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Impact of phosphorus on the functional properties of extracellular polymeric substances recovered from sludge

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ABSTRACT

Extracellular Polymeric Substances (EPS) are ubiquitous in biological wastewater treatment (WWT) technologies like activated sludge systems, biofilm reactors, and granular sludge systems. EPS recovery from sludge potentially offers a high-value material for the industry. It can be utilized as a coating in slow-release fertilizers, as a bio-stimulant, as a binding agent in building materials, for the production of flame retarding materials, and more. P recovered within the extracted EPS is an intrinsic part of the recovered material that potentially influences its properties and industrial applications. P is present in EPS in different speciation (e.g., P esters, poly-P, ortho-P, etc.). Such P species are already intensively used in the chemical industry to enhance thermal stability, viscoelasticity, emulsification, water-holding capacity, and many other properties of some natural and petroleum-derived polymers. The translation of this knowledge to EPS is missing which prevents the full utilization of phosphorus in EPS. This knowledge could allow us to engineer EPS via phosphorus for specific target properties and applications. In this review, we discuss how P could affect EPS properties based on experiences from other industries and reflect on how these P species could be influenced during the EPS extraction process or in the WWTPs.

1. Introduction

Extracellular polymeric substances (EPS) constitute an essential element within the matrix of microbial biofilms in diverse ecological contexts, particularly in wastewater treatment technologies, e.g., activated sludge systems, biofilm reactors, and granular sludge systems (L. Huang et al., 2022a). EPS has potential applications across industrial sectors such as agriculture, construction, textiles, paper industry, and flame retardants production (E. van der Knaap et al., 2019.; Henze et al., 2020; Feng et al., 2019; Kim et al., 2020; Lin et al., 2015). The Netherlands has established the world's first two demonstration facilities for EPS extraction from aerobic granular sludge systems (Bahgat et al., 2023; E. van der Knaap et al., 2019). These developments signify a pioneering advance in commercializing EPS recovered from wastewater sludge, emphasizing their potential significance in industrial contexts.

EPS extracted from waste sludge contains significant amounts of P (Zhang et al., 2013; Zeng et al., 2019). Bahgat et al., 2023 reported that 20% of the total P load to AGS WWTPs ultimately becomes incorporated into the EPS. P recovered within the extracted EPS is an intrinsic part of the recovered material that potentially influences its properties and industrial applications. P is already intensively used in various contexts to modify polymers to improve or endow many properties. For instance, chemical phosphorylation of polyesters and cotton in textiles and polyvinyl chloride in construction materials make these materials flame-resistant (Liang et al., 2013; Salmeia et al., 2016; ESPP webinar, 2020). Similarly, protein phosphorylation is applied in food industries to change the properties of substances such as egg white, potatoes, and rice to enhance wettability, dispersibility, and water absorption capacity (Y. Hu et al., 2023a; Z. Hu et al., 2019a; P. Li et al., 2020; Miedzianka & Pęksa, 2013a). So, phosphorus could be a way to engineer EPS by
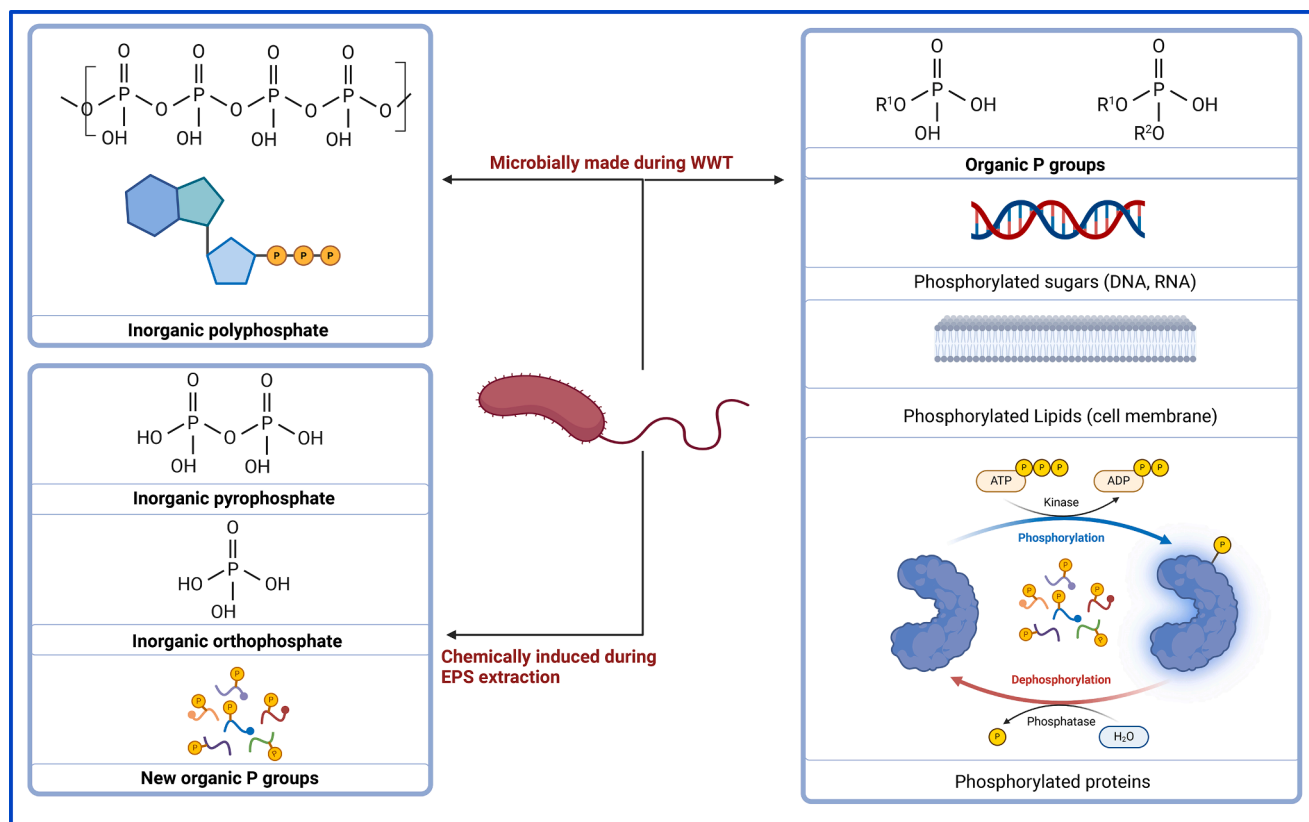
Fig. 1. Expected P species in EPS either microbially produced or chemically induced during the EPS extraction.

influencing its properties analogous to these polymers, thereby enhancing the significance of phosphorus within EPS.

Different P species would be expected to be found in EPS that are either microbially produced before extraction or chemically induced during EPS extraction processes. Expected species are organophosphorus groups in phosphorylated protein, sugars, and lipids and could also be polyphosphates, pyrophosphates, and orthophosphates (García Becerra et al., 2010; Huang et al., 2015; N.T. Bahgat et al., 2024 and Bahgat et al., 2023). All these possible P species are not only relevant for engineering EPS properties but could also be considered to be potential high-value phosphorus groups that could be extracted from WWTPs. These phosphorus compounds could replace some of the elemental phosphorus, P_4, derivatives that are necessary to make flame retardants, lubricants, detergents, and others in the industrial sector, representing 2% of the P rock demand (Blank, 2023; ESPP webinar, 2020; Jupp et al., 2021). So, utilizing these P groups incorporated in recovered EPS to engineer and design its properties would create an industrial niche for recovered P and EPS from WWTPs.

