Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 9, No. 12, 148-174
2025
Publisher: Learning Gate
DOI: 10.55214/2576-8484.v9i12.11298

Microbial innovations in phosphorus removal: Advancing biological phosphorus removal for sustainable environmental management and resource recovery

Geeta Verma[1], Debasis Mitra[1], Anju Rani[1*], Priya Chaudhary[2]
[1]Department of Microbiology, Graphic Era (Deemed to be University), Dehradun, Uttarakhand, India; geetav911@gmail.com (G.V.), debasismitra3@gmail.com (D.M.) anju.teotia@gmail.com, anjurani@geu.ac.in (A.R.).
[2]Centre for Herbal Pharmacology and Environmental Sustainability, Chettinad Hospital and Research Institute, Chettinad, Academy of Research and Education, Kelambakkam, Chennai-603103, Tamil Nadu, India; priyachaudhary@care.edu.in (P.C.).

Abstract: Understanding the microbial basis of phosphorus (P) removal is essential for enhancing biological treatment systems. Among various approaches, the Enhanced Biological Phosphorus Removal (EBPR) process utilizes polyphosphate-accumulating organisms (PAOs) that take up and store phosphorus as intracellular polyphosphate under aerobic conditions and release it anaerobically, facilitating practical phosphorus cycling. This cyclical mechanism plays a central role in phosphorus removal and is driven by the distinct metabolism of these microbes. However, the presence of glycogen-accumulating organisms (GAOs) can reduce phosphorus removal efficiency. Extensive research over the past few decades has improved our understanding of how microbial interactions, metabolic pathways, and environmental controls influence system performance. This review traces the progression of PAO-centered strategies, explores the biochemical and ecological mechanisms involved, and highlights recent advances in molecular techniques used for the oversight and optimization of EBPR processes. It also identifies persistent gaps in our understanding of PAO physiology and considers how improved recovery methods could support more sustainable, resource-efficient wastewater treatment.

Keywords: *Enhanced biological phosphorus removal (EBPR), Glycogen-accumulating organisms (GAOs), Phosphorus, Polyphosphate-accumulating organisms (PAOs), Wastewater treatment systems.*

1. Introduction

Phosphorus (P) is a critical macronutrient for growth and development and is second only to nitrogen in its ecological importance. It is a key structural component of cellular membranes and plays an essential role in the biosynthesis of macromolecules, such as DNA, RNA, and proteins. In aquatic environments, phosphorus can be found in several forms, including orthophosphates, polyphosphates, and organic phosphorus compounds. Among these, orthophosphate species (PO_4^{3-}, HPO_4^{2-}, $H_2PO_4^-$, H_3PO_4) are the most soluble and accessible to organisms, allowing direct assimilation without chemical modification [1]. P is an indispensable macronutrient for growth and development, ranking second only to nitrogen in its ecological significance. It constitutes a pivotal structural element in cellular membranes and is integral to the biosynthesis of macromolecules, including DNA, RNA, and proteins. In aquatic ecosystems, phosphorus exists in various forms, including orthophosphate, polyphosphate, and organic phosphorus compounds. Among these, orthophosphate species (PO_4^{3-}, HPO_4^{2-}, $H_2PO_4^-$, H_3PO_4) are the most soluble and readily available to organisms, facilitating direct assimilation without necessitating chemical alteration [2]. Anthropogenic sources, such as municipal wastewater, agricultural runoff, and industrial effluent, are the primary pathways by which excessive phosphorus enters water bodies, including rivers, estuaries, and marine environments. These elevated phosphorus concentrations in aquatic ecosystems lead to nutrient enrichment, resulting in a cascade of ecological imbalances. These include algal blooms,

History: Received: 7 October 2025; Revised: 18 November 2025; Accepted: 24 November 2025; Published: 3 December 2025
* Correspondence: anju.teotia@gmail.com

hypoxia, and biodiversity loss, which collectively cause eutrophication [3]. In response, environmental regulatory agencies, such as the U.S. Environmental Protection Agency (EPA) and the Central Pollution Control Board (CPCB), have implemented stringent regulations on phosphorus discharge to safeguard water quality (Table 1).

Table 1.
Phosphorus Standards According to Different International Regulatory Agencies.

Country	**Regulatory body**	**Phosphorus standard**	**Treatment requirements**	**References**
United Kingdom	Environmental Agency (EA)	≤ 0.1 mg/L	Advanced treatment methods	Ashekuzzaman and Jiang [4]
European Union	European Commission	≤ 2 mg/L (Council Directive 91/271/EEC)	Comprehensive treatment and monitoring	Dalahmeh and Baresel [5]
Germany	Federal Ministry for the Environment	1 mg/L	Advanced biological and chemical treatments	Oleszkiewicz and Barnard [6]
Sweden	Swedish Environmental Protection Agency	0.3-0.5 mg/L	Advanced tertiary treatments	Preisner et al. [7]
Denmark	Danish Environmental Protection Agency	0.05-0.1 mg/L	Advanced biological nutrient removal and chemical treatments	Preisner et al. [7]
Netherlands	Rijkswaterstaat	< 0.15 mg/L	Innovative nutrient removal technologies	Hendriks and Langeveld [8]
Poland	Polish Ministry of Environment	1 mg/L	Mix of traditional and advanced treatment technologies	Preisner et al. [7]
Korea	Ministry of Environment	0.2, 0.3, 0.5 mg/L depending on district	Advanced biological and chemical treatments	Park et al. [9]
India	Central Pollution Control Board (CPCB)	1 mg/L (inland waters), 2 mg/L (public sewers)	Standardized treatment processes	Rosario et al. [10]
United States	Environmental Protection Agency (EPA)	≤ 0.10 mg/L, with 0.01-0.06 mg/L for sensitive areas	Advanced tertiary treatment processes	Gu [11]

Wastewater treatment plants employ a range of removal techniques to reduce the environmental threats posed by excess phosphorus. Physical methods such as screening, sedimentation, gas transfer, and filtration utilize mechanical forces to eliminate particulate matter. The chemical treatment process involves precipitating soluble phosphates by introducing metal salts, such as ferric chloride, alum, lime, or silica, followed by coagulation and flocculation to further aggregate phosphorus. Although these chemical treatments are effective and swift, they may result in unintended effects such as increased sludge generation, disruption of microbial populations in activated sludge systems, and higher operational expenses [12]. To overcome these inherent drawbacks, researchers have increasingly focused on Biological Phosphorus Removal (BPR). These systems rely on naturally occurring microbial consortia comprising bacteria, algae, fungi, and actinomycetes to assimilate phosphorus biologically. A widely used approach is Enhanced Biological Phosphorus Removal (EBPR), which operates by cycling under anaerobic and aerobic conditions. This process encourages the growth of polyphosphate-accumulating organisms (PAOs), which absorb excess phosphorus, and limits the activity of glycogen-accumulating organisms (GAOs) (Figure 1). EBPR is a more sustainable and biologically driven alternative to conventional treatment methods [13]. During the anaerobic phase, PAOs take up volatile fatty acids (VFAs) and convert them into intracellular storage polymers, such as polyhydroxybutyrate (PHB) and polyhydroxyalkanoates (PHAs), powered by the hydrolysis of stored polyphosphates. In the subsequent aerobic phase, these stored carbon compounds are metabolized, enabling PAOs to reaccumulate polyphosphates from wastewater. Many EBPR setups use Sequencing Batch Reactors (SBRs), which facilitate cyclical conditions within a single, time-controlled treatment unit [14]. This review explores EBPR not only as an advanced wastewater treatment method but also as a sustainable approach to managing nutrient pollution. However, the efficiency of the EBPR system is significantly influenced by microbial competition, particularly between PAOs and GAOs. While PAOs contribute to phosphorus removal, GAOs compete for the same carbon sources during anaerobic phases, thereby hindering phosphorus removal. This imbalance can compromise the system's efficiency. Research suggests that adjusting environmental factors, such as pH, temperature, and substrate type, can promote PAO dominance and improve overall phosphorus removal in EBPR systems [15]. Enhancing the performance of EBPR systems requires a deeper understanding of metabolic processes and ecological interactions among key microbial groups. Recent advances in genomics, systematics, and computational biology have enabled the identification of new PAO species and clarified their phosphorus uptake mechanisms. These tools are vital for closing knowledge gaps and advancing the design of robust EBPR systems. In the current global context, interdisciplinary collaboration is increasingly focused on the biological recovery of phosphorus from wastewater. This approach not only supports a circular economy but also helps secure a sustainable supply of phosphorus for agricultural fertilizers, thereby reducing the economic burden on farmers and contributing to global food security [16].

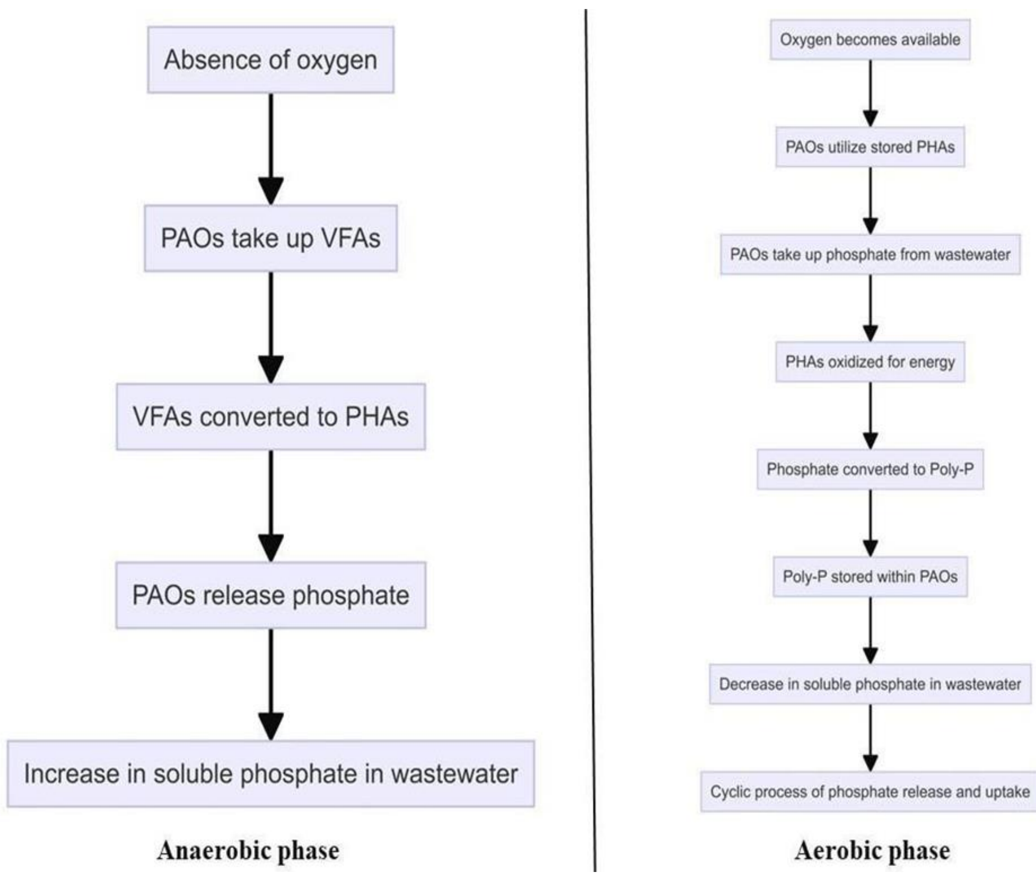

Figure 1.
Mechanism of phosphorus absorption in EBPR.

The methodology employed in this critical review encompasses several key steps aimed at comprehensively evaluating the efficacy of EBPR and the metabolic diversity of PAOs and GAOs. Initially, an exhaustive literature search was conducted on reputable databases such as PubMed, Scopus, and Web of Science, utilizing keywords like "biological phosphorus removal," "polyphosphate-accumulating organisms," "glycogen-accumulating organisms," and "wastewater treatment," "phosphorus removal," "biological wastewater treatment," "efficiency enhancement," "sustainable environmental management," "advancements," "wastewater treatment practices," "environmental sustainability," "nutrient removal," "water quality improvement," "environmental management strategies." Relevant studies were identified and systematically reviewed to extract data on the metabolic capabilities of PAO and GAO, as well as on the influence of environmental factors on PAO and GAO populations. A comprehensive understanding of the phosphorus removal process can be achieved by integrating genomic data provided by previous studies. This will help the scientific community in the strong and insightful analysis of enhanced biological phosphorus removal by PAOs. Figure 2 summarizes the search tools and strategies used in this study.

