recovery from WWTPs [53].

Phosphorus recovery from the liquid phase of anaerobic reactors in EBPR-based WWTPs has been explored only for orthophosphate recovery or chemical release of bound phosphorus [66,67]. However, polyphosphate is a valuable product already recovered from food industry byproducts at laboratory scale [68]. Implementing direct recovery of polyphosphate from PAOs offers a promising avenue for future research [69].

Conclusions

Attention to phosphorus removal and recovery has significantly grown in recent years. Strategic EBPR implementation effectively removes phosphorus from wastewater and enhances recovery from digester supernatant and dewatered, digested SS fractions. Full-scale WWTPs designed for EBPR report stable performance and a diverse community of PAOs that offers functional resilience ensuring efficient phosphorus removal. Given the significant recovery potential from vivianite or SSA, evaluating the synergistic benefits of these technologies with EBPR at pilot or full-scale implementation is essential to develop a robust techno-economic perspective.

CRediT authorship contribution statement

SR: Conceptualization, Writing – original draft. **JFP**: Conceptualization, Writing – original draft. **SM**: Conceptualization, Writing – original draft. **ZK**: Conceptualization, Writing – original draft. **MvL:** Conceptualization, Writing – review and editing, Funding acquisition. **TW**: Conceptualization Writing – review and editing, Funding acquisition. **PHN**: Conceptualization, Writing – review and editing, Funding acquisition.

Data Availability

Data will be made available on request.

Declaration of Competing Interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us. We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing, we confirm that we have followed the regulations of our institutions concerning intellectual property. We understand that the Corresponding Author(s) is the sole contact for the Editorial process (including Editorial Manager and direct communications with the office). The Corresponding Author(s) are responsible for communicating with the other authors about progress, submissions of revisions, and final approval of proofs.

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