The current status of aerobic granular sludge plants equipped with EPS extraction systems is that they focus solely on EPS recovery, with no attention given to phosphorus recovery. While there is little literature addressing the influence of P on EPS properties and possible industrial applications, the influence of P has been extensively reported in other contexts. In this review,

- **Chapter 2** focuses on the fundamentals, including the phosphorus species commonly found in wastewater and EPS, and provides an explanation of the phosphorylation concept and its thermodynamics.
- **Chapter 3** elaborates on how these different phosphorus species reported in Chapter 2 could influence the properties of various polymers, as this knowledge has not yet been explored in the context of EPS.

- **Chapter 4** offers suggestions on how phosphorus affects EPS and outlines the further research required in this area.

2. P species in EPS

Phosphorus compounds are essential for the emergence of life on Earth. Phosphorus is a group 15 element that has five electrons in its outer shell. By donating its electrons, phosphorus can form five covalent bonds, e.g., by combining with four oxygen atoms to form orthophosphate. Orthophosphate is chemically versatile and can form mono-, di- and tri-esters (P-O-C) with alkyl and aryl hydroxyl groups, as well as acid anhydrides (P-O-P). The ability of phosphate to form esters (nucleic acids in RNA, DNA, and phosphoproteins) and anhydrides (ATP, polyphosphate) that are stable at ambient temperatures in water made it ideal for the generation of biological molecules that predominate in living organisms (Hunter, 2012). It was reported by Stephanopoulos et al., 1998 that the dry biomass of E. coli consists of about 55% protein (with P as a minor modification), 20% RNA (about 3.5% (w/w) P), 9% lipids mainly phospholipids (about 2% P), 6% cell wall constituents (very minor P), 4% free metabolites (as pH buffer, as pyrophosphate, or as activation group (e.g., glucose-6-P)), 3% DNA (about 3.5% (w/w) P), and 3% storage polymer (no P). However, the phosphorous composition of bacteria differs mainly when large amounts of energy-rich phosphate bonds are used for energy storage like in polyphosphate (Blank, 2012). Fig. 1 shows the possible P species to be found in EPS either microbially made or chemically induced during EPS extraction. Phosphorus content in EPS can differ according to sludge source, and the conditions used to extract EPS as extraction conditions would influence phosphorus chemistry and speciation (N.T. Bahgat et al., 2024 and Bahgat et al., 2023). For example, Huang et al., 2015 reported no organic phosphorus in EPS extracted by EDTA–ultrasound from aerobic granular sludge. Zhang et al., 2013 also reported no organic phosphorus in EPS extracted by cation exchange resin (CER) from WAS. Zeng et al., 2019 reported
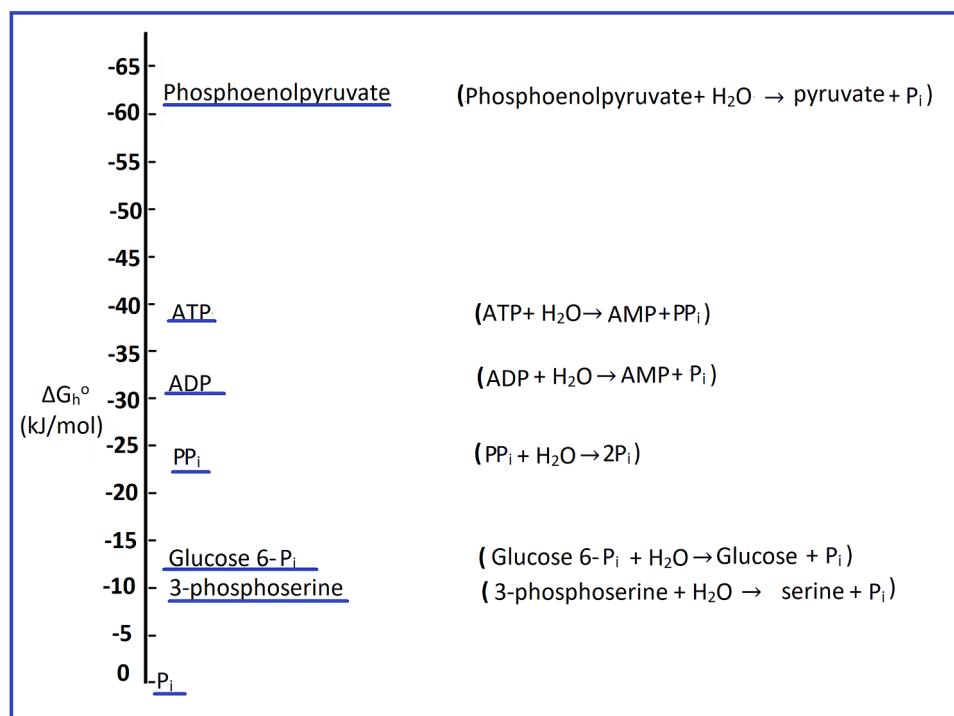
Fig. 2. Gibbs free energy of hydrolysis (kJ/mol) of some high and low-energy phosphorus compounds compared to their cleavage products (adapted from Jakubowski et al., 2022; Smith, 2016).

polyphosphates and orthophosphates were the dominant forms with negligible organic P in EPS extracted by formaldehyde-NaOH from waste activated sludge. N.T. Bahgat et al., 2024 reported that organic phosphorus represents 51%-54% of total phosphorus in EPS extracted by alkaline high-temperature conditions combined with acidic precipitation step from aerobic granular sludge, followed by orthophosphates as the second highest percentage with little to no polyphosphates.

2.1. Organic P
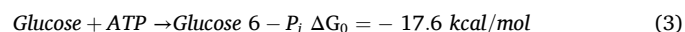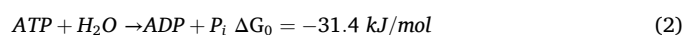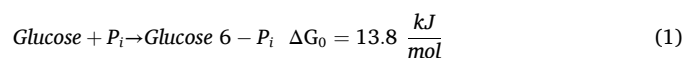
2.1.1. RNA, DNA, phospholipids

Organic P-ester groups found in DNA, RNA, and phospholipids could end up in EPS. Phosphodiester bonds make up the backbones of DNA and RNA, genetic information carriers, as the phosphate is attached to the 5′ carbon of one nucleoside and the 3′ carbon of the adjacent nucleoside. RNA is not stable so it will possibly hydrolyze and form mono-ribonucleotides during the alkaline extraction of EPS as done in Epe and Zutphen installations (Lemire et al., 2016). Phospholipids are also diester compounds that have both a hydrophobic and a hydrophilic component and contribute to the structure and function of cell membranes. Phospholipids from cell membranes could end up in extracted EPS or could split to free fatty acids and glycerophosphoric acid under alkaline conditions (Hartman et al., 1980), saponification reaction, during the alkaline extraction of EPS (Bahgat et al., 2023).