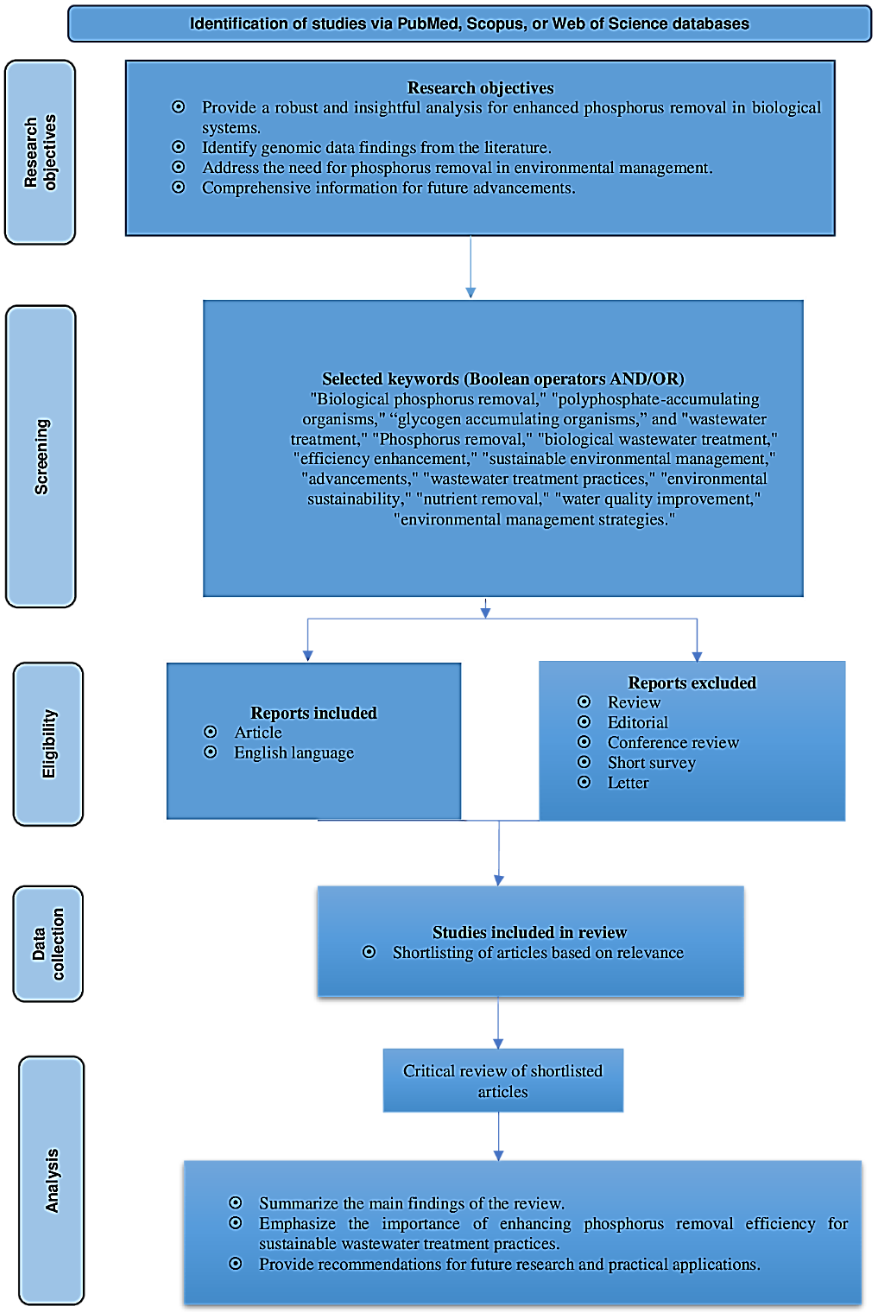

Figure 2.
Step-wise representation of the search strategy.

2. Substrate and Carbon Source Preferences

Substrates play an important role in Enhanced Biological Phosphorus Removal (EBPR) by influencing metabolic pathways and selection pressures that affect the overall community structure. The type, availability, and stoichiometry of carbon sources directly affect phosphorus cycling dynamics, PAO enrichment, and competition with glycogen-accumulating organisms (GAOs). Recent studies have explored a range of carbon sources, from short-chain volatile fatty acids to industrial byproducts and

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 9, No. 12: 148-174, 2025
DOI: 10.55214/2576-8484.v9i12.11298

alternative electron donors, revealing diverse ways that shape PAO enrichment and function Zhang et al. [17]. Oehmen et al. [18] investigated the impact of various volatile fatty acids (VFAs) on polyphosphate-accumulating organisms (PAOs) using a membrane bioreactor at the University of Cape Town. A comparative analysis of acetate and propionate revealed that propionate significantly enhanced phosphorus removal. Specifically, when propionate accounted for 76% of the total chemical oxygen demand (COD), phosphorus release was 155 ± 17.7 mg P/L, and uptake was 213.7 ± 11.4 mg P/L. In contrast, acetate performed poorly. These findings highlight the effect of propionate on PAO metabolism and suggest its potential as a preferred carbon source for optimizing the efficiency of biological phosphorus removal (EBPR) in wastewater treatment systems. The potential of glycerol in EBPR systems was also assessed. Elahinik et al. [19] examined glycerol-fed aerobic granular sludge reactors and observed an initial accumulation of 1,3-propanediol (1,3-PDO) during glycerol fermentation, which was not anaerobically utilized by PAOs. However, with continued operation, 1,3-PDO production decreased, and anaerobic COD uptake improved. Metagenomic and metaproteomic analyses identified fermentative bacteria, such as *Tessaracoccus* and *Micropruina*, as mediators of glycerol transport and metabolism, indirectly supporting PAO activity through a syntrophic food chain. The system was stabilized with effective phosphorus removal, indicating the feasibility of using glycerol as a substrate in granular systems with adapted consortia. The temporal dynamics of carbon loading and their metabolic implications for PAOs were further elucidated by Crnek et al. [20] who demonstrated that aerobic-phase acetate spikes disrupted phosphorus removal, but the withdrawal of acetate restored function, even within the same cycle. When polyphosphate was limited, the PAO-rich sludge exhibited greater glycogen recovery and higher polyhydroxyvalerate (PHV) production. The researchers proposed that PAOs, under anaerobic conditions, might switch to alternative metabolic pathways, such as glyoxylate and methylmalonyl-CoA, potentially utilizing segments of the tricarboxylic acid (TCA) cycle. These results indicate that PAOs can adapt their metabolism to fluctuating conditions, thereby sustaining phosphorus removal, even when typical resources are scarce. Ni et al. [21] isolated a novel strain, *Acinetobacter lwoffii* (P5), and evaluated its phosphorus removal capacity at varying phosphorus-to-carbon (P/C) ratios. When the P/C ratio was 1/40 or higher, the bacteria utilized polyphosphate-based metabolism, achieving up to 82% phosphorus removal. Conversely, at lower P/C ratios (1/100 or less), bacteria shifted to glycogen-based metabolism, resulting in reduced phosphorus removal. This indicates that polyphosphate-accumulating organisms (PAOs) can alter their metabolic pathways in response to nutrient levels, highlighting the importance of balancing phosphorus and carbon for optimal performance in enhanced biological phosphorus removal (EBPR) systems. The interaction between carbon and alternative electron donors also influences substrate-driven selection. Guo et al. [22] investigated the effect of elemental sulfur addition on microbial competition in carbon-limited EBPR systems. They examined various carbon-to-sulfur (C/S^0) ratios and found that at very high (0.28) or very low (0.07–0) ratios, sulfur-utilizing bacteria outcompete glycogen-accumulating organisms (GAOs), leading to improved phosphorus removal. However, at a medium ratio (0.14), GAOs predominated owing to their faster acetate uptake. This study demonstrated that sulfur addition can shift the microbial balance in favor of organisms that enhance phosphorus removal. Wei et al. [23] examined PAO behavior in real wastewater treatment plants by conducting batch experiments with EBPR sludge using acetate and propionate as carbon sources. Their findings revealed that PAOs primarily utilized glycogen for energy under anaerobic conditions, with some contribution from the tricarboxylic acid (TCA) cycle. Acetate is associated with polyhydroxyvalerate (PHV) production via a modified metabolic pathway. These results are in good agreement with laboratory-based PAO models, indicating that such models can also explain the processes in full-scale systems. This supports the notion that laboratory research can aid in predicting and enhancing the real-world phosphorus removal performance.

3. Community Assembly and Microbial Ecology

The stability and efficiency of Enhanced Biological Phosphorus Removal (EBPR) systems are contingent upon the structure of the microbial communities, particularly the growth and maintenance of polyphosphate-accumulating organisms (PAOs). These communities are influenced by environmental conditions, operational settings, and inter-microbial competition. Recent advancements in high-throughput sequencing and ecological theory have provided scientists with improved tools for investigating how deterministic and stochastic processes influence PAO selection. These insights are crucial for enhancing the long-term performance and reliability of EBPR systems for practical wastewater treatment applications. Tatari et al. [24] investigated the impact of allylthiourea (ATU), a nitrification inhibitor, on microbial communities within an EBPR system over a 288-day period. The reactor was enriched with *Tetrasphaera*-containing PAOs. The introduction of ATU resulted in a more stable and predictable microbial community, with a shift in the dominant *Tetrasphaera* group from Clade III to Clade I. Genes associated with phosphorus removal exhibited increased activity, whereas those related to nitrification and bacteria such as *Nitrosomonas* were diminished. However, the removal of ATU led to the overgrowth of filamentous bacteria, such as *Thiothrix*, disrupting the system. This indicates that although ATU can guide microbial growth, its discontinuation can lead to significant issues. Ge et al. [25] examined the temporal evolution of microbial communities during the start-up of an enhanced biological phosphorus removal (EBPR) system in a laboratory-scale sequencing batch reactor. Over six weeks, the system achieved 97.8% phosphorus removal and 97.5% total organic carbon (TOC) removal, with polyphosphate-accumulating organisms (PAOs) gradually becoming the dominant microbes. Using fluorescence in situ hybridization (FISH) and denaturing gradient gel electrophoresis (DGGE) techniques, researchers observed increasing microbial diversity, including groups such as α-, β-, and γ-Proteobacteria and genera such as *Rhodococcus* and *Pseudomonas.* The findings demonstrate that EBPR startup is a dynamic process in which PAO population growth is linked to improved system stability and performance over time. Ruiz-Haddad et al. [26] conducted a comprehensive genomic review of diverse PAOs. They identified key groups, such as *Candidatus accumulibacter* and *Candidatus dechloromonas*, as proficient in producing polyhydroxyalkanoates (PHAs) and utilizing polyphosphate for energy. Other genera such as *Tetrasphaera* and *Phycicoccus* employ different enzymes and transport systems, including polyphosphate-glucokinase. This study revealed significant variability among PAOs in substrate utilization, energy conservation methods, and redox strategies. The authors also emphasized the utility of FISH-Raman spectroscopy, a technique that facilitates the detection of polyphosphate granules within cells, thereby enabling the correlation of genetic traits with actual microbial activity in EBPR systems. Zilles et al. [27] investigated the microbial communities in two full-scale Orbital wastewater treatment plants operating under aerated–anoxic conditions, as opposed to the conventional anaerobic–aerobic cycle used in EBPR. Using flow cytometry and FISH, they found that *Candidatus sccumulibacter* phosphatis was present but not dominant. Instead, a diverse array of PAOs was observed, which were likely adapted to the unique operational conditions. The absence of a single dominant PAO group suggests that alternative EBPR systems can support diverse microbial communities, which may contribute to system stability and functionality by allowing different microbes to share or partition roles in phosphorus removal.

4. Metabolic Flexibility and Functional Behavior

This study investigated the adaptive mechanisms of *Candidatus accumulibacter* under prolonged anaerobic conditions with limited carbon availability. Typically, these organisms utilize stored glycogen for energy; however, researchers have discovered that they can also degrade their own extracellular polymeric substances (EPS) when glycogen is depleted. This capability enables them to continue synthesizing polyhydroxyalkanoates (PHAs) and releasing phosphate, thereby enhancing their survival under stress conditions. This study demonstrated that EPS, previously considered stable and inactive, actively contributes to the functionality of polyphosphate-accumulating organisms (PAOs) under challenging conditions. This finding elucidates a critical survival strategy in carbon-limited environments for enhanced biological phosphorus removal (EBPR) [28]. Expanding on intra-PAO diversity, Gu et al. [29] used genome-resolved metagenomics and bioreactor experimentation to compare two clades of *Candidatus accumulibacter*. Although both clades functioned as typical PAOs, they exhibited distinct

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 9, No. 12: 148-174, 2025
DOI: 10.55214/2576-8484.v9i12.11298

genetic characteristics. One clade demonstrated the ability to perform denitrification and utilize a broader range of carbon sources, whereas the other exhibited a more restricted metabolism. These findings suggest that different clades of *Accumulibacter* may occupy unique ecological niches within EBPR systems. Selecting specific clades can optimize system performance based on treatment objectives, such as simultaneous phosphorus and nitrogen removal through denitrification. Behavioral plasticity among PAOs was further elucidated by Kristiansen et al. [30], who identified phenotypic variability within individual microbial cells in a full-scale EBPR plant. Employing single-cell Raman microspectroscopy, they observed that while some cells exhibited typical PAO behavior, others stored PHA without concomitant phosphate release. This variation occurs even within genetically similar groups, indicating that minor environmental changes or stochastic metabolic shifts can generate subpopulations with distinct functional roles. These findings underscore the complexity of PAO communities, with individual cells exhibiting differential responses based on internal and external conditions. In a systems biology study, Rubio-Rincón et al. [31] developed a genome-scale metabolic model for *Candidatus accumulibacter* to simulate the effects of process configuration on intracellular polymer cycling. This model facilitated predictions regarding the influence of various process setups on PAO energy storage and utilization. It revealed that PAOs can modulate their metabolism depending on whether they are supplied with acetate or propionate. The model also identified a trade-off: when PAOs prioritize PHA synthesis, they may exhibit reduced phosphate uptake, and vice versa. These insights provide valuable guidance for optimizing EBPR system performance by understanding how PAOs balance their internal processes under varying conditions. Researchers contributed to understanding the dynamics of storage polymers, specifically polyphosphate, PHA, and glycogen, during cycling in *Candidatus accumulibacter*. Through batch tests and targeted metabolite assays, they demonstrated that the organism can adjust its intracellular polymer ratios in response to carbon availability and operational phase. This plasticity in polymer management underpins PAOs' metabolic resilience, enabling them to maintain phosphorus removal efficiency even under suboptimal conditions. Roy et al. [16] used proteomics to study the metabolic activity of *Tetrasphaera* using a lab-scale EBPR system. They found high levels of proteins involved in amino acid breakdown, glycolysis, and the regulation of polyphosphates. These results suggest that *Tetrasphaera* uses a different metabolic strategy than *Accumulibacter*, which is often considered the standard PAO model. This supports the idea that metabolic flexibility is not only a property of specific clades but also a general trait distributed across diverse PAO taxa. This broader understanding could aid in designing systems that support a wider range of efficient phosphorus-removing organisms.

5. Stress Response and Environmental Inhibitors

The efficacy and stability of Enhanced Biological Phosphorus Removal (EBPR) systems are significantly influenced by environmental stressors, including temperature fluctuations, pH variations, toxic compounds, and limitations in electron acceptors. Zeng et al. [32] provided early insights into the temperature sensitivity of Polyphosphate-Accumulating Organisms (PAOs). Through batch experiments, they demonstrated that both anaerobic phosphate release and aerobic uptake rates declined markedly at temperatures exceeding 30°C. The authors also observed increased acetate uptake lag times and reduced glycogen consumption under thermal stress. Such physiological challenges diminish PAO competitiveness and may lead to washout or proliferation of non-PAO organisms, particularly in tropical environments or during summer operations. Researchers investigated the impact of low oxygen levels on EBPR systems, focusing on *Candidatus accumulibacter*. They found that this organism can still take up phosphate and cycle polyhydroxyalkanoates (PHAs) under microaerobic conditions. However, when the dissolved oxygen levels fell below 0.1 mg/L, phosphorus removal became less effective. This suggests that PAOs can manage both aerobic respiration and denitrification under low-oxygen conditions but only within a narrow range. These findings help define the optimal oxygen levels for stable EBPR operation. To address chemical toxicity, Li et al. [33] examined the effect of heavy metals, particularly Cu and Zn, on the metabolic functions of *Candidatus accumulibacter*. They found that even low levels of these metals reduced bacteria's ability to store polyphosphate and disrupted PHA production. The study suggested that metal toxicity disrupts the cell's redox balance, possibly by inhibiting essential enzymes, such as polyphosphate kinase and NADH-dependent dehydrogenases. These findings highlight PAOs' vulnerability to trace-level contaminants commonly found in industrial wastewater. Feng et al. [34] explored the effects of pH and free nitrous acid (FNA) on the stability of PAO populations. They observed that acidic conditions ($pH < 6.5$) and elevated FNA levels inhibited phosphate release and PHA synthesis during the anaerobic phase, with a significant disruption in the activity of glycogen-degrading enzymes. These findings suggest that FNA acts as a metabolic inhibitor by targeting key enzymatic steps in PAO metabolism, particularly under suboptimal pH conditions, thus destabilizing the EBPR performance. Researchers employed meta-omics approaches to study community shifts under nitrite and nitrate stress from a broader ecological perspective. They reported that high nitrate loading enriched denitrifying Glycogen-Accumulating Organisms (GAOs), which outcompeted PAOs under certain conditions. Functional gene analysis revealed the downregulation of polyphosphate metabolism genes and increased expression of stress-response proteins, including chaperones and efflux pumps. These findings indicate an intricate link between nitrogen stress, community restructuring, and EBPR failure modes. Collectively, these studies underscore that while PAOs possess mechanisms to cope with mild environmental fluctuations, they remain vulnerable to extreme temperature, pH, redox potential, and chemical toxicity. More importantly, stress factors often operate synergistically, exacerbating their effects and tipping the balance toward less desirable microbial communities. Designing EBPR systems that minimize stress exposure through tight control of operational parameters and mitigation of inhibitory compounds can safeguard both microbial functionality and long-term phosphorus removal.

6. Alternative or Unconventional PAOs

Although *Candidatus accumulibacter* phosphatis has historically been the central focus of enhanced biological phosphorus removal (EBPR) research, recent studies have identified additional microorganisms capable of polyphosphate accumulation. These alternative polyphosphate-accumulating organisms (PAOs) often thrive under atypical environmental conditions by employing distinct metabolic strategies. Andres and Carvallo [35] investigated EBPR systems for treating high-strength wastewater using anaerobic-anoxic-aerobic sequencing batch reactors. Notably, despite the low abundance of typical PAOs, this system effectively removes phosphorus. The microbial community is predominantly composed of facultative anaerobes and fermenters. Through metaproteomics, researchers identified enzymes associated with polyphosphate production, indicating that other, potentially unknown, or hybrid organisms facilitated phosphorus removal. Welles et al. [36] discovered that *Tetrasphaera*-like organisms play a significant role in phosphorus removal in full-scale wastewater treatment plants. Unlike *Accumulibacter*, which stores polyhydroxyalkanoates (PHAs) under anaerobic conditions, *Tetrasphaera* employs a different strategy: it ferments sugars and amino acids, stores glycogen anaerobically, and removes phosphate aerobically. This finding challenges the notion that PHA storage is essential for the EBPR. The study also demonstrated that plants with higher *Tetrasphaera* populations maintained stable phosphorus removal even when *Accumulibacter* levels were low. Kristiansen et al. [30] utilized fluorescence in situ hybridization (FISH) and metagenomic analysis to reveal that *Tetrasphaera* species were highly abundant in Danish wastewater treatment plants. These bacteria were found to harbor key genes, such as polyphosphate kinases and phosphate transporters, confirming their role in phosphorus removal, despite not exhibiting the classic carbon metabolism observed in *Accumulibacter*. Their ability to thrive under nutrient-rich but unstable conditions suggests a strong adaptive advantage. This supports the hypothesis that *Tetrasphaera* is a significant PAO, particularly in systems under environmental stress or operational imbalance. Researchers contributed to the understanding of PAO diversity by identifying PAO-like traits in *Chloroflexi* and *Planctomycetes* that are not typically associated with phosphorus removal. In laboratory-scale reactors, these microbes contain polyphosphate granules and express phosphate transporter genes, indicating their participation in phosphorus cycling. Their role became more pronounced under conditions such as extended sludge retention times and low organic load. This suggests that EBPR systems may harbor overlooked microbial groups with critical metabolic functions and that phosphorus removal is supported by a broader range of organisms than traditionally recognized (Figure 3).

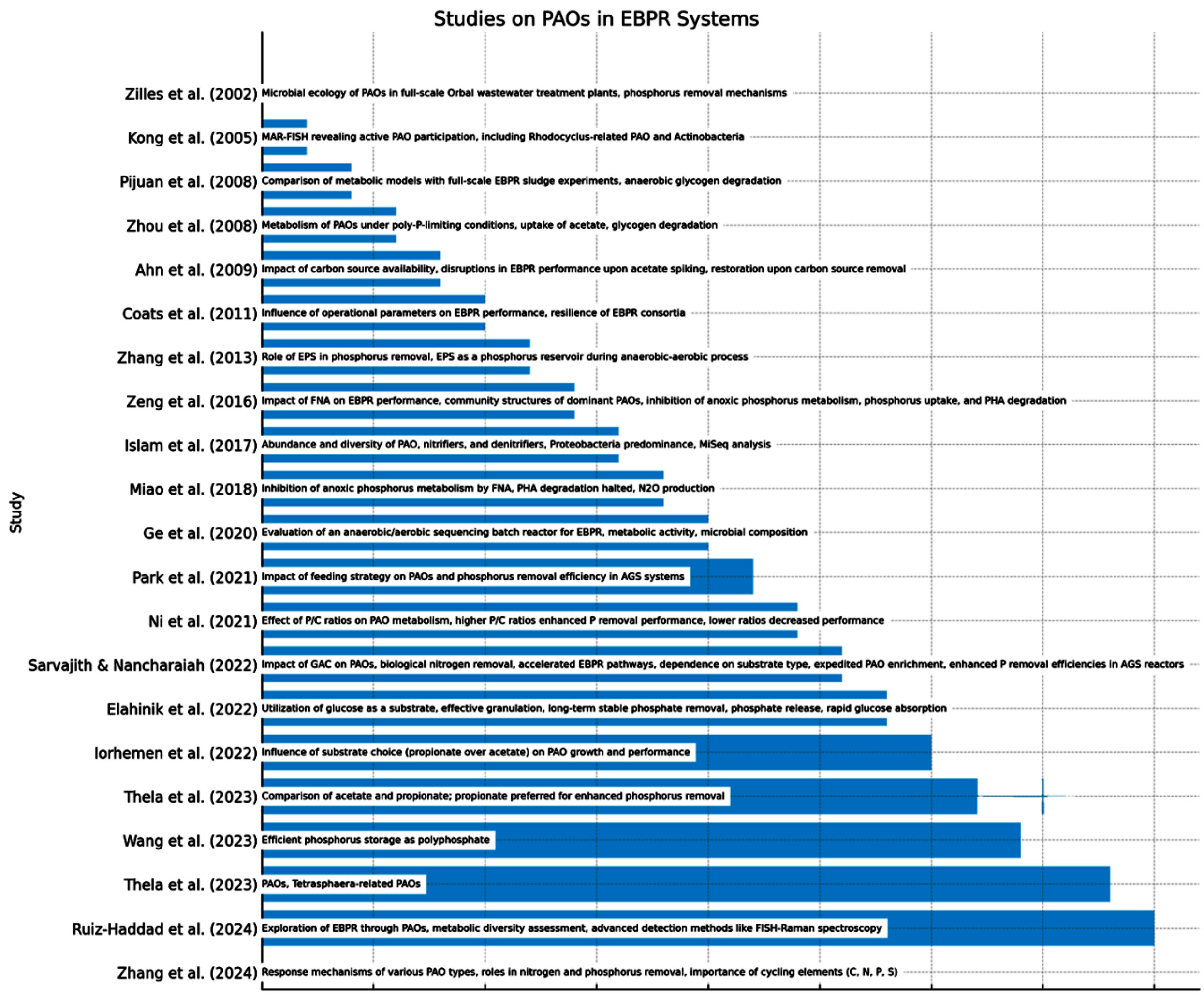

Figure 3.
Studies on PAOs in EBPR systems.
Source: Zilles et al. [27], Kong et al. [37], Pijuan et al. [38], Zhou et al. [39], Ahn et al. [40], Coats et al. [41], Zhang et al. [42], Zeng et al. [43], Islam et al. [44], Miao et al. [45], Ge et al. [25], Park et al. [46], Ni et al. [47], Sarvajith and Nancharaiah [48], Elahinik et al. [19], Iorhemen et al. [49], Thela et al. [50], Wang et al. [51], Thela et al. [52], Ruiz-Haddad et al. [26] and Zhang et al. [53].

7. Denitrification in EBPR Systems

Polyphosphate-accumulating organisms (PAOs) have been primarily studied for their role in phosphorus removal; however, their capacity for denitrification in anoxic environments has attracted growing interest. The potential for simultaneous nitrogen and phosphorus removal through denitrifying PAOs (DPAOs) is particularly beneficial for energy-efficient wastewater treatment systems. Nonetheless, leveraging this dual functionality depends on the complex interplay between microbial characteristics, clade-level diversity, and reactor operational strategies. Peng et al. [54] investigated the feasibility of achieving endogenous partial denitrification (EdPD) in an A/O/A (anaerobic–aerobic–anoxic) enhanced biological phosphorus removal (EBPR) system. By initially restricting PAO growth under anaerobic/anoxic conditions, the system promoted the proliferation of *Competibacter*, a glycogen-accumulating organism (GAO), which increased the nitrite transformation rate (NTR) from 29.4% to 82.8%. Upon re-establishing aerobic conditions, both *Competibacter* and *Accumulibacter* coexisted, resulting in 88.6% phosphorus removal efficiency and a sustained NTR of 67.7%. This study demonstrated that modulating the balance between PAOs and GAOs can facilitate EdPD, thereby creating conditions conducive to efficient nitrogen removal via downstream anammox processes. Expanding on the role of GAOs in denitrification, Bin et al. [55] cultivated GAO-dominated granules in a laboratory-scale sequencing batch reactor and demonstrated their effective denitrifying capabilities. These granules, rich in tetrad-forming organisms (TFOs), achieve complete nitrate reduction, with some nitrite accumulation and nitrogen gas as the primary end product. Microscopic and fluorescence in situ hybridization (FISH) analyses confirmed the strong presence of GAOs throughout the granules, while PAOs were significantly inhibited. Although this system was not optimized for phosphorus removal, it presents a promising strategy for energy-efficient nitrogen elimination through the selective enrichment of denitrifying GAOs. Zou et al. [56] evaluated a novel two-sludge EBPR system designed to cultivate denitrifying PAOs under anaerobic and anoxic conditions. Laboratory tests revealed robust phosphorus release and uptake, and analysis of the biomass after the anoxic phase indicated a phosphorus content of approximately 12.3% of the dry weight. Molecular tools, such as FISH and polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE), revealed that *Accumulibacter* constituted nearly 70% of the microbial community. This study demonstrated that denitrifying PAOs can be successfully enriched using this configuration and showed that separating nitrification and denitrification processes can enhance microbial control and overall EBPR system performance. However, not all PAOs exhibit a consistent denitrifying potential. Rubio-Rincón et al. [31] focused on *Candidatus accumulibacter* clade IC, which was previously hypothesized to perform anoxic phosphorus uptake by using nitrate as an electron acceptor. Despite achieving a highly enriched EBPR culture composed of approximately 97% clade IC, the study observed minimal anoxic phosphorus uptake activity, at most 13% of the aerobic rate. Genome-resolved metagenomics has identified a new species, *C. Accumulibacter* delftensis, which lacks respiratory nitrate reductase (nar) genes, possesses only assimilatory (nas) and periplasmic (nap) nitrate reductases. These findings suggest an energetic limitation that impedes effective nitrate respiration, underscoring the need to differentiate between PAO clades when predicting denitrification potential.

8. Competition Dynamics Between PAOs and GAOs

In enhanced biological phosphorus removal (EBPR) systems, competition between polyphosphate-accumulating organisms (PAOs) and glycogen-accumulating organisms (GAOs) is critical for process stability and nutrient removal efficiency. Although PAOs are essential for phosphorus uptake, GAOs may prevail under specific conditions, thereby disrupting the phosphorus cycling. Nevertheless, recent studies have indicated that this interaction is neither static nor universally adversarial. Instead, results are determined by complex interactions among environmental factors, carbon availability, microbial community composition, and operational strategies that affect both competition and coexistence.