2.1.2. Phosphorylation

Phosphorylated proteins and sugars are essential components of microbial cells, involved in energy metabolism, signal transduction, enzymatic regulation, cellular structure, and stress adaptation, among other functions (Ardito et al., 2017). The only negatively charged amino acids, Asp and Glu, whose carboxyl side chains only have a single negative charge and a smaller hydrated shell. The phosphate group, with its large, hydrated shell and negative charge greater than 1, makes amino acids in phosphoproteins act as new chemical entities that do not resemble any natural amino acid and provide a means of diversifying the chemical nature of protein surfaces (Hunter, 2012). Phosphorylated

proteins and/or sugars inside cells could also end up in EPS but might also be induced chemically along the extraction process as discussed in Section 4.2. Understanding biochemical phosphorylation in cells and pure chemical phosphorylation of polymers can help us evaluate the possible presence of phosphorylation and organic P species in EPS and is discussed in the following two sections.

2.1.2.1. Biochemical phosphorylation. Enzymatic phosphorylation in biochemistry is a highly endothermic reaction that uses a phosphate donor and an energy carrier and occurs at a physiological pH of 7.4. ATP is a famous energy carrier in biochemistry, containing a phosphate anhydride bond (P-O-P), and it is often referred to as high-energy phosphate because it produces high free energy upon hydrolysis. That high free energy is needed so that the overall reaction of phosphorylation proceeds with a negative ΔG^o for example glucose phosphorylation shown in Eqs. (1), (2), and (3). Fig. 2 illustrates the free energies of hydrolysis (kJ/mol) for various phosphorylated compounds in cellular biology (Jakubowski et al., 2022; Smith, 2016). Thermodynamically favored reactions are determined by the energy levels, such as the utilization of ATP (higher energy compound) to produce Glucose 6-Pi and 3-phosphoserine (lower energy compound). To maintain the energy source in the cells, ADP is phosphorylated to make ATP again using a proton gradient that provides the mechanical energy needed for phosphorylation. This reaction proceeds by the isolation of the ADP in close proximity to the phosphate, which results in a localized environment with very high phosphate/ADP activity coupled with low water activity, which is important to drive phosphorylation according to le chatelier's principal (Pasek, 2020).

$$Glucose + P_i \rightarrow Glucose\ 6 - P_i \quad \Delta G_0 = 13.8\ \frac{kJ}{mol} \tag{1}$$

$$ATP + H_2O \rightarrow ADP + P_i \quad \Delta G_0 = -31.4\ kJ/mol \tag{2}$$

$$Glucose + ATP \rightarrow Glucose\ 6 - P_i \quad \Delta G_0 = -17.6\ kcal/mol \tag{3}$$

2.1.2.2. Chemical phosphorylation. Chemical phosphorylation is widely
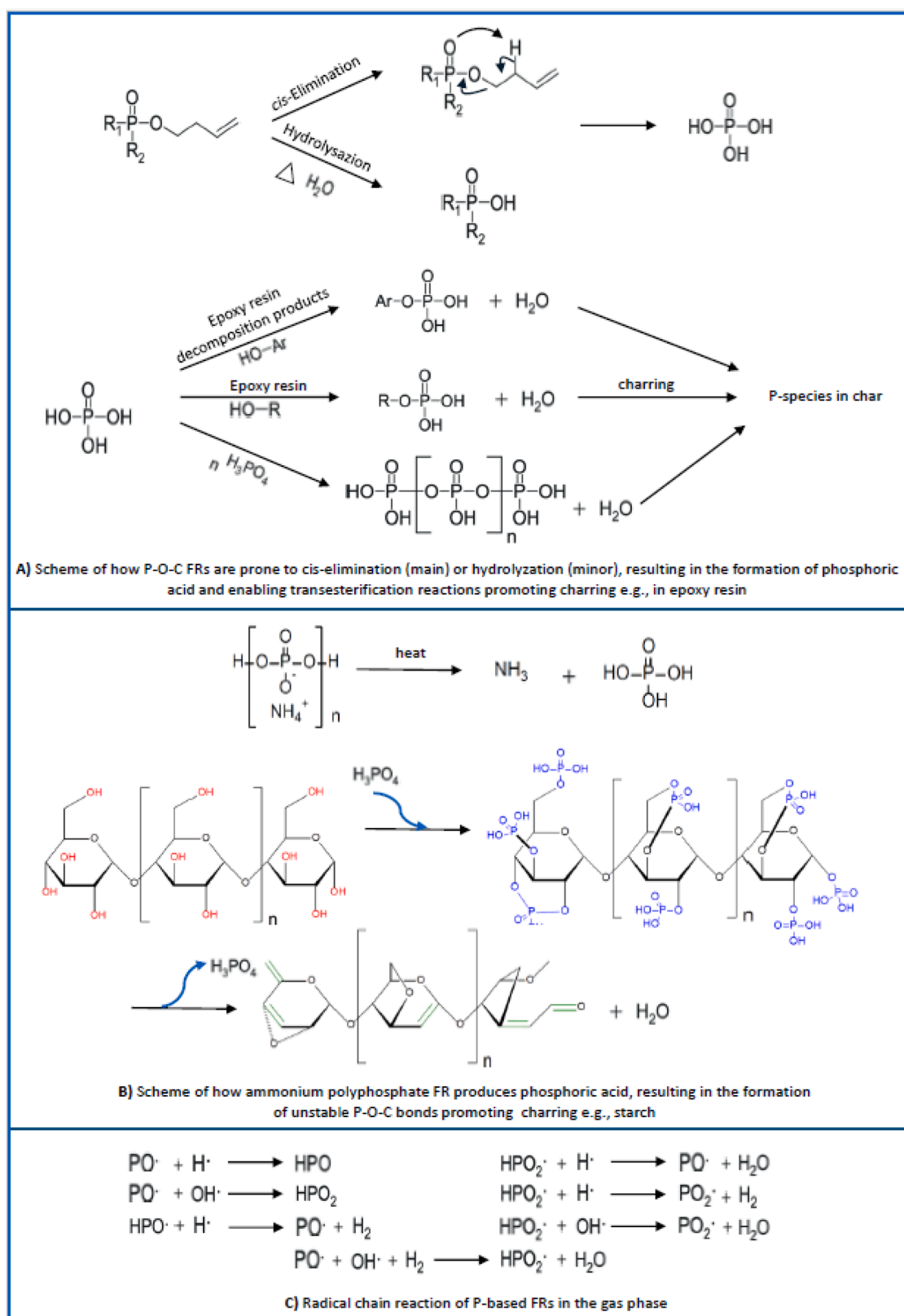
Fig. 3. Examples from the literature of organophosphorus and poly-P FRs work in the condensed and gas phases A) (Markwart et al., 2019), B) (Maqsood & Seide, 2019), C) (Scharte, 2010).

used in various industries for both natural and synthetic polymers to modify their properties, and it is primarily non-enzymatic (J. Chen et al., 2020; Y. Hu et al., 2022). The thermodynamics of chemical phosphorylation are driven by two main factors: 1) the abundance of the leaving group, and 2) the efficiency of the leaving group in providing the energy needed for phosphorylation (Pasek, 2020). For example, phosphorylation by H_3PO_4 is a condensation reaction with H_2O as a leaving group. To push this reaction forward, the environment must have a high activity of the two reactants (phosphate and organic substrate) and an extremely low water activity. Therefore, this reaction requires dry conditions (Damer & Deamer, 2015) or the application of condensing agents that react with water to form new compounds, achieving the low water activity needed for phosphorylation to take place (Hulshof & Ponnamperuma, 1976). Additionally, phosphate (Pi) is also very low in energy, as shown in Fig. 2, so extremely high temperatures are needed as a source of energy to induce phosphorylation (Gajewski et al., 1986). In contrast, salts containing high-energy phosphate anhydride bonds (P-O-P) are prevalent in the chemical industry. This route requires much lower activities of the P reagent and lower temperatures, and it occurs in aqueous environments because: 1) these salts have a high net energy, 2) these salts are activated by the leaving groups as they withdraw electrons from the electrophilic P center, making it even more electron-poor and reactive (Klein, 2012a; Pasek, 2020), and 3) the leaving group is rarer than water, making phosphorylation more favorable. During phosphorylation, phosphate groups graft onto the structure by reacting with the side chain groups of the polymer, such as the hydroxyl groups of serine, threonine, and tyrosine, the amino groups of lysine, the 1 and 3 nitrogen atoms of the histidine ring, and the nitrogen atom of the arginine guanidine group in proteins. This forms different organic-P bonds, such as esters (P-O-C) and amidates (P-N-C) (Hadidi et al., 2021; Illy et al., 2015a; S. Liang et al., 2013; Schwenke et al., 2000). The extraction conditions of EPS, compared to the phosphorylation conditions reported in the literature, could induce phosphorylation chemically as discussed in detail in Section 4.2.

2.2. Polyphosphates, pyrophosphates and orthophosphates

Polyphosphate (polyP) is a linear polymer of a few to many hundreds of phosphates (Pi) linked by a high-energy phosphate anhydride (P-O-P) bond (Akbari et al., 2021). Polyphosphate is used by many organisms instead of, or complementary to polysaccharides or lipids for energy storage, and the amount of polyphosphate can be as high as 30% of total cell mass (Blank, 2012). The phenomenon of intracellular Poly-P storage in phosphorus-accumulating microorganisms (PAOs) via luxury P uptake occurs in enhanced biological phosphorus removal (EBPR) system in WWTPs. The excess sludge produced contains around 90% of the total P load of the WWTP (Bahgat et al., 2023), mainly as polyphosphates. Polyphosphates can be hydrolyzed into pyrophosphate and subsequently pyrophosphate to orthophosphates along the EPS extraction process over time or under changing pH or temperature conditions (CHANG & RACZ, 1977; Hirota et al., 2010; Kuroda et al., 2002; McBeath et al., 2007). Pyrophosphate (PPi) is the simplest form of polyphosphate that contains two phosphates. Orthophosphate (Pi) is the simplest and most stable form of phosphorus as it has higher resonance stabilization and greater delocalization of electrons.

Orthophosphate minerals such as struvite ($MgNH_4PO_4 \cdot 6H_2O$), calcium phosphate, and vivianite ($Fe_3(PO_4)_2 \cdot 8H_2O$) have been identified in WWTPs and sludge (Manas et al., 2011; Doyle & Parsons, 2002; Wilfert et al., 2018). These minerals may also end up in EPS based on the pH of the extraction process and the solubility of these minerals at that pH. Additionally, throughout the EPS extraction process, phosphate minerals could form due to orthophosphate and other cations released from sludge into solution thus inducing supersaturation of these minerals, often in combination with changes in pH, thus accumulating within the EPS.

3. P and functional properties

EPS showed significant potential as a flame retardant, agricultural biostimulant, and coating material (E. van der Knaap et al., 2019; L. M. Chen et al., 2024; N. K. Kim et al., 2022). EPS can benefit significantly from the incorporation of phosphorus groups into its structure to enhance its properties for these applications. EPS's viscoelastic and water-holding properties are pivotal to further develop EPS a biostimulant in agriculture to enhance soil quality. EPS versatility extends to coatings for seeds, slow-release fertilizers, and papers, as well as adhesive applications, with viscoelastic and emulsifying properties crucial for coating efficacy and durability. In the following sections, we look into how important the phosphorus groups described in Section 2 could be for EPS properties to maximize its potential usage in those applications by gathering insights from different fields.

3.1. Thermal properties

Thermal stability is important to develop flame retardants out of EPS. To understand how P could influence the thermal stability of EPS, first, the general mechanisms of P-containing FR will be discussed focusing on the P chemical groups expected to be found in EPS discussed earlier in the Section 2. Then we look closer into biobased FRs (e.g., DNA, Casein, phytic acid) as they are closely related to EPS in nature. Finally, we focus on EPS to reflect what the existing knowledge means for EPS and its industrial application as a flame retardant.

3.1.1. Mechanisms of P-based flame retardants

P-based FRs are widely used due to their nontoxic degradation during the burning process, unlike halogenated FRs which are now considered global contaminants (Markwart et al., 2019). P-based FRs can be organic (phosphoesters, phosphonates, phosphinates) or inorganic (e.g., polyphosphates, phosphate minerals) (Aaronson, 1992; Naiker et al., 2023; Z. Liang et al., 2022). Flame retardants (FRs) act primarily in the condensed phase (forming a protective layer or char) but also in the gas phase (releasing flame-inhibiting gases) (Gaan et al., 2009; Naiker et al., 2023a; Salmeia, Jovic, et al., 2016). P-based FRs act in the condensed phase by enhancing the formation and stabilization of carbonaceous char on the material which isolates and protects the polymer from the flames. The char-forming products by the end of the burning process are difficult to generalize for all P-based FRs as it depends on multiple parameters, e.g., chemical structure, chemical bonds, intermolecular forces, rigidity, the oxidation state of P, combustion temperature, etc (Hu & Wang, 2019; Naiker et al., 2023; Schmitt, 2007; Cho et al., 2011; Schartel et al., 2016). P-based FRs can also act in the gas phase by forming P radicals such as $PO\bullet$, $PO_2\bullet$ and $HPO_2\bullet$ upon heating (Scharte, 2010). These radicals trap the highly reactive radicals $H\bullet$ and $OH\bullet$, the principal flame-propagating radicals, to terminate the exothermic reactions of the combustion cycle (Salmeia et al., 2015), as shown in Fig. 3(C).

A chain reaction creates water molecules and other non-flammable compounds dilute the gas phase which reduces the oxygen concentration (Levchik & Weil, 2005; Naiker et al., 2023; Shen et al., 2022). All these reactions together in the gas and the condensed phases cool down the system.