8.1. Environmental Factors Modulating PAO-GAO Competition

Temperature, substrate type, and pH are critical environmental parameters influencing the competitive dynamics between polyphosphate-accumulating organisms (PAOs) and glycogen-accumulating organisms (GAOs). Wang [57] investigated the effects of temperature, pH, and carbon source on the competition between PAOs and GAOs in enhanced biological phosphorus removal (EBPR) systems, particularly at elevated temperatures exceeding 30 °C. This study revealed that the type of carbon source and the specific GAO species present were significant factors. *Accumulibacter* PAOs demonstrated superior performance to *Defluviicoccus vanus* GAOs when butyrate was utilized, whereas Competibacter GAOs exhibited enhanced performance with propionate. The PAOs showed a preference for a combination of acetate, propionate, and butyrate, which facilitated increased volatile fatty acid (VFA)

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 9, No. 12: 148-174, 2025
DOI: 10.55214/2576-8484.v9i12.11298

uptake and phosphorus removal. Butyrate also inhibits GAO metabolism and promotes the development of novel polyhydroxyalkanoate (PHA) production pathways in PAOs, thereby enhancing their dominance and improving overall system performance. Similarly, Weissbrodt et al. [58] emphasized the role of pH and temperature in granular sludge reactors. Alkaline pH values (>7.3) and lower mesophilic temperatures (<20 °C) were found to enrich *Accumulibacter*, leading to improved nutrient removal performance selectively. Under these conditions, GAOs were less favored, indicating that pH and temperature act as co-regulators of microbial selection in the EBPR systems. The availability and distribution of organic carbon significantly affected PAO-GAO dynamics. Carvalheira et al. [59] reported that under carbon-limiting conditions, GAOs exhibit a biomass decay rate nearly four times higher than that of PAOs. The ability of PAOs to retain residual PHAs under aerobic conditions supported sustained maintenance metabolism and conferred a strategic advantage in systems with fluctuating or low influent carbon levels. Acevedo et al. [60] examined the metabolic adaptability of PAOs under nutrient stress, using a dynamic metabolic model. They discovered that PAOs could transition from a polyphosphate-based to a glycogen-based VFA uptake pathway when internal polyphosphate reserves are depleted, effectively adopting a GAO-like phenotype. This metabolic plasticity may enable PAOs to endure unfavorable conditions, although it also suggests functional convergence between PAOs and GAOs under specific stress scenarios.

8.2. Synchronicity in Full-Scale Systems

Contrary to the conventional perception that GAOs are solely detrimental, several comprehensive studies indicate that their presence does not invariably compromise EBPR performance. Stokholm-Bjerregaard et al. [13] conducted a 9-year study across 18 Danish wastewater treatment facilities, revealing that, despite a significant prevalence of GAOs such as *Defluviicoccus*, *Micropruina*, and *Propionivibrio*, phosphorus removal remained effective. In numerous instances, Tetrasphaera, a PAO from the Actinobacteria group, has emerged as the primary contributor to EBPR. These findings highlight the significance of phylogenetic diversity, metabolic complementarity, and community structure over mere abundance ratios in determining EBPR outcomes. Studies of tropical EBPR further corroborate this understanding. Law et al. [61] demonstrated that *Accumulibacter* maintains its activity and competitiveness at elevated ambient temperatures following operational modifications. Although the GAO levels also increased, phosphorus removal improved overall. Metagenomic and transcriptomic analyses revealed that *Accumulibacter* exhibited a higher expression of key metabolic genes associated with glycolysis and the TCA cycle, indicating their adaptive capacity. These findings challenge the prevailing notion that tropical temperatures invariably favor GAOs over PAOs in EBPR systems. Recent evidence suggests that PAOs and GAOs may engage in functional cooperation under specific conditions. Rubio-Rincón et al. [31] discovered that in enriched cultures, GAOs could perform partial denitrification by converting nitrate into nitrite, which PAOs then utilize to uptake phosphorus under anoxic conditions. This cooperative interaction enhanced the overall nutrient removal. These findings indicate that GAOs may indirectly support the PAO function, particularly in systems targeting the removal of both nitrogen and phosphorus.

9. Process-Level Strategies to Modulate Competition

To improve the EBPR performance, new strategies are being designed to support PAOs while limiting the growth of GAOs. Chen et al. [28] tested an alternating anoxic/oxic/anoxic (A/O/A) setup in constructed wetlands using the SNEDPR process. This approach causes GAOs to oxidize their stored glycogen during the early anoxic phase, leaving them with less carbon for later use. As a result, PAOs have a better chance of growing and performing phosphorus removal. This research demonstrated significant advantages: phosphorus removal improved, and the proportion of GAOs to PAOs decreased. Wang et al. [62] showed that side-stream EBPR (S2EBPR) can effectively boost PAO growth. In this system, sludge is directed to a separate anaerobic tank where it ferments, producing more volatile fatty acids (VFAs). These enriched conditions help PAOs thrive. Compared with traditional A2O setups, S2EBPR systems showed greater microbial diversity, more stable PAO activity during long anaerobic phases, and better overall phosphorus removal. This study highlights how adjusting the system design, such as adding a side-stream fermenter, can shift the microbial balance and enhance EBPR efficiency.

10. Genomic Insights into Phosphorus-Accumulating Organisms (PAOs)

Recent advances in genomic sequencing have significantly enhanced our understanding of polyphosphate-accumulating organisms (PAOs), particularly within the genus *Tetrasphaera*, a predominant group in enhanced biological phosphorus removal (EBPR) systems, as shown in Table 2. Shimada et al. [63] and earlier research by Van Dien and Keasling [64] provided crucial insights into the genetic regulation of phosphate uptake, identifying PhoU as a pivotal negative regulator of the Pho regulon, a gene system integral to phosphorus metabolism. Their findings elucidated a finely balanced interaction between phosphate transport and polyphosphate storage, establishing a foundational understanding of how genetic regulation directly influences phosphorus cycling in microbial communities that are essential for wastewater treatment. Kawakoshi et al. [65] sequenced the complete genome of *Microlunatus phosphovorus* and identified key genes involved in phosphate and polyphosphate metabolism, including pit/pst transporters, ppk, and ppx. Additionally, they identified genes associated with amino acid metabolism and potential denitrification, highlighting the organism's metabolic versatility. Albertsen et al. [66] employed large-scale metagenomic sequencing of full-scale EBPR sludge, generating over 18 GB of data. Their genome assembly techniques have facilitated the recovery of near-complete genomes from abundant microbes, including *Tetrasphaera*-like PAOs. This culture-independent approach has unveiled novel, functionally diverse PAOs, reshaping our understanding of EBPR microbiomes. Subsequently, Kristiansen et al. [30] conducted detailed genome annotations of four *Tetrasphaera* isolates and identified the genes responsible for amino acid fermentation, phosphate uptake, and storage. Their analysis revealed that, unlike *Accumulibacter*, *Tetrasphaera* lacks the classical polyphosphate kinase gene (ppk1) and instead utilizes alternative pathways for phosphate metabolism, indicating a distinct physiological strategy. This functional divergence among PAOs underscores the unique contributions of different organisms to phosphorus removal, emphasizing the role of niche differentiation, where multiple PAOs coexist and complement each other's functions, thereby stabilizing microbial communities in EBPR systems under various environmental conditions. Stokholm-Bjerregaard et al. [13] utilized genome-resolved metagenomics to investigate *Tetrasphaera* populations in Danish EBPR systems. They discovered that *Tetrasphaera* populations with denitrifying and fermentative capabilities were dominant and stable even under fluctuating operational conditions. This confirms *Tetrasphaera's* resilience and pivotal role as a core PAO in temperate climates. Recently, Wang et al. [67] examined the response of *Tetrasphaera*-enriched cultures to anaerobic urea treatment (ATU). Their genomic and transcriptomic analyses demonstrated increased expression of genes involved in phosphate transport and metabolism, illustrating the genus's robust metabolic flexibility and rapid adaptation to environmental changes. Xie et al. [68] employed comparative pangenomics to analyze 36 *Tetrasphaera* metagenome-assembled genomes (MAGs), categorizing them into distinct clades and identifying clade IIC as particularly significant for EBPR. Their research indicated that horizontal gene transfer (HGT) substantially influenced the gene content of different clades, especially for traits related to polyphosphate and nitrogen metabolism. This suggests that HGT is a crucial driver of *Tetrasphaera's* ecological success, enabling specific lineages to adapt to the nutrient-rich dynamic conditions prevalent in wastewater systems. These findings underscore the role of genetic exchange in metabolic versatility and stability within EBPR microbial communities.

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 9, No. 12: 148-174, 2025
DOI: 10.55214/2576-8484.v9i12.11298

Table 2.
Genetic insights into phosphorus removal by PAOs.

Genetic analysis	Organism	Key genes/elements	Reference
Whole genome sequence	*Microlunatus phosphovorus* NM-1T	Diverse taxa of microorganisms differentially express homologues of genes for poly-P degradation (Polyphosphate kinases (ppks), polyphosphate-dependent glucokinases (ppgks), phosphate transporters (pits), exopolyphosphatase gene (ppx)) under different incubation conditions in the presence of anaerobic conditions.	Kawakoshi et al. [65]
Metagenomic analysis and community composition using Illumina sequencing and qFISH.	*Candidatus accumulibacter*	The *Accumulibacter* clade IIA strain UW-1 genome (NC-013194) was utilized as a reference for mapping 87 ppk1 genes, employing CLC's reference mapping algorithm.	Albertsen et al. [66]
Purified library de novo assembly sequenced using an Illumina GAII, CLC Genomics, and ABySS in a merged manner.	*Tetrasphaera* (*T. australiensis*, *T. japonica*, *T. elongata*, *T. jenkinsii*)	PPK1, PPK2, PPX, PAP, ADK. Functional validation of identified genes for metabolic pathway analysis related to polyphosphate accumulation, denitrification, and fermentation.	Kristiansen et al. [30]
16S rRNA gene amplicon sequencing of microbiomes in full-scale wastewater treatment plants, the library pool sequenced on a MiSeq (Illumina).	*Tetrasphaera*, *Dechloromonas*, *Microlunatus*, *Tessaracoccus*, *Micropruina*, sbr-gs28	Ppk gene-clade type is involved in P removal efficiency, and it may also be associated with traits like Pit, which helps in inorganic phosphate transfer, community stability, PAO, and GAO dynamics.	Stokholm-Bjerregaard et al. [13]
Advanced genomic and bioinformatics techniques for the assessment of selective enrichment and the identification of novel PAO species.	*Tetrasphaera* PAOs, *Microlunatus* PAOs, *EBPR-ASV0001*	All *Accumulibacter* organisms were found to contain three essential metabolic genes associated with PAO: K03306 (pit, a transporter for inorganic phosphate with low affinity), K22486 (ppk2, the enzyme poly-P kinase 2), and K01895 (acs, which synthesizes acetyl coenzyme A [acetyl-CoA]).	Wang et al. [69]
Genomic and metatranscriptomic analysis identified 124 genes acquired through HGT.	*Candidatus accumulibacter*	Polyphosphate kinase 2 (PPK2) converts phosphate into polyphosphate during high availability of phosphorus and releases it during scarcity.	Xie et al. [68]

11. Factors affecting PAOs and GAOs in phosphorus removal

11.1. Temperature

Temperature variations are pivotal in influencing the competitive dynamics and metabolic efficiency of polyphosphate-accumulating organisms (PAOs) and glycogen-accumulating organisms (GAOs) in enhanced biological phosphorus removal (EBPR) systems. Ren et al. [70] investigated the impact of abrupt temperature fluctuations during EBPR initiation. They revealed that such perturbations diminished PAO performance, while conferring a competitive advantage to GAOs, particularly tetrad-forming organisms (TFOs). Notably, some TFO-like cells contained polyphosphate granules, indicating potential role-switching or trait flexibility under stress conditions. This observation challenges the conventional dichotomy of PAOs and GAOs as distinct entities and underscores the intricate adaptive microbial responses to environmental changes such as temperature variations. Wang et al. [69] explored the response of *Defluviicoccus vanus*-type GAOs to elevated temperatures (30 °C) and their competition with Accumulibacter PAOs under varying volatile fatty acid (VFA) conditions. Although GAOs are generally presumed to prevail in warmer settings, this study demonstrated that DvGAOs exhibited

DOI: 10.55214/2576-8484.v9i12.11298

significantly lower VFA uptake rates than PAOs, particularly when butyrate and iso-butyrate were provided. Conversely, PAOs exhibit robust adaptation, characterized by enhanced growth and elevated expression of critical metabolic genes. These findings suggest that GAO performance at higher temperatures is contingent upon the specific clade and that selecting appropriate carbon sources can favor PAOs. Ni et al. [47] examined the influence of seasonal temperature fluctuations on PAO-GAO competition within a biofilm sequencing batch reactor (BSBR). During warmer periods (27–30 °C), some GAOs became competitive, resulting in reduced phosphorus removal efficiency. This decline was attributed to the decreased activity of essential PAO enzymes, such as polyphosphate kinase (PPK) and phosphate hydrolase (PPX). However, under cooler conditions (15–22 °C), PAOs re-established dominance, leading to improved phosphorus removal. This study also identified glycolysis as the primary pathway supplying energy for polyhydroxyalkanoate (PHA) production during these intervals (Figure 4).