3.1.1.1. Organic P.
Organophosphorus FRs are associated with the production of phosphoric acid when the system is heated and its role as a catalyst in the dehydration process of hydroxyl groups (Shen et al., 2022). Markwart et al., 2019 described that upon heating, P-ester bonds in organic P-based FRs are quickly decomposed to phosphoric acid via *cis*-elimination (main reaction) or hydrolyzation (minority reaction), as shown in Fig. 3(A). During the burning process, enough energy is released to attack a non-bonding electron of the oxygen from the phosphoryl group (P=O) on the hydrogen of the second carbon from the

alkoxy group. This reaction gives an alkene and a phosphorus compound with a hydroxy group. This reaction can be repeated as many times as there are alkoxy group bonds to the phosphorus. Then, the reaction of phosphoric acid in char formation depends on the polymer and its structure. For example, in the epoxy resin, as shown in Fig. 3(A), the hydroxy group (P-OH) of the phosphoric acid is esterified with the hydroxy group of the undecomposed resin (HO-R) or with the aromatic alcohol (HO-Ar) which is the resin decomposition product, or polymerization takes place forming a polyphosphoric acid (inorganic glass). All these dehydration reactions create char precursors (Markwart et al., 2019; Naiker et al., 2023a; Weil & et Sergei V. Levchik, 2017). Structural arrangements might also take place to facilitate the P-esters formation and consequently the char formation. Levchik & Weil, 2005; Velencoso et al., 2018 reported that aromatic phosphate flame retardants, such as Resorcinol bis (diphenyl phosphate), first catalyze the Fries rearrangement during the burning process in polycarbonate/acrylonitrile butadiene styrene (PC/ABS) polymer to convert phenolic esters into hydroxy aryl ketones so phosphoric acid can react with the polymer by transesterification, forming P-ester groups.

3.1.1.2. Polyphosphates and pyrophosphates.

Polyphosphates and pyrophosphates- based flame retardants were reported to behave similarly as the organic P-based flame retardants. Upon heating, phosphoric acid is also produced and acts as an acid catalyst in the dehydration process to form char e.g, ammonium polyphosphates (APP), and pyrophosphate piperazine (PAPP). Maqsood & Seide, 2019 investigated APP combined with cornstarch and reported APP decomposition to phosphoric acid, and ammonia. Phosphoric acid catalyzed the dehydration process of hydroxyl groups present in the starch, forming P-esters on that can later decompose, releasing carbon dioxide and water due to dehydration of the starch as described in Fig. 3(B). Sun et al., 2023 also reported a similar mechanism for pyrophosphate piperazine (PAPP) that forms polyphosphoric acid substances upon heating when tested on Ethylene Vinyl Acetate polymer.

3.1.1.3. Orthophosphate minerals.

Orthophosphate minerals such as $NH_4H_2PO_4$, $MgNH_4PO_4 \cdot 6H_2O$ (struvite), and hydroxyapatite were also reported in the firefighting industry (Z. Liang et al., 2022; Guo et al., 2018; A. H. Kim et al., 2021). Z. Liang et al., 2022 reported struvite efficiency as a fire extinguisher on burning oil samples as it acts both in the condensed phase by forming $Mg_2P_2O_7$, pyrophosphate, also called heat-resistant ceramic as a final pyrolysis product, and in the gas phase by releasing water, ammonia, and phosphorus oxygen which can absorb OH· and H· radicals in the flame. This mechanism contrasts with A. H. Kim et al., 2021's proposal, wherein struvite suspended with cellulose carriers was tested on wood. Struvite was proposed to act similarly to ammonium polyphosphate (APP), generating phosphoric acid under burning conditions, dehydrating alcohols, and forming unstable esters that decompose into char, as also reported by H. Guo et al., 2020 in their study on the flame retardancy mechanism of struvite mineralization treatment. Guo et al., 2018 also prepared cellulose nanofiber (CNF)-calcium phosphate hydroxyapatite (HAP) composite foams that showed excellent flame retardancy as HAP was thermally stable.

3.1.1.4. Additives that show synergy effects with P-FR.

Some additives show synergy effects with P-FRs further enhancing the thermal stability of these flame retardants e.g., P-metal-based FR in the presence of multivalent cations or P-N-based FR in the presence of nitrogen-containing compounds. For example, Laoutid et al., 2021 reported calcium-based minerals such as calcium hydroxides combined with APP in ethylene vinyl acetate (EVA) polymers form a thicker homogenous char and significantly reduce the peak of heat release rate (PHRR) compared to when additives have been used separately thanks to the interactions between additives during combustion process. The presence of calcium hydroxide assisted in forming cohesive residues by deposition

of calcium carbonate during the combustion induced by the chemical reaction between calcium hydroxide and the polymer (Laoutid et al., 2013; Rothon, 1999). It is also reported that when nitrogen is combined with phosphorus, it increased considerably the flame retardancy effect through the formation of more char (Vothi et al., 2019) as the result of intermolecular coupling via a transesterification reaction which continues until it removes all the hydrocarbons and forms a phosphorus-nitrogen compound-rich char which is thermally stable (Nguyen & Kim, 2008). Also, nitrogen-containing compounds release incombustible gases, such as nitrogen oxides (NOx), during combustion, which dilute the concentration of flammable gases, reduce the availability of oxygen, and suppress the spread of fire as for ammonium polyphosphates or melamine polyphosphate flame retardants (G. Wang & Bai, 2017).

3.1.2. Phosphorylated polymers

P-based FRs can be used as an additive (coating the material with a flame retardant as in section Mechanisms of P-based flame retardants 3.1.1) or can be obtained by inducing phosphorylation of the material (performing chemical phosphorylation described in Section 2.1.2.2) to make the material inherently flame-retardant (Velencoso et al., 2018). Some naturally phosphorylated polymers, due to their inherent flame-retardant properties, have gained significant interest for flame retardancy applications because they are naturally phosphorylated, and elemental P_4 is not needed for their production as it is done for commercial P-based FRs. This section explores examples of naturally phosphorylated biopolymers e.g., DNA, Casein, and phytic acid, as they are analogous to the EPS nature.

3.1.2.1. DNA.

Deoxyribonucleic acid (DNA) has been reported as a flame retardant for different polymeric matrices as ethylene-vinyl-acetate (EVA), polypropylene (PP), and polyamide 6 (PA6) and 10 wt. % DNA showed good performances and a reduction in the PHRR of more than 50% in all polymeric matrices (Alongi et al., 2016). DNA is an effective flame retardant because its chemical structure contains P-esters as a phosphoric acid source, charring deoxyribose carbohydrates, and nitrogen-heterocyclic building blocks as blowing components forming ammonia and nitrogen inert gases during combustion (Alongi et al., 2013, 2015, 2016; Koedel et al., 2021). ATR-FTIR and TGA analysis showed the separation of sugar (phosphorus part) and nucleobase (nitrogen part) via scission. Phosphodiester sugars underwent dehydration, polymerization, and reorganization through pyrolysis to also form char as described earlier. Nucleobases reacted with each other, leading to dehydration and polymerization, forming char. This suggests that there is a synergy effect between phosphorus and proteins contributing to the flame retardancy of DNA. DNA as a flame retardant for cellulose acetate (CA) polymer was also investigated by Koedel et al., 2021, and it was reported that flame-retarding effects of DNA on CA are concentration-dependent. The 10 wt.% DNA mixture exhibited the strongest PHRR reduction by 25.5%, twice as high as the PHRR reduction of 5 wt.% DNA mixture.

3.1.2.2. Casein.

Casein has also been proposed as a bio-based flame retardant. Phosphorus is present inside micellar casein (0.7–0.9 wt.%); its structure is unclear, but it has a high P content in the form of colloidal CaP and P-ester bonds (phosphor-serine) (Uddin et al., 2020; Hindmarsh & Watkinson, 2017; Léonil et al., 2013). In earlier studies, casein exerted flame-retardant effects on cotton fabric (Carosio et al., 2014; Malucelli et al., 2014; Xu et al., 2019). Xu et al., 2019 have modified casein by introducing more P atoms to reach 7.2 wt.% to expand its application as a flame retardant in textiles. The P atoms were first introduced into casein by chemical phosphorylation to synthesize the casein-based FR, which was then grafted onto cotton fabrics by covalent bonds. During the burning process, the casein-based flame retardant changed the conventional pyrolysis route of cellulose as phosphoric acid was

released and dehydration reactions occurred to form a strong char layer. This study did not report specifically if colloidal CaP contributed to the flame retardancy effect or not; however, we propose that calcium would contribute to the flame retardancy of casein. Calcium phosphate could have formed hydroxyapatite while burning forming a thermal stable layer as mentioned earlier in Section 3.1.1.3. Similarly, Uddin et al. 2020 reported that magnesium produced a thermally stable insulating magnesium oxide layer in casein-magnesium composites.

3.1.2.3. Phytic acid. Phytic acid (PA), or inositol hexaphosphate acid, is another phosphorus-rich natural substance explored as an eco-friendly FR. With a phosphorus content of 28 wt.%, PA serves as the primary phosphorus source in plant tissues, mainly in seeds, roots, and stems. Cheng et al., 2016 used PA to make poly (lactic acid) (PLA) fabric less flammable and reported that higher concentrations of PA led to a greater reduction in PHRR of the treated fabric. Liu et al., 2023 reported that an effective approach to maximize the flame retardancy of PA involves blending PA with other elements to create a compound or mixture, establishing a synergistic flame-retardant system such as a phytate metal system. Since PA has 6 negatively charged phosphate groups in its structure, it has a strong affinity for metals. It can form complexes with metal ions (magnesium, sodium, potassium), further enhancing the char layer formation.

3.1.3. Insights into flame retardancy in EPS

EPS, extracted from excess sludge, can replace traditional flame retardants in the chemicals industries. EPS is a gel-forming polymer that consists of a mixture of proteins, free amino acids, sugars, and glyco-conjugates (including glycoproteins and glycolipids) and other inorganic metals such as iron, calcium, phosphorus, etc (Bahgat et al., 2023; L. M. Chen et al., 2024; Komazin et al., 2019).

EPS extracted from AGS was reported to have 2–2.5% dry weight of phosphorus, with 25–30% of total P as free orthophosphates in the liquid fraction and 75%-70% is bound P (possibly organic, polyphosphates, pyrophosphate, mineral precipitates or others) (Bahgat et al., 2023). Kim et al., 2022 reported EPS as a possible bio-based flame retardant since polyvinyl alcohol (PVA) composites fully decomposed at 800 °C, whereas approximately 31.7% residue remained in the EPS/PVA composite, and the EPS/PVA composite exhibited a 36.4% reduction in PHRR compared to PVA alone. Kim et al., 2020 also reported that EPS-AGS had better results than EPS-flocs. They contributed the difference to the phosphorus in the EPS because carbonated hydroxyapatite was a dominant composite in the remaining residue of EPS-AGS coated flax fabric after burning, unlike the residue of EPS-flocs coated flax fabric in which only amorphous phosphate was detected, suggesting that different P groups would contribute differently to the thermal stability. Carbonated hydroxyapatite is a thermally stable barrier contributing to the flame retardancy effect as explained in Sections 3.1.1.3 and 3.1.1.4, and its formation could be explained that calcium phosphates, either present initially in EPS or formed during heating, in EPS react with CO_2 produced while burning. Another possible mechanism is that organic P or poly-P in EPS releases H_3PO_4 upon heating and acts as a catalyzer forming the char layer as explained in Sections 3.1.1.1 and 3.1.1.2. Also, EPS contains other components that could contribute to flame retardancy such as nitrogen-containing compounds (similarly to P-N-based flame retardants and reports on DNA), charring sugars (similarly to reports on DNA), metals such as calcium, magnesium, iron (similarly to reports on phytic acid). All these components besides phosphorus could be a synergistic effect contributing to the flame retardancy of EPS. Therefore, although there is evidence that phosphorus components play a role, the exact mechanism of flame retardancy is still unknown and needs further investigation.

3.2. Physiochemical properties

The intercellular structures formed by EPS possess the inherent capability to retain moisture through two binding mechanisms: electrostatic interactions and hydrogen bonds (Neyens et al., 2004). EPS also carries a negative charge and selectively binds to divalent cations forming a stable gel that prevents water seepage from flocs pores which makes it a choice as a biostimulant in agriculture, horticulture, and forestry (Mangrum & Jenkins, 2020). Viscoelastic properties and water-holding capacity properties are important to develop EPS as a bio-stimulant. EPS can also be applied as a coating for seeds, slow-release fertilizers, and paper and as an adhesive material. Emulsifying properties are important for coatings applications to improve the performance and extend the shelf life of the coating, and viscoelastic properties are important to ensure the ability of making thin coatings and films out of EPS. For any industrial applications where an EPS system is used as a gel it would be important to know and even be able to influence the viscoelastic properties. P could contribute to these properties and could be a way to tune the properties. So, in this section, we explore the mechanisms by which P influences these properties in different matrices as in food industries, such as in walnuts, egg whites, ovalbumin, potatoes, and rice proteins to understand how this knowledge translates to EPS (Y. Hu et al., 2023; Cen et al., 2022; Wang et al., 2019; Anjaneyulu et al., 1989; Hsu & Chung, 2001).

3.2.1. Influencing the intermolecular forces of polymers via P salt addition

The combination of intermolecular forces of polymers leads to a well-developed structure causing specific polymer properties. For example, the three-dimensional protein structures are maintained by intermolecular forces such as covalent bonds (e.g. disulfide bonds), noncovalent bonds (e.g. hydrogen bonds), hydrophobic interactions, and electrostatic interactions (Castellanos et al., 2014; Hong et al., 2017). The properties of proteins can be modified by influencing these intermolecular forces via polyphosphates and pyrophosphates salts addition by introducing organic P groups (chemical phosphorylation, described in Section 2.1.2.2) that increase the polymers' electronegativity or influence the physiochemical environment (changes in pH and ionic strength).