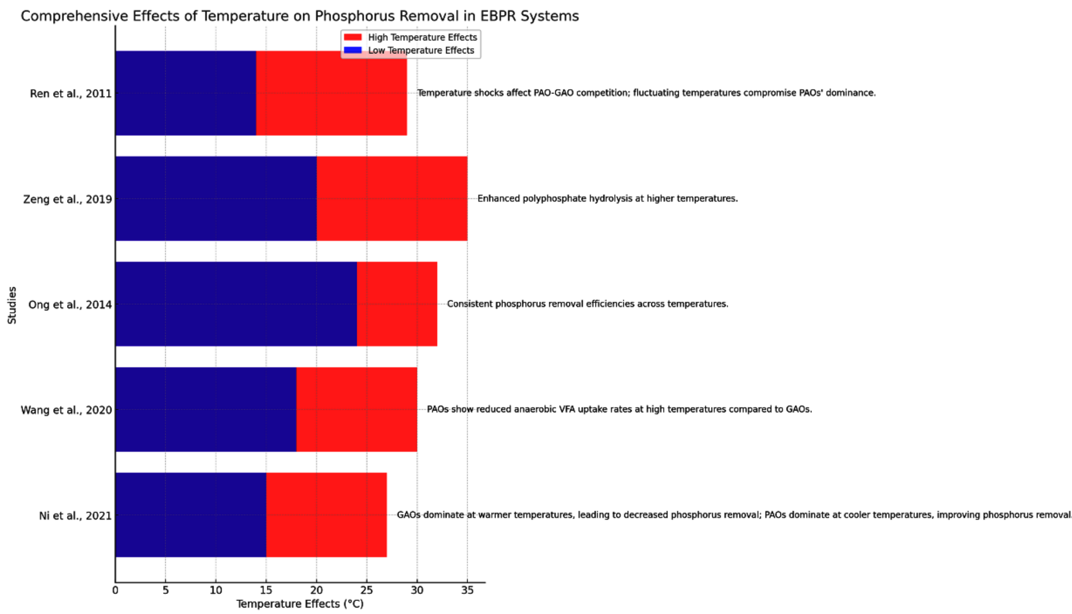

Figure 4.
Comprehensive effects of temperature on phosphorus removal in EBPR systems.
Source: Ren et al. [70], Zeng et al. [32], Ong et al. [71], Wang et al. [72], and Ni et al. [21].

Contrary to the assumption that phosphorus removal efficiency declines at elevated temperatures, Ong et al. [71] demonstrated stable enhanced biological phosphorus removal (EBPR) performance within a temperature range of 24 °C to 32 °C. Despite a significant reduction in *Accumulibacter* abundance and an increase in *Competibacter* populations at 32 °C, phosphorus removal efficiency remained high. Notably, a single thermotolerant lineage, *Accumulibacter* clade IIF, remained active and was likely responsible for maintaining high phosphorus removal efficiency, as shown in Table 3. This finding suggests that temperature-induced habitat filtering can enrich resilient polyphosphate-accumulating organism (PAO) lineages capable of sustaining EBPR under thermal stress. Zeng et al. [32] investigated the effect of temperature on phosphorus release from anaerobic sludge. Their findings indicated that higher temperatures enhanced polyphosphate hydrolysis and altered the behavior of extracellular polymeric substances (EPS), shifting phosphorus from non-reactive to more bioavailable forms. Interestingly, biological phosphorus release peaked at 15 °C but declined sharply at 5 °C due to reduced microbial activity. This study underscores the sensitivity of intracellular processes, such as enzyme-driven polyphosphate breakdown, and extracellular dynamics, such as EPS reactivity, to temperature variations.

DOI: 10.55214/2576-8484.v9i12.11298

These insights elucidate why phosphorus removal efficiency can fluctuate with seasonal or operational temperature changes in EBPR systems.

Table 3.
Overview of Key Studies Investigating Factors Influencing PAO–GAO Dynamics in EBPR Systems.

Study	Investigated Factor(s)	Key Findings
Ren et al. [70]	pH and carbon source	PAO enrichment was enhanced at higher pH (8.0) and with acetate-based carbon sources.
Bassin et al. [73]	Temperature and carbon source	Lower temperatures favored PAOs; acetate and propionate impacted PAO–GAO ratios.
Ong et al. [71]	Sludge microbiome and VFA usage	GAOs could coexist with PAOs, but high GAO abundance compromised P removal.
Saad et al. [74]	Operational mode (SBR vs. CSTR)	Dynamic SBR favored PAO dominance; CSTRs enabled GAO proliferation.
Li et al. [75]	pH and P-release carbon profile	Optimal phosphorus removal observed at pH 7.5 with mixed carbon (acetate + propionate).
Zeng et al. [32]	Carbon source and microbial profiling	Revealed shifts in PAO/GAO dominance based on carbon composition.
Wang et al. [69]	Long-term reactor operation	Showed operational conditions drove dynamic PAO–GAO succession.
Meng et al. [76]	Phosphate levels, stoichiometry	Higher influent phosphorus enhances PAO competition over GAOs.
Yuan et al. [77]	Propionate/acetate ratio	A higher propionate-to-P ratio promoted PAO activity and improved P removal.
Ni et al. [47]	Temperature and carbon dosage	Moderate temperatures and carbon levels favored stable PAO enrichment.
Nguyen et al. [78]	Electron donor and pH in the anaerobic phase	Specific pH ranges and carbon profiles promoted the dominance of *Accumulibacter*.

11.2. pH

Li et al. [75] investigated the influence of pH on denitrifying polyphosphate-accumulating organisms (PAOs) within a sequencing batch reactor, focusing on variations in intracellular polymers during anaerobic and anoxic phases. Their findings indicated a consistent improvement in phosphorus removal efficiency over a pH range of 6.5 to 8.5, with optimal performance at pH 8.0. At this optimal pH, the rates of anaerobic phosphorus release and anoxic uptake were 20.95 and 23.29 mg/(g·h), respectively, accompanied by increased intracellular polyhydroxybutyrate (PHB) and polyphosphate accumulation. However, when the pH exceeded 8.0, chemical precipitation became the predominant mechanism for phosphorus removal, thereby diminishing the system's reliance on biological processes and slightly reducing overall efficiency. This underscores the critical role of pH regulation in optimizing enhanced biological phosphorus removal (EBPR) systems. Nguyen et al. [78] examined the impact of pH on the behavior of *Tetrasphaera*-enriched cultures, focusing on their phosphorus cycling kinetics across a pH range of 6.0 to 8.0. The study demonstrated that both phosphorus release and uptake rates increased significantly with increasing pH, with release rates exceeding threefold and uptake rates more than doubling at pH 8.0 compared to pH 6.0. Notably, *Tetrasphaera* exhibited stable polyhydroxyalkanoate (PHA) production and substrate uptake, indicating its robust metabolic stability. These findings parallel those observed in *Accumulibacter*, suggesting that a slightly alkaline pH can enhance the activity of multiple PAO groups and improve overall EBPR performance.

11.3. Sulfide

Investigation of the impact of sulfide, commonly generated through sulfate reduction in anaerobic environments, on the activity of polyphosphate-accumulating organisms (PAOs) in enhanced biological phosphorus removal (EBPR) systems. In short-term activity assays, they observed a 50% reduction in acetate uptake, a critical step in phosphorus release, at a concentration of only 60 mg H_2S/L. PAO activity

DOI: 10.55214/2576-8484.v9i12.11298

was notably impaired at 275 mg SO_4/L at pH 6.5 and 1200 mg SO_4/L at pH 7.8. These findings highlight the detrimental effects of sulfide on PAOs and emphasize the necessity of managing sulfate reduction and sulfide accumulation in EBPR operations to sustain efficient phosphorus removal. The study further revealed that elevated H_2S levels led to higher phosphate release-to-acetate uptake ratios, suggesting that PAOs require additional energy for detoxification. Moreover, the introduction of seawater exacerbated sulfide stress. When more than 10% of freshwater was substituted with seawater at pH 6.5, or more than 45% at pH 7.8, the EBPR performance significantly declined. This decline is likely attributable to the combined stress caused by salinity and sulfide. These results highlight the importance of site-specific strategies in wastewater treatment facilities, particularly those that process sulfate-rich or saline influents, to avert microbial inhibition and maintain effective phosphorus removal [74].

11.4. Salinity

Numerous studies have documented PAOs' increased sensitivity to saline conditions, particularly in their competitive dynamics with GAOs, with implications for system performance and stability. Bassin et al. [73] evaluated both short- and long-term salinity effects in an aerobic granular sludge (AGS) system under varying NaCl concentrations. Ammonium removal remained stable up to 33 g/L NaCl. However, nitrite accumulation became evident above 22 g/L, coinciding with the loss of *Nitrospira* spp. Phosphorus removal was significantly impaired as the salinity increased. At 33 g/L NaCl, Candidatus *Accumulibacter phosphatis* was no longer detectable, and biological phosphorus removal ceased. Although batch experiments confirmed that residual phosphorus could be removed using 30 g/L NaCl, prolonged exposure was detrimental. High-salinity conditions shifted the competitive balance toward GAOs, which became dominant at 33 g/L, as evidenced by PCR-DGGE and FISH analyses. Further work by Yuan et al. [77] assessed moderate salinity levels (0.5–0.75% wt) in a simultaneous partial nitrification, denitrification, and phosphorus removal (SPNDPR) reactor operating under anaerobic/microaerobic conditions. The results demonstrated enhanced granule formation and increased EPS production, with a corresponding increase in the abundance of ammonia-oxidizing bacteria (AOB). However, PAOs and denitrifying PAOs were outcompeted by GAOs under salinity stress, particularly at 0.75% salinity, resulting in reduced phosphorus removal. *Candidatus competibacter*, a known GAO, increased in relative abundance from 4.86% to 15.34%, while the partial nitrification-denitrification performance improved. These findings suggest that, while salinity enhances granulation and nitrogen removal, it impairs EBPR by inhibiting PAOs. It exerts selective pressure favoring GAO dominance across concentrations, necessitating treatment strategies to balance the nitrogen-phosphorus trade-offs and address PAO vulnerability.

11.5. Carbon Source

The availability and concentration of carbon in wastewater significantly influence the competitive dynamics between polyphosphate-accumulating organisms (PAOs) and glycogen-accumulating organisms (GAOs), as shown in Table 4. Substrates such as acetate, propionate, glucose, and starch elicit distinct metabolic responses, particularly when combined with varying carbon-to-phosphorus (C/P) ratios. Meng et al. [76] investigated this interaction by examining PAO-GAO competition under conditions of elevated acetate levels and a high C/P ratio of 100:1. Notably, PAOs transitioned from their typical phosphorus-centric metabolism (PAM) to glycogen-based metabolism (GAM), yet they continued to outcompete GAOs. Elevated acetate concentrations are more toxic to GAOs than to PAOs, allowing *Candidatus accumulibacter* (clades IIC and IID) and *Thiothrix caldifontis* to flourish. These results indicate that PAOs possess metabolic flexibility, enabling them to adapt to high-carbon environments while maintaining dominance and stable phosphorus removal in enhanced biological phosphorus removal (EBPR) systems, even under changing environmental conditions. The choice of carbon source not only influences microbial competition but also affects phosphorus release and uptake kinetics. Acetate and propionate generally favor PAOs, enhancing phosphorus release and polyhydroxyalkanoate (PHA) storage, whereas carbon sources such as glucose may promote GAO activity. Furthermore, the ratio and timing of carbon addition affect the formation of key metabolic intermediates, such as polyhydroxybutyrate (PHB) and glycogen, thereby affecting system stability.

Table 4.
Carbon sources' impact on PAOs and GAOs in the EBPR system.

C sources	Key findings	Reference
Acetate	The combination of acetate, propionate, and butyrate proved to be a superior carbon source for enhancing PAO-GAO dynamics. This mixture resulted in a 46% increase in the overall VFA uptake rate, leading to a 38.4% improvement in aerobic phosphorus removal efficiency. Additionally, it significantly reduced glycogen recovery by over 63%.	Zhou et al. [39]
Acetate	Acetate spiking in EBPR systems temporarily boosted phosphorus uptake but led to long-term performance disruptions by favoring GAOs over PAOs, reducing phosphorus removal efficiency by up to 30-50%.	Ahn et al. [40]
Acetate	Optimal conditions for PAOs include high pH levels (7.4–8.4) and low acetate concentrations, while GAOs flourish in environments with low pH (6.4–7.0) and high acetate levels. Maintaining appropriate pH and acetate levels is essential for successful EBPR. Under standard SBR operations with swift acetate introduction, PAOs became the dominant microorganisms in the system.	Tu and Schuler [79]
VFAs	PAOs dominate at high acetate and neutral pH, GAOs at low pH. PAOs' metabolic shifts are based on poly-P levels, achieving 85–95% phosphorus removal in EBPR systems.	Acevedo et al. [60]
Propionate, acetate	PAOs favor propionate; rapid depletion occurs as PAOs preferentially consume propionate, improving EBPR with a 75% acetate, 25% propionate feed. The metabolic model accurately predicted carbon uptake, with aerobic kinetics unaffected by VFA compositions.	Carvalheira et al. [59]
Acetic acid	In environments with high carbon to phosphorus (C/P) ratios (100:1), a reduced concentration of acetic acid (HAC) enabled the glycogen-accumulating metabolism (GAM) of phosphate-accumulating organisms (PAOs) to outperform glycogen-accumulating organisms (GAOs). Low levels of acetate favor PAOs under high C/P conditions. Additionally, changes in the incoming acetate concentration had a more pronounced effect on GAOs than on PAOs.	Meng et al. [76]
Acetate, propionate, butyrate, iso-butyrate	As the carbon source was gradually shifted from acetate to butyrate, a significant change in microbial population was observed. The abundance of *Candidatus accumulibacter* phosphatis decreased from 37.4% to 13.9%, while *Rhodocyclaceae* increased from 2.0% to 14.5%. Both of these organisms were likely crucial for phosphorus removal. The introduction of butyrate led to improved phosphorus removal driven by PAOs, with 155 ± 17.7 mg P/L released and 213.7 ± 11.4 mg P/L taken up. Additionally, butyrate inhibited GAOs, reducing *Competibacter* from 27.3% to 6.2%.	Wang [57]
Granular Activated Carbon (GAC)	Exhibited fine-grained stability and successful biological processing of authentic urban sewage across a temperature range of 15 to 30°C. GAC addition enhances phosphorus removal (up to 70%) by enriching PAOs in biofilms, accelerating EBPR establishment, and improving nutrient removal in AGS reactors.	Sarvajith and Nancharaiah [48]
Propionate, acetate	The choice of feeding strategy is crucial to determining the effectiveness of AGS bioreactors for phosphorus removal. These strategies enable the accumulation of active PAOs while substantially reducing GAOs within the bioreactor. Specifically, the techniques of anaerobic slow feeding and pulse feeding combined with anaerobic mixing have demonstrated exceptional phosphorus removal rates exceeding 97%, effectively promoting PAO growth while suppressing GAO development.	Iorhemen et al. [49]
Glucose	Studies have shown that aerobic granular sludge systems can effectively process effluents containing glucose, demonstrating that glucose serves as an appropriate substrate for efficient phosphate elimination. Research indicates that aerobic granular sludge fed with glycerol achieved phosphorus removal rates exceeding 90%, with the microbial community	Elahinik et al. [19]

	predominantly composed of *C. accumulibacter*, despite this organism's inability to directly metabolize glycerol.	
Acetate, propionate	For improved phosphorus elimination, polyphosphate-accumulating organisms (PAOs) favor propionate, a more intricate volatile fatty acid (VFA), over the simpler acetate. This aligns with earlier research that has expanded our knowledge of PAO metabolism. Propionate usage resulted in enhanced phosphorus removal, with 155 ± 17.7 mg P/L released and 213.7 ± 11.4 mg/L absorbed. High concentrations of Ca^{2+} negatively impact both enhanced biological phosphorus removal (EBPR) and membrane effectiveness.	Thela et al. [80]

12. Technological Innovations for Phosphorus Removal

Recent technological advancements have markedly enhanced the efficiency and sustainability of phosphorus removal from wastewater treatment systems (Figure 5). This progress is particularly evident through an improved understanding of polyphosphate-accumulating organisms (PAOs), advancements in bioreactor design, and the development of novel cultivation techniques. The following studies illustrate a diverse array of approaches aimed at addressing the limitations of conventional Enhanced Biological Phosphorus Removal (EBPR) systems and optimizing nutrient recovery. Klein et al. [81] developed a miniature model system for aerobic granular sludge (AGS) utilizing a machine-assisted microfluidic cultivation platform that allowed precise control of oxic and anoxic cycles. This platform facilitates in situ sampling and microscale analysis of biofilms, significantly increasing the presence of PAOs (family *Rhodocyclaceae*) within the biome. Their setup presented new opportunities for analogous research and refinement of operational parameters to enhance AGS-based phosphorus removal. Tomás-Martínez et al. [82] examined the role of extracellular polymeric substances (EPS) in PAO communities using ^{13}C-labeled acetate under stable EBPR conditions. They discovered that proteins and polysaccharides within EPS decayed at rates similar to those of general biomass and were not selectively consumed by the surrounding microbes. This finding suggests that EPS remains relatively stable in controlled systems, providing new insights into its turnover, biosynthesis, and contribution to EBPR community structure. A novel approach to improving phosphorus recovery through sunlight-driven treatment was demonstrated by Trebuch et al. [83], who introduced PAOs into established photogranular cultures. By employing feast/famine cycling in photobioreactors, phosphorus removal increased sixfold from 5.4 to 30 mg/L/day. However, diurnal light cycles reduce phototroph populations, leading to decreased biomass growth and nitrogen removal. Although promising for sustainable phosphorus recovery, the system requires further optimization for consistent performance in large-scale wastewater treatment. Villard et al. [84] concentrated on the development of EBPR biofilms using the Hias process, which integrates moving-bed biofilm reactor (MBBR) technology with oxic-anoxic transitions. Although the system achieved >95% phosphorus removal efficiency within 16 weeks, known PAOs were not initially dominant, indicating complex spatiotemporal layering within the biofilm. Their study proposed a new model for biofilm growth, highlighting the critical yet still enigmatic microbial mechanisms underpinning phosphorus removal. Gong et al. [85] proposed a pilot-scale bioreactor employing an alternating anaerobic/oxic/anoxic (A/O/A) regime that synergizes denitrifying phosphorus removal (DPR) and endogenous denitrification (ED), achieving COD, NH_4^+-N, TN, and PO_4^{3-}-P removal efficiencies of up to 91.5%, 97.3%, 89.9%, and 96.7%, respectively, without requiring external carbon sources. The A/O/A configuration outperformed the conventional A/A/O system, revealing microbial synergy between the DPR organisms and endogenous denitrifiers. Temperature and substrate fluctuations were the focus of Fanta et al. [86], who studied a sequencing batch moving bed biofilm reactor (SB-MBBR) under industrially influenced wastewater conditions. They found that low temperatures (10°C) and the absence of volatile fatty acid (VFA) dosing reduced PAO performance and hypothesized that excessive extracellular polymeric substance production further inhibited phosphorus release, especially under thick biomass conditions in colder systems.

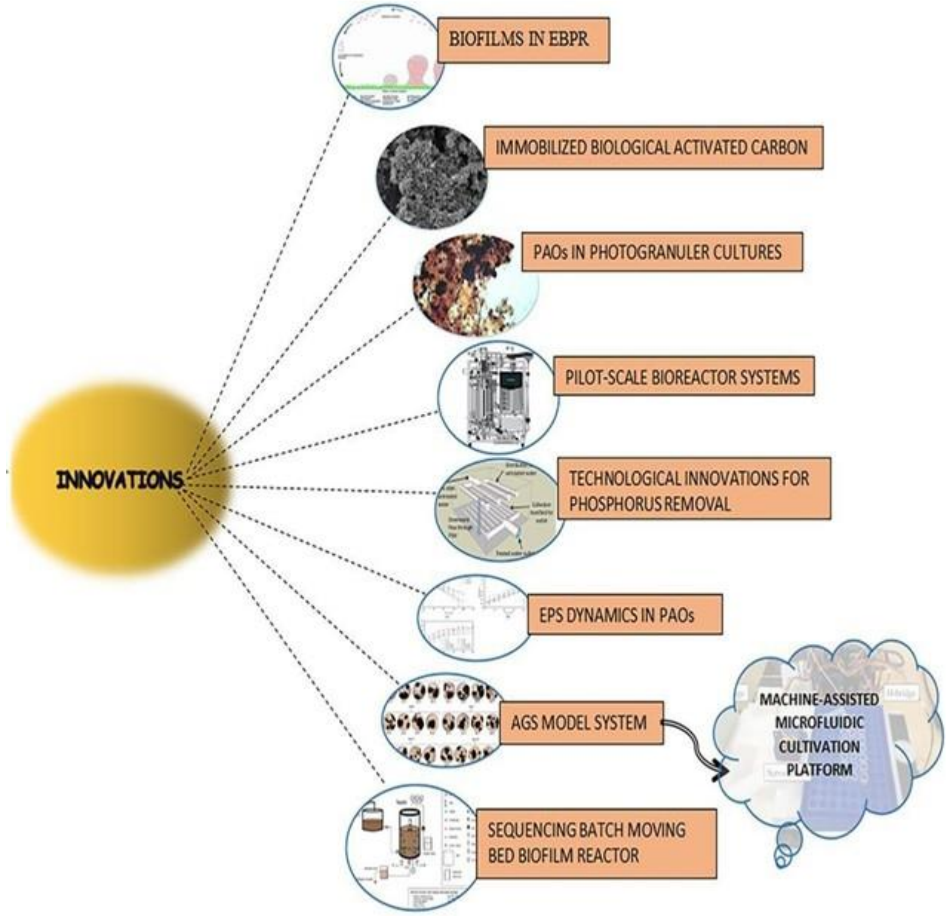

Figure 5.
By using advanced technologies, there are multiple advantages in wastewater phosphorus reduction.

This study evaluated feeding strategies aimed at maximizing the activity of polyphosphate-accumulating organisms (PAO) within aerobic granular sludge (AGS) systems by testing three distinct reactor configurations. The study found that anaerobic slow feeding (R1) and pulse feeding with anaerobic mixing (R2) achieved high phosphorus removal efficiencies of 97.6% and 98.3%, respectively, whereas simple pulse feeding (R3) was less effective. Notably, the microbial communities remained compositionally stable, predominantly consisting of Betaproteobacteria and PAO genera, such as *Paracoccus* and *Thauera*, despite variations in performance. In a related study, Wang et al. [69] employed genome-resolved metagenomics and metatranscriptomics to investigate denitrification in systems enriched with *Candidatus accumulibacter*. Their findings revealed higher expression of nitrite reductase genes than of nitrous oxide reductase genes, suggesting potential N_2O emissions. Interestingly, non-PAO-flanking microbes expressed key genes, such as norB and norZ, indicating cooperative detoxification of intermediates. These insights contribute to understanding microbial interactions and suggest potential strategies for reducing greenhouse gas emissions in enhanced biological phosphorus removal (EBPR) systems. Furthermore, Petriglieri et al. [87] identified two novel PAO species, *Candidatus dechloromonas* phosphoritropha and *Candidatus dechloromonas* phosphorivorans, in Danish EBPR systems. Utilizing metagenome-assembled genomes and FISH-Raman techniques, they demonstrated that these bacteria cycle polyphosphate, glycogen, and polyhydroxyalkanoates (PHA) under feast-famine conditions. Their findings confirmed the PAO function of these organisms and suggested a possible role in denitrification, thereby expanding the known phylogenetic and metabolic diversity within P-removing microbial communities. Collectively, these studies underscore a significant transformation in phosphorus removal technologies, shifting from merely enhancing process efficiency to achieving comprehensive optimization across systems through advanced microbial ecology, bioreactor engineering, and metabolic modeling. Because wastewater treatment facilities aim for carbon neutrality and resource recovery, these advancements support the development of sustainable and integrated nutrient management approaches (Table 5).

Table 5.
Summary of some of the Technological Innovations in Phosphorus Removal.

Study	Innovation Focus	Key Findings	Significance
Klein et al. [81]	Microfluidic cultivation platform for AGS	Enabled precise oxic-anoxic control and in situ sampling; enriched PAOs from *Rhodocyclaceae.*	High-resolution model to optimize process parameters in AGS systems.
Tomás-Martínez et al. [82]	EPS turnover in PAO-enriched cultures	EPS is not selectively degraded by flanking microbes; general biomass decay dominates.	Clarifies EPS dynamics under stable EBPR; implications for sludge stability.
Trebuch et al. [83]	PAO-enriched photogranules	Phosphorus removal increased sixfold while maintaining nitrogen and carbon removal; the light cycle affects biomass.	Promotes sunlight-based nutrient recovery; needs diurnal cycle optimization.
Villard et al. [84]	Hias MBBR process for biofilm-based EBPR	>95% P-removal achieved; inner PAO-rich layer forms slowly; removal not fully explained by known PAOs	Proposed new biofilm establishment model; highlights hidden mechanisms.
Gong et al. [85]	A/O/A bioreactor combining DPR & ED	96.7% PO_4^{3-}-P removal without external carbon source; synergistic microbial activity.	Cost-effective, high-standard nutrient removal without added carbon.
Fanta et al. [86]	SB-MBBR under low-temp, industrial VFA cycling	Reduced PAO efficiency at low temperature & VFA; excess EPS suspected to inhibit activity.	Highlights operational challenges in cold climates and low-substrate regimes.
Wang et al. [69]	Feeding strategies in AGS systems	Anaerobic slow feeding (R1) and mixed pulse (R2) achieved >97% phosphorus removal; the community remained stable.	Demonstrates the performance benefits of optimized feeding regimes.
Wang et al. [69]	Meta-omics analysis of denitrifying PAOs	Differential expression of nirS versus norB/norZ; cooperative N_2O reduction with flanking taxa.	Reveals gene-level basis of N_2O accumulation and microbial cooperation
Petriglieri et al. [87]	Novel PAOs (*Dechloromonas* spp.) characterization	Identified two novel PAO species with dynamic P, glycogen, and PHA cycling.	Expands the known diversity of PAOs and confirms their roles in denitrification.

13. Conclusion and Limitations of the Study

This review highlights substantial progress in Enhanced Biological Phosphorus Removal (EBPR) and focuses on the microbial mechanisms that underpin its effectiveness. Central to these processes are polyphosphate-accumulating organisms (PAOs), whose enrichment, metabolic adaptability, and competition with glycogen-accumulating organisms (GAOs) influence system performance. We examined how substrate preferences, environmental stressors, and community interactions determine PAO dominance and the overall efficacy of EBPR. Additionally, attention has been directed towards non-canonical and denitrifying PAOs, which broaden the ecological and functional scope of these systems. Collectively, these findings signify a shift from basic operational models to more sophisticated ecology-oriented frameworks. Translating these ecological insights into robust full-scale EBPR operations remains a persistent challenge. Operational instabilities, variable influent conditions, and stress-induced shifts in community structure continue to undermine EBPR performance. Addressing these challenges requires integrated strategies that combine process engineering, high-resolution microbial monitoring, and adaptive control systems. Notably, the future of EBPR lies not only in efficient phosphorus removal but also in its reconceptualization as a platform for phosphorus recovery. Advancing sustainable methods for phosphorus extraction, reuse, and valorization from EBPR biomass is crucial to align wastewater treatment with circular economic objectives. Continued interdisciplinary efforts are essential in establishing EBPR as a robust, resilient, and resource-oriented biotechnology for long-term environmental stewardship. Despite the comprehensive scope of this study, several limitations should be acknowledged. First, reliance on previously published studies introduces the possibility of inheriting methodological or interpretative biases from these sources. Additionally, this review primarily focuses on Enhanced Biological Phosphorus Removal (EBPR), which may limit the scope of discussion regarding alternative phosphorus removal or recovery strategies. While every effort was made to include the most relevant and recent literature, certain developments may have been overlooked because of publication lag or limited indexing. Furthermore, language and access constraints may have led to the exclusion of pertinent non-English publications, potentially narrowing the analysis's global perspective. Finally, the absence of a quantitative meta-analysis restricts the ability to statistically assess trends or effect sizes across studies, which could have added further analytical depth to the findings.