3.2.1.1. Organic P. The properties of polymers can be modified by influencing intermolecular forces by introducing phosphate groups on the side chains of the polymers by phosphorylation. These new organic-P bonds increase the electronegativity of the polymer which enhance the ionic interaction between phosphate groups and $-NH_3^+$ of amino acids (Criado-Gonzalez et al., 2020; T. Huang et al., 2019; Kaewruang et al., 2014b) and can form salt bridges in the presence of metal ions bridging between negatively charged P groups on the polymer molecules (Bryant & McClements, 2000; Lv et al., 2022;Arfat & Benjakul, 2013). Arfat & Benjakul, 2013 reported the enhancement in the gelation of phosphorylated protein isolate of yellow stripe trevally by the addition of zinc sulfate as zinc served as a cross-linker via a salt bridge between the negatively charged phosphate groups introduced by phosphorylation.

3.2.1.2. pH and ionic strength. P salt addition can also influence the intermolecular forces and polymer properties via physicochemical changes, pH, and ionic strength. When the pH of the polymer environment is close to the isoelectric point (pI) (pH point where a particular molecule carries no net electrical charge, equally positive and negative charges), the static repulsion between polymer molecules decreases, and it aggregates. However, at pH > pI, the polymer will have a higher negative charge; at pH < pI, the polymer will have a higher positive charge. That creates electrostatic repulsion forces and exposes the hydrophobic groups inside the polymer, influencing the intermolecular forces causing the swelling of the polymer chains (Duan et al., 2013; Lv et al., 2022). Ionic strength also influences intermolecular forces such as

pH. Salt addition increases the negative charge of the polymer due to anion preferential binding, increases the electrostatic repulsion forces, and exposure of the hydrophobic groups. Cations from the salt addition also form an ion cloud around the polymer, resulting in local concentration difference and increased osmotic pressure, causing further swelling of the proteins filaments (Offer et al., 1989a; Puolanne et al., 2001). However, excessive salt can cause the ionic and hydrogen bonds between protein molecules to break and cause the system to collapse (Sow & Yang, 2015); hence, controlled pH and ionic strength are necessary to have the optimum effect on polymer properties upon salt addition. P salts are also commonly used in the meat industry because P has multiple negative charge which induces higher electronegativity and physiochemical changes with little salt addition. Also, it forms a sticky gel layer upon heating that greatly reduces water losses after cooking compared to other salts (Cao et al., 2020; Feiner, 2006; Offer & Trinick, 1983; SIEGEL & SCHMIDT, 1979; S. Q. Xu et al., 2009).

3.2.2. Viscoelastic properties

EPS exhibits both viscous and elastic characteristics when deformed under stress. As discussed in the earlier section, phosphorus influences the intermolecular forces of polymers and consequently their viscoelastic properties. Cen et al., 2022 phosphorylated fish gelatin (FG) using TSPP (50 Celsius, 30 mins, pH 7), and Kaewruang et al., 2014a did the same using STPP (65 Celsius, 1–3 hours, pH 5–11) and reported enhanced gelation and better gel strength of gelatine. The samples were dialyzed in both studies to confirm that the better gelation effect was due to bound phosphorus to the FG. Both studies showed that excessive phosphorylation (organic bound) or addition of orthophosphates (free) weakened the gel strength due to electrostatic repulsions between protein molecules. Similarly Yu et al., 2024 reported that phosphorylated ovalbumin (OVA) addition to pork myofibrillar protein (MP) increased the gel hardness by 2.67-fold higher compared to the control; however, excess addition of phosphorylated OVA decreased the gel hardness which was attributed to the fact that excessive amounts of P-OVA weakened the interaction between MP and P-OVA and resulted in depolymerization (Walayat et al., 2021). Hence, the degree of phosphorylation is a crucial parameter to optimize, and residual free phosphate concentrations might have to be prevented or eliminated to prevent negative influences on gelation. Xiong & Ma, 2017 reported stronger intermolecular cross-linking, improved rheological properties, and increased rigidity of phosphorylated ovalbumin (OVA) using STPP within pH 5–9 and at 45 °C for 12 hours. MALDI-TOF/MS confirmed that phosphate groups were successfully grafted onto the OVA backbone through covalent interactions and C-O-P bond formation, and the number of phosphorylated peptides was similar over the pH range. Kaewruang et al., 2014a also reported that gelatin phosphorylation occurred over pH 5–11 but the alkaline pH range had the highest gel strength, which was interpreted due to a higher phosphorylation degree at alkaline pH. Miedzianka & Pęksa, 2013 also reported a higher phosphorylation effect at alkaline pH compared to lower pH while phosphorylating potato protein isolates at a pH range of 5.2–10.5 at room temperature for 30 mins. Phosphorylation at alkaline pH was also reported by SUNG et al., 1983 for phosphorylation of soybean protein (pH 10.5–12.5, 25–45 °C, for 3 h). Also, Woo et al., 1982 obtained stable phosphorylated bovine protein at pH 8.5, and the ^{31}P NMR spectral data suggested that protein lysine and histidine residues have been phosphorylated. Hence, pH also plays a role in optimizing phosphorylation, and alkaline conditions seem to induce higher phosphorylation degree than acidic conditions. To understand the influence of pH on phosphorylation, it is important to think about the activity of the nucleophilic groups and the stability of the leaving groups (Klein, 2012). Ideally, the pH should be such that the nucleophile is in its deprotonated, more active form, while the leaving group remains protonated enough to leave easily. OH- is only active in alkaline conditions, where it is in its deprotonated form (Illy et al., 2015b; Li et al., 1997; Petreuş et al., 2003b). The unprotonated form of NH_2 groups is a good

nucleophile as it has a pair of lone electrons on the nitrogen. Alkaline conditions will make it highly reactive (deprotonated form), whereas acidic conditions will make it less active due to NH_3^+ formation, although it can still participate in nucleophilic attacks (Miedzianka & Pęksa, 2013; SUNG et al., 1983). Therefore, the pH should be optimized to balance nucleophile activity and leaving group stability, making slightly alkaline conditions more optimal for higher phosphorylation than acidic conditions.

3.2.3. Water-holding capacity

Water holding capacity (WHC) is the ability of the polymer to hold water during the application of force, pressure, centrifugation, or heating (Ikeuchi, 2011; Zayas, 1997) Viscoelastic properties and WHC are related concepts as intermolecular forces influence both properties (Albarracín et al., 2011; Poornima & Dean, 1995) and the development of a three-dimensional network structure, capable of capturing water molecules and trapping them, leading to better WHC (Hughes et al., 2014; X. Wang et al., 2017). Miedzianka & Pęksa, 2013 reported improved WHC in phosphorylated potato protein isolate after STMP treatment and dialysis to confirm that improved WHC was due to new bound phosphorus. Increased water holding capacity of phosphorylated soy protein isolate using STMP was also observed by SUNG et al., 1983 who confirmed phosphoesterification of serine residues and the phosphoramidation of lysine residues in soy protein and shift of pI (isoelectric point) of the protein approximately 0.8 pH unit because of the introduced negative phosphate groups. Yu et al. 2024 also reported that phosphorylated ovalbumin (OVA) addition to pork myofibrillar protein improved the WHC up to 76% due to the construction of more compact network structures, which facilitated the capture of water molecules and increased the WHC of the MP gel.