Abbreviations:

A/B: Adsorption/bio-oxidation.
AGS: Aerobic Granular Sludge
AOB: Ammonia-oxidizing bacteria
ATU: Allylthiourea
DGGE: Denaturing Gradient Gel Electrophoresis
DPAOs: Denitrifying phosphate-accumulating organisms.
EBPR: Enhanced Biological Phosphorus Removal
EPS: Extracellular Polymeric Substances
FISH: Fluorescence in situ hybridization
FNA: Free Nitrous Acid
GAC: Granular Activated Carbon
GAM: Glycogen-Accumulating Metabolism
GAOs: Glycogen-Accumulating Organisms.
GLWA: Great Lake Water Authority.
HPO-AS: High-purity oxygen-activated sludge.
MAR-FISH: Micro autoradiography-fluorescence in situ hybridization
PAM: Phosphate-accumulating Metabolism
PAOs: Polyphosphate-Accumulating Organisms.
PHA: Polyhydroxyalkanoates
qPCR: quantitative Polymerase Chain Reaction
SBR: Sequencing Batch Reactor.
SPNDPR: Simultaneous Partial Nitrification, Denitrification, and Phosphorus Removal
SRT: Solid Retention time.
TN: Total Nitrogen
TFO: Thio-fed organisms
VFA: Volatile Fatty Acids
WRRF: Water Resource Recovery Facility

Transparency:

The authors confirm that the manuscript is an honest, accurate, and transparent account of the study; that no vital features of the study have been omitted; and that any discrepancies from the study as planned have been explained. This study followed all ethical practices during writing.

Acknowledgments:
The authors are thankful to Graphic Era (Deemed to be University), India, for its support. We would like to thank Paperpal (https://paperpal.com/) and Cactus Communications Services Pte Ltd., Singapore, for their invaluable online assistance in refining the language and grammar of our manuscript.

References
[1] F. Khan, A. B. Siddique, S. Shabala, M. Zhou, and C. Zhao, "Phosphorus plays key roles in regulating plants' physiological responses to abiotic stresses," *Plants*, vol. 12, no. 15, p. 2861, 2023.

[2] N. Bechtaoui, M. K. Rabiu, A. Raklami, K. Oufdou, M. Hafidi, and M. Jemo, "Phosphate-dependent regulation of growth and stresses management in plants," *Frontiers in Plant Science*, vol. 12, p. 679916, 2021.

[3] X. Álvarez, E. Valero, R. M. Santos, S. Varandas, L. S. Fernandes, and F. A. L. Pacheco, "Anthropogenic nutrients and eutrophication in multiple land use watersheds: Best management practices and policies for the protection of water resources," *Land Use Policy*, vol. 69, pp. 1-11, 2017.

[4] S. M. Ashekuzzaman and J.-Q. Jiang, "Improving the removal of phosphate from the secondary effluents of domestic wastewater treatment plants," presented at the Conference on Environmental Science and Technology (CEST2017), 2017.

[5] S. Dalahmeh and C. Baresel, "Reclaimed wastewater use alternatives and quality standards from global to country perspective : Spain versus Abu Dhabi swedish environmental research institute," 2014. https://www.ivl.se/english/ivl/publications/publications/reclaimed-wastewater-use-alternatives-and-quality-standards---from-global-to-country-perspective-spain-versus-abu-dhabi-emirate.html

[6] J. A. Oleszkiewicz and J. L. Barnard, "Nutrient removal technology in North America and the European Union: A review," *Water Quality Research Journal*, vol. 41, no. 4, pp. 449-462, 2006. https://doi.org/10.2166/wqrj.2006.048

[7] M. Preisner, E. Neverova-Dziopak, and Z. Kowalewski, "An analytical review of different approaches to wastewater discharge standards with particular emphasis on nutrients," *Environmental Management*, vol. 66, pp. 694-708, 2020. https://doi.org/10.1007/s00267-020-01344-y

[8] A. T. W. M. Hendriks and J. G. Langeveld, "Rethinking wastewater treatment plant effluent standards: Nutrient reduction or nutrient control," *Environmental Science & Technology*, no. 9, pp. 4735–4737, 2017. https://doi.org/10.1021/acs.est.7b01186

[9] H.-Y. Park, S.-M. Park, K.-C. Lee, O.-S. Kwon, S.-J. Yu, and S.-J. Kim, "Survey of physicochemical methods and economic analysis of domestic wastewater treatment plant for advanced treatment of phosphorus removal," *Journal of Korean Society of Environmental Engineers*, vol. 33, no. 3, pp. 212-221, 2011.

[10] P. Rosario *et al.*, "Potential pitfalls in wastewater phosphorus analysis and how to avoid them," *Environmental Health Insights*, vol. 15, p. 11786302211019218, 2021. https://doi.org/10.1177/11786302211019218

[11] A. Z. Gu, "Phosphorus fractionation and removal in wastewater treatment - implications for minimizing effluent phosphorus," *Water Intelligence Online*, p. 13, 2014. https://doi.org/10.2166/9781780406893

[12] A. G. Sheik, S. B. N. Krishna, R. Patnaik, S. R. Ambati, F. Bux, and S. Kumari, "Digitalization of phosphorous removal process in biological wastewater treatment systems: Challenges, and way forward," *Environmental Research*, vol. 252, p. 119133, 2024. https://doi.org/10.1016/j.envres.2024.119133

[13] M. Stokholm-Bjerregaard, S. J. McIlroy, M. Nierychlo, S. M. Karst, M. Albertsen, and P. H. Nielsen, "A critical assessment of the microorganisms proposed to be important to enhanced biological phosphorus removal in full-scale wastewater treatment systems," *Frontiers in Microbiology*, vol. 8, p. 718, 2017. https://doi.org/10.3389/fmicb.2017.00718

[14] M. Bolla, M. Pettinato, P. F. Ferrari, B. Fabiano, and P. Perego, "Polyhydroxyalkanoates production from laboratory to industrial scale: A review," *International Journal of Biological Macromolecules*, p. 143255, 2025.

[15] R. Diaz, B. Mackey, S. Chadalavada, P. Heck, and R. Goel, "Enhanced Bio-P removal: Past, present, and future–A comprehensive review," *Chemosphere*, vol. 309, p. 136518, 2022.

[16] S. Roy, Q. Guanglei, R. Zuniga-Montanez, R. B. Williams, and S. Wuertz, "Recent advances in understanding the ecophysiology of enhanced biological phosphorus removal," *Current Opinion in Biotechnology*, vol. 67, pp. 166-174, 2021. https://doi.org/10.1016/j.copbio.2021.01.011

[17] Y. Zhang *et al.*, "A review of the phosphorus removal of polyphosphate-accumulating organisms in natural and engineered systems," *Science of The Total Environment*, vol. 912, p. 169103, 2024. https://doi.org/10.1016/j.scitotenv.2023.169103

[18] A. Oehmen, Z. Yuan, L. L. Blackall, and J. Keller, "Comparison of acetate and propionate uptake by polyphosphate accumulating organisms and glycogen accumulating organisms," *Biotechnology and bioengineering*, vol. 91, no. 2, pp. 162-168, 2005. https://doi.org/10.1002/bit.20500

[19] A. Elahinik *et al.*, "Glycerol conversion by aerobic granular sludge," *Water Research*, vol. 227, p. 119340, 2022. https://doi.org/10.1016/j.watres.2022.119340

[20] V. Crnek, V. M. Tomić, M. Vuković, J. Ćurko, and M. Matošić, "Influence of high acetate concentrations on enhanced biological phosphorus removal," *International Journal of Environmental Science and Technology*, vol. 21, no. 12, pp. 7897-7906, 2024. https://doi.org/10.1007/s13762-024-05555-2

[21] M. Ni, Y. Pan, Y. Chen, X. Zhang, Y. Huang, and Z. Song, "Effects of seasonal temperature variations on phosphorus removal, recovery, and key metabolic pathways in the suspended biofilm," *Biochemical Engineering Journal*, vol. 176, p. 108187, 2021. https://doi.org/10.1016/j.bej.2021.108187

[22] G. Guo *et al.*, "Advances in elemental sulfur-driven bioprocesses for wastewater treatment: From metabolic study to application," *Water Research*, vol. 213, p. 118143, 2022.

[23] J. Wei *et al.*, "Effect of different carbon sources on the biological phosphorus removal by a sequencing batch reactor using pressurized pure oxygen," *Biotechnology & Biotechnological Equipment*, vol. 28, no. 3, pp. 471-477, 2014. https://doi.org/10.1080/13102818.2014.924200

[24] K. Tatari, A. Gülay, B. Thamdrup, H.-J. Albrechtsen, and B. F. Smets, "Challenges in using allylthiourea and chlorate as specific nitrification inhibitors," *Chemosphere*, vol. 182, pp. 301-305, 2017. https://doi.org/10.1016/j.chemosphere.2017.05.005

[25] Y. Ge, J. Lan, C. Zhan, Y. Zhou, C. Ma, and L. Zhao, "Biological removal of phosphorus and diversity analysis of microbial community in the enhanced biological phosphorus removal (EBPR) system," *Water and Environment Journal*, vol. 34, no. 4, pp. 563-574, 2020. https://doi.org/10.1111/wej.12485

[26] L. Ruiz-Haddad, M. Ali, M. Pronk, M. C. van Loosdrecht, and P. E. Saikaly, "Demystifying polyphosphate-accumulating organisms relevant to wastewater treatment: A review of their phylogeny, metabolism, and detection," *Environmental Science and Ecotechnology*, vol. 21, p. 100387, 2024. https://doi.org/10.1016/j.ese.2024.100387

[27] J. L. Zilles, J. Peccia, and D. R. Noguera, "Microbiology of enhanced biological phosphorus removal in aerated—anoxic Orbal processes," *Water Environment Research*, vol. 74, no. 5, pp. 428-436, 2002. https://doi.org/10.2175/106143002X140224

[28] J. Chen *et al.*, "Strengthening anoxic glycogen consumption in SNEDPR-CW as a strategy to control PAO–GAO competition under carbon limited condition," *Chemosphere*, vol. 288, p. 132617, 2022. https://doi.org/10.1016/j.chemosphere.2021.132617

[29] J. Gu *et al.*, "Recent advances in convolutional neural networks," *Pattern Recognition*, vol. 77, pp. 354-377, 2018. https://doi.org/10.1016/j.patcog.2017.10.013

[30] R. Kristiansen *et al.*, "A metabolic model for members of the genus Tetrasphaera involved in enhanced biological phosphorus removal," *The ISME Journal*, vol. 7, no. 3, pp. 543-554, 2013. https://doi.org/10.1038/ismej.2012.136

[31] F. Rubio-Rincón, C. Lopez-Vazquez, L. Welles, M. Van Loosdrecht, and D. Brdjanovic, "Cooperation between Candidatus Competibacter and Candidatus Accumulibacter clade I, in denitrification and phosphate removal processes," *Water Research*, vol. 120, pp. 156-164, 2017. https://doi.org/10.1016/j.watres.2017.05.001

[32] F. Zeng, W. Jin, and Q. Zhao, "Temperature effect on extracellular polymeric substances (EPS) and phosphorus accumulating organisms (PAOs) for phosphorus release of anaerobic sludge," *RSC Advances*, vol. 9, no. 4, pp. 2162-2171, 2019. https://doi.org/10.1039/C8RA10048A

[33] M. Li *et al.*, "Insights on nitrogen and phosphorus removal mechanism in a single-stage Membrane Aeration Biofilm Reactor (MABR) dominated by denitrifying phosphorus removal coupled with anaerobic/aerobic denitrification," *Journal of Water Process Engineering*, vol. 52, p. 103583, 2023. https://doi.org/10.1016/j.jwpe.2023.103583

[34] S. Feng *et al.*, "Correlates of protection against symptomatic and asymptomatic SARS-CoV-2 infection," *Nature Medicine*, vol. 27, no. 11, pp. 2032-2040, 2021. https://doi.org/10.1038/s41591-021-01540-1

[35] C. Andres and O. Carvallo, "Identity and physiology of glycogen accumulating organisms in activated sludge (May issue)," 2018. https://digital.library.adelaide.edu.au/dspace/handle/2440/114264

[36] L. Welles *et al.*, "Accumulibacter clades Type I and II performing kinetically different glycogen-accumulating organisms metabolisms for anaerobic substrate uptake," *Water Research*, vol. 83, pp. 354-366, 2015. https://doi.org/10.1016/j.watres.2015.06.045

[37] Y. Kong, J. L. Nielsen, and P. H. Nielsen, "Microautoradiographic–fluorescence in situ hybridization (MAR-FISH) analysis of aerobic and anaerobic substrate uptake by glycogen-accumulating organisms (GAOs) and polyphosphate-accumulating organisms (PAOs) in activated sludge," *Environmental Microbiology*, vol. 7, no. 10, pp. 1560–1570, 2005.

[38] M. Pijuan, A. M. Saunders, A. Guisasola, J. A. Baeza, and Z. Yuan, "Characterizing the biochemical activity of full-scale enhanced biological phosphorus removal systems: A comparison with metabolic models," *Biotechnology and Bioengineering*, vol. 99, pp. 170–179, 2008.

[39] Y. Zhou, M. Pijuan, R. J. Zeng, H. Lu, and Z. Yuan, "Could polyphosphate-accumulating organisms (PAOs) be glycogen-accumulating organisms (GAOs)?," *Water Research*, vol. 42, no. 10, pp. 2361-2368, 2008. https://doi.org/10.1016/j.watres.2008.01.003

[40] C. H. Ahn, J. K. Park, and L. Whang, "Altered carbon flow by polyphosphate-accumulating organisms during enhanced biological phosphorus removal," *Water Environment Research*, no. 2, pp. 184–191, 2009. https://doi.org/10.2175/106143008X325836
[41] E. R. Coats, C. W. Robinson, and H. S. Lee, "Influence of operational parameters on EBPR performance and resilience of EBPR consortia," *Water Environment Research*, vol. 83, no. 2, pp. 187–196, 2011.
[42] J. Zhang, X. Liu, and H. Chen, "Role of extracellular polymeric substances (EPS) in phosphorus removal, EPS as a phosphorus reservoir during anaerobic–aerobic process," *Water Research*, vol. 47, no. 7, pp. 2450–2458, 2013.
[43] R. J. Zeng, Z. Yuan, and J. Keller, "Impact of free nitrous acid (FNA) on EBPR performance, community structures of dominant PAOs, inhibition of anoxic phosphorus metabolism, phosphorus uptake, and PHA degradation," *Water Research*, vol. 100, pp. 161–169, 2016.
[44] M. S. Islam *et al.*, "Abundance and diversity of PAO, nitrifiers, and denitrifiers in EBPR systems," *RSC Advances*, vol. 7, pp. 45938–45948, 2017.
[45] Y. Miao, Q. Zhou, Z. Yuan, and L. L. Blackall, "Inhibition of anoxic phosphorus metabolism by FNA: PHA degradation halted, N2O production," *Water Research*, vol. 145, pp. 213–222, 2018.
[46] S. Park, D. Kim, and S. Jeong, "Impact of feeding strategy on PAOs and phosphorus removal efficiency in AGS systems," *Chemical Engineering Journal*, vol. 418, p. 129450, 2021.
[47] M. Ni *et al.*, "Effects of P/C ratios on the growth, phosphorus removal and phosphorus recovery of a novel strain of highly efficient PAO," *Process Biochemistry*, vol. 111, pp. 109-117, 2021. https://doi.org/10.1016/j.procbio.2021.08.010
[48] M. Sarvajith and Y. Nancharaiah, "Enhanced biological phosphorus removal in aerobic granular sludge reactors by granular activated carbon dosing," *Science of The Total Environment*, vol. 823, p. 153643, 2022. https://doi.org/10.1016/j.scitotenv.2022.153643
[49] O. T. Iorhemen, S. Ukaigwe, H. Dang, and Y. Liu, "Phosphorus removal from aerobic granular sludge: Proliferation of polyphosphate-accumulating organisms (PAOs) under different feeding strategies," *Processes*, vol. 10, no. 7, p. 1399, 2022. https://doi.org/10.3390/pr10071399
[50] H. Thela, Z. Wang, and Z. Yuan, "Comparison of acetate and propionate; propionate preferred for enhanced phosphorus removal," *Water Research*, vol. 228, p. 119484, 2023.
[51] Z. Wang *et al.*, "Efficient phosphorus storage as polyphosphate in Accumulibacter," *Applied and Environmental Microbiology*, vol. 89, no. 8, p. e00771-23, 2023.
[52] H. Thela, Z. Wang, and Z. Yuan, "PAOs, Tetrasphaera-related PAOs in EBPR systems," *Frontiers in Microbiology*, vol. 14, p. 123456, 2023.
[53] T. Zhang, W. Song, Z. Wang, and Z. Yuan, "Response mechanisms of various PAO types; roles in nitrogen and phosphorus removal; cycling elements (C, N, P, S)," *Water Research*, vol. 227, p. 119734, 2024.
[54] Z. Peng, Q. Zhang, X. Li, S. Gao, C. Jiang, and Y. Peng, "Achieving rapid endogenous partial denitrification by regulating competition and cooperation between glycogen accumulating organisms and phosphorus accumulating organisms from conventional activated sludge," *Bioresource Technology*, vol. 393, p. 130031, 2024. https://doi.org/10.1016/j.biortech.2023.130031
[55] Z. Bin *et al.*, "Denitrifying capability and community dynamics of glycogen accumulating organisms during sludge granulation in an anaerobic-aerobic sequencing batch reactor," *Scientific Reports*, vol. 5, no. 1, p. 12904, 2015. https://doi.org/10.1038/srep12904
[56] H. Zou, X. Lu, and S. Abualhail, "Characterization of denitrifying phosphorus removal microorganisms in a novel two-sludge process by combining chemical with microbial analysis," *Journal of Chemistry*, vol. 2014, no. 1, p. 360503, 2014. https://doi.org/10.1155/2014/360503
[57] L. Wang, "The metabolism of polyphosphate accumulating organisms (PAOs) and glycogen accumulating organisms (GAOs) in enhanced biological phosphorus removal (EBPR) system under the tropical climate," Doctoral Dissertation, Nanyang Technological University, 2021.
[58] D. G. Weissbrodt, G. S. Schneiter, J.-M. Fuerbringer, and C. Holliger, "Identification of trigger factors selecting for polyphosphate-and glycogen-accumulating organisms in aerobic granular sludge sequencing batch reactors," *Water Research*, vol. 47, no. 19, pp. 7006-7018, 2013. https://doi.org/10.1016/j.watres.2013.08.043
[59] M. Carvalheira, A. Oehmen, G. Carvalho, and M. A. Reis, "Survival strategies of polyphosphate accumulating organisms and glycogen accumulating organisms under conditions of low organic loading," *Bioresource Technology*, vol. 172, pp. 290-296, 2014. https://doi.org/10.1016/j.biortech.2014.09.059
[60] B. Acevedo, L. Borrás, A. Oehmen, and R. Barat, "Modelling the metabolic shift of polyphosphate-accumulating organisms," *Water Research*, vol. 65, pp. 235–244, 2014. https://doi.org/10.1016/j.watres.2014.07.028
[61] Y. Law *et al.*, "Integrative microbial community analysis reveals full-scale enhanced biological phosphorus removal under tropical conditions," *Scientific Reports*, vol. 6, no. 1, p. 25719, 2016. https://doi.org/10.1038/srep25719
[62] D. Wang *et al.*, "Side-stream enhanced biological phosphorus removal (S2EBPR) process improves system performance-A full-scale comparative study," *Water Research*, vol. 167, p. 115109, 2019. https://doi.org/10.1016/j.watres.2019.115109

[63] T. Shimada, N. Fujita, K. Yamamoto, and A. Ishihama, "Novel roles of cAMP receptor protein (CRP) in regulation of transport and metabolism of carbon sources," *PloS One*, vol. 6, no. 6, p. e20081, 2011. https://doi.org/10.1371/journal.pone.0020081

[64] S. J. Van Dien and J. Keasling, "Effect of polyphosphate metabolism on the Escherichia coli phosphate-starvation response," *Biotechnology Progress*, vol. 15, no. 4, pp. 587-593, 1999. https://doi.org/10.1021/bp990067u

[65] A. Kawakoshi *et al.*, "Deciphering the genome of polyphosphate accumulating actinobacterium Microlunatus phosphovorus," *DNA Research*, vol. 19, no. 5, pp. 383-394, 2012. https://doi.org/10.1093/dnares/dss020

[66] M. Albertsen, L. B. S. Hansen, A. M. Saunders, P. H. Nielsen, and K. L. Nielsen, "A metagenome of a full-scale microbial community carrying out enhanced biological phosphorus removal," *The ISME Journal*, vol. 6, no. 6, pp. 1094-1106, 2012. https://doi.org/10.1038/ismej.2011.176

[67] H. Wang, L. Lin, L. Zhang, P. Han, and F. Ju, "Microbiome assembly mechanism and functional potential in enhanced biological phosphorus removal system enriched with Tetrasphaera-related polyphosphate accumulating organisms," *Environmental Research*, vol. 233, p. 116494, 2023. https://doi.org/10.1016/j.envres.2023.116494

[68] X. Xie *et al.*, "Integrated genomics provides insights into the evolution of the polyphosphate accumulation trait of Ca. Accumulibacter," *Environmental Science and Ecotechnology*, vol. 20, p. 100353, 2024. https://doi.org/10.1016/j.ese.2023.100353

[69] Z. Wang *et al.*, "Expanding the diversity of Ca. Accumulibacter with a novel type and deciphering the transcriptional and morphological features among co-occurring strains," *Applied and Environmental Microbiology*, vol. 89, no. 8, p. e00771-23, 2023.

[70] N. Ren, H. Kang, X. Wang, and N. Li, "Short-term effect of temperature variation on the competition between PAOs and GAOs during acclimation period of an EBPR system," *Frontiers of Environmental Science & Engineering in China*, vol. 5, pp. 277-282, 2011. https://doi.org/10.1007/s11783-010-0226-x

[71] Y. H. Ong, A. S. M. Chua, T. Fukushima, G. C. Ngoh, T. Shoji, and A. Michinaka, "High-temperature EBPR process: the performance, analysis of PAOs and GAOs and the fine-scale population study of Candidatus "Accumulibacter phosphatis"," *Water Research*, vol. 64, pp. 102-112, 2014. https://doi.org/10.1016/j.watres.2014.06.038

[72] L. Wang, A. Oehmen, C. Le, J. Liu, and Y. Zhou, "Defluviicoccus vanus glycogen-accumulating organisms (dv GAOs) are less competitive than polyphosphate-accumulating organisms (PAOs) at high temperature," *ACS ES&T Water*, vol. 1, no. 2, pp. 319-327, 2020. https://doi.org/10.1021/acsestwater.0c00092

[73] J. Bassin, M. Pronk, G. Muyzer, R. Kleerebezem, M. Dezotti, and M. Van Loosdrecht, "Effect of elevated salt concentrations on the aerobic granular sludge process: Linking microbial activity with microbial community structure," *Applied and Environmental Microbiology*, vol. 77, no. 22, pp. 7942-7953, 2011. https://doi.org/10.1128/AEM.05016-11

[74] S. A. Saad, L. Welles, C. M. Lopez-Vazquez, M. C. van Loosdrecht, and D. Brdjanovic, "Sulfide effects on the anaerobic metabolism of polyphosphate-accumulating organisms," *Chemical Engineering Journal*, vol. 326, pp. 68-77, 2017. https://doi.org/10.1016/j.cej.2017.05.074

[75] W. Li, H. Zhang, H. Sun, F. Zeng, Y. Gao, and L. Zhu, "Effect of pH on short-cut denitrifying phosphorus removal," *Water Science and Engineering*, vol. 11, no. 1, pp. 17–22, 2018.

[76] Q. Meng, W. Zeng, B. Wang, Z. Fan, and Y. Peng, "New insights in the competition of polyphosphate-accumulating organisms and glycogen-accumulating organisms under glycogen accumulating metabolism with trace Poly-P using flow cytometry," *Chemical Engineering Journal*, vol. 385, p. 123915, 2020. https://doi.org/10.1016/j.cej.2019.123915

[77] C. Yuan, Y. Peng, B. Wang, X. Li, and Q. Zhang, "Facilitating sludge granulation and favoring glycogen accumulating organisms by increased salinity in an anaerobic/micro-aerobic simultaneous partial nitrification, denitrification and phosphorus removal (SPNDPR) process," *Bioresource Technology*, vol. 313, p. 123698, 2020. https://doi.org/10.1016/j.biortech.2020.123698

[78] P. Y. Nguyen, R. Marques, H. Wang, M. A. M. Reis, G. Carvalho, and A. Oehmen, "The impact of pH on the anaerobic and aerobic metabolism of Tetrasphaera-enriched polyphosphate accumulating organisms," *Water Research X*, vol. 19, p. 100177, 2023. https://doi.org/10.1016/j.wroa.2023.100177

[79] Y. Tu and A. J. Schuler, "Low acetate concentrations favor polyphosphate-accumulating organisms over glycogen-accumulating organisms in enhanced biological phosphorus removal from wastewater," *Environmental Science & Technology*, vol. 47, no. 8, pp. 3816-3824, 2013. https://doi.org/10.1021/es304846s

[80] N. Thela, D. Ikumi, T. Harding, and M. Basitere, "Growing an enhanced culture of polyphosphate-accumulating organisms to optimize the recovery of phosphate from wastewater," *Water*, vol. 15, no. 11, p. 2014, 2023. https://doi.org/10.3390/w15112014

[81] E. Klein *et al.*, "Enrichment of phosphate-accumulating organisms (PAOs) in a microfluidic model biofilm system by mimicking a typical aerobic granular sludge feast/famine regime," *Applied Microbiology and Biotechnology*, vol. 106, no. 3, pp. 1313-1324, 2022. https://doi.org/10.1007/s00253-022-11759-8

[82] S. Tomás-Martínez *et al.*, "Turnover of the extracellular polymeric matrix of granules performing biological phosphate removal," *Applied Microbiology and Biotechnology*, vol. 107, pp. 1997-2009, 2023. https://doi.org/10.1007/s00253-023-12421-7

[83] L. M. Trebuch *et al.*, "Enhancing phosphorus removal of photogranules by incorporating polyphosphate accumulating organisms," *Water Research*, vol. 235, p. 119748, 2023. https://doi.org/10.1016/j.watres.2023.119748

[84] D. Villard *et al.*, "Spatiotemporal succession of phosphorous accumulating biofilms during the first year of establishment," *Water Science & Technology*, vol. 88, no. 2, pp. 381-391, 2023. https://doi.org/10.2166/wst.2023.214
[85] B. Gong, K. Duan, S. Chen, and Y. Wang, "Enhanced nutrients removal and microbial mechanisms in a pilot-scale anaerobic-oxic-anoxic (A/O/A) system: Synergistic roles of denitrifying polyphosphate accumulating organisms and endogenous denitrifiers," *Process Safety and Environmental Protection*, vol. 179, pp. 47-56, 2023. https://doi.org/10.1016/j.psep.2023.08.085
[86] A. B. Fanta, A. M. Nair, S. Sægrov, and S. W. Østerhus, "Phosphorus removal from industrial discharge impacted municipal wastewater using sequencing batch moving bed biofilm reactor," *Journal of Water Process Engineering*, vol. 41, p. 102034, 2021. https://doi.org/10.1016/j.jwpe.2021.102034
[87] F. Petriglieri, C. Singleton, M. Peces, J. F. Petersen, M. Nierychlo, and P. H. Nielsen, "“Candidatus Dechloromonas phosphoritropha” and “Ca. D. phosphorivorans”, novel polyphosphate accumulating organisms abundant in wastewater treatment systems," *The ISME Journal*, vol. 15, no. 12, pp. 3605-3614, 2021. https://doi.org/10.1038/s41396-021-01029-2

DOI: 10.55214/2576-8484.v9i12.11298