The combined effect of P salts via phosphorylation and physiochemical properties on WHC could also be concluded from the meat industry literature and knowledge on actomyosin ATPase cycle in nature. Anjaneyulu et al., 1989 studied the effect of SPP, SAPP STPP, and SHMP on the physicochemical properties of raw and cooked buffalo meat and patties. They reported a WHC increase of 3–4-fold depending on the type of phosphate and its concentration. Pyrophosphate was found to be significantly superior in increasing WHC and moisture retention % in raw and cooked buffalo patties than other polyphosphates due to its greater contribution in raising the pH of meat compared to polyphosphate salts (Kondaiah et al., 1985; Molins, 2018; Zayas, 1997). Gadekar et al., 2014 also reported a similar trend of enhanced WHC, moisture retention %, and decreased cooked losses in raw and cooked patties using TPP and TSPP in goat meat. The increase of WHC in raw patties is associated with physiochemical changes, as discussed in 3.2.1.2, but also due to phosphorylation as discussed in section 3.2.1.13.2.1.1. Although phosphorylation was not verified directly in those studies, based on the methodology used (heat treatment during cooking, high pH, P-O-P salts addition), this should have induced phosphorylation, which makes P-O-P salts superior in meat applications compared to other salts (e.g., NaCl). This can be confirmed by Trout & Schmidt, 1986 who reported that the improvement in protein functionality in meat products by P-O-P salts could not be explained only by pH or ionic strength changes as it also forms a sticky gel on the top of the cooked meat, binding meat pieces together (Offer et al., 1989; Offer & Trinick, 1983). P-O-P salts act to dissociate actomyosin via myosin phosphorylation, which frees myosin (increases its solubility) to participate in a greater number of molecular interactions (Jolley & Offer, 1984; SIEGEL & SCHMIDT, 1979b). Myosin was reported to be the main responsible protein for the binding together of meat pieces as it forms a sticky exudate on the surface of meat products. This effect is similar to the role of ATP in the actomyosin ATPase cycle, a fundamental process in muscle contraction, when it binds to myosin head and detaches it from the actin (L. Cao et al., 2020; Kodera & Ando, 2014; Cecchini et al., 2008). So, P-O-P salts added to meat products not only influence pH and ionic strength but also bind to myosin

(phosphorylation concept kicks in), causing actin-myosin dissociation which enhances the gelling, texture, tenderness, and WHC of the meat. Since EPS could contain different P species as discussed in Section 2, organic P species could influence the intermolecular interactions between EPS proteins, or in general different P species could influence pH and ionic strength and consequently influence EPS viscoelastic and WHC properties.

3.2.4. Emulsifying properties

Phosphate esters are anionic emulsifiers that have many industrial applications (e.g., detergents, cleaners) because of their excellent surface properties, chemical stability, and biocompatibility (Zhao et al., 2020). These esters have a negative charge on the phosphate group acting as the hydrophilic part and the hydrocarbon chain is the hydrophobic part (Cooper, 1963). Enhancing the emulsifying properties of proteins through phosphorylation is also reported in food literature to enhance their stability against environmental stress (Chen et al., 2019; (Y. Hu et al., 2023). For example, Ovalbumin, the most abundant protein in egg white, treated with STPP with dry heating exhibited higher stability against flocculation, coalescence and phase separation compared to the native Ovalbumin due to the formation of phosphopeptide monoesters revealed by FTIR, XPS and ^{31}P NMR (Xiong et al., 2016). Z. Hu et al., 2019 also reported better stability and storage for phosphorylated rice bran protein under wide range of environmental conditions and phosphate esters and changes in the secondary and tertiary structure of the protein was observed upon the phosphorylation with STMP. Miedzianka & Pęksa, 2013 also reported better emulsifying activity for phosphorylated potato protein isolates over pH range of 5.2 to 10.5 and higher emulsifying activity at the alkaline range. As mentioned in Section 2.1.1, phospholipids (P-diesters) are part of the cell membranes that might end up in the EPS. Phospholipids were also reported to reduce the surface tension to levels ranging from 25 mN/m to 45 mN/m (Veldhuizen et al., 1998). García Becerra et al., 2010 reported that the surface tension of alkaline extracts of sludge decreased from 70 mN to around 35 mN/m increasing the pH from 7 to 13 due to the presence of lipids, phospholipids as detected by ^{31}P NMR, which are released due to cell lysis. So, organic P might also be one of the factors contributing to EPS emulsifying activity and stability over time which boosts its possible applications as a coating material.

4. Outlook: EPS engineering via P

4.1. Translating literature knowledge to EPS

The previous sections show that different P species that are expected to be present in EPS or after extraction of the EPS are likely to influence properties such as flame retardancy, viscoelastic properties, water holding capacity, emulsifying properties etc. The main influences of P groups in EPS that we anticipate are the following:

- For thermal stability: organic P, polyphosphates, and pyrophosphates in EPS could contribute significantly to its flame retardancy by forming phosphoric acid upon heating and its role as a catalyzer for the dehydration reactions forming the char layer. Orthophosphate minerals, if initially present in EPS or formed during burning, could also contribute to flame retardancy by forming a thermal stable layer e.g., hydroxyapatite. Or if all these P species were present in EPS, a synergic P and multivalent cation flame retardant could be anticipated. The presence of glycoproteins and amino acids in EPS could also contribute to flame retardancy along P species creating a synergic P-N based flame retardant. Future research should elaborate on the possible mechanisms of phosphorus and their binding modes in EPS and the effects on flame retardants.
- For viscoelastic properties, water-holding capacity, and emulsifying properties: Organic P in EPS could contribute significantly to these properties, as it changes the surface chemistry by increasing its

electronegativity and influencing the intermolecular forces. Polyphosphates, pyrophosphate salts or any other salts that could influence the pH or the ionic strength of the polymer would also influence the intermolecular forces and consequently the polymer properties. Around 15–20% TS of EPS extracted from AGS is salt (primary from the chemical additions in the extraction process, ends up as ash), as estimated by Bahgat et al., 2023. Such a high salt content could contribute significantly to these properties, so, washing experiments are recommended for the EPS to evaluate the influence of these salt on viscoelastic and water-holding capacity properties and if it's recommended to wash it or not for industrial applications. These washing experiments could also be designed to differentiate the influence of anions and cations to EPS. Since EPS is reported to be mostly negatively charged (L. Huang et al., 2022b; Sudmalis et al., 2020), it is expected that cations could also have a significant influence.

4.2. Engineering P species in EPS

Understanding and influencing the role of P species in EPS will open a new direction towards how to potentially maximize the effect of P on EPS by engineering certain P species in EPS, for instance, by inducing phosphorylation. Based on the phosphorylation conditions reported in Sections 2.1.2.2 and 3.2, EPS extraction conditions reported by Bahgat et al., 2023 (mainly the alkaline solubilization step) are very promising to induce purely chemical phosphorylation (N.T. Bahgat et al., 2024): 1) the sludge treated at this stage already contains significant amounts of polyphosphates (high energy P-O-P salt if not stored anaerobically in the buffer tank to provide the energy needed for phosphorylation), 2) the temperature is 80 Celsius (high temperatures promote the endothermic phosphorylation reaction), 3) the pH is alkaline ≈9 (potentially promoting higher phosphorylation degree compared to acidic conditions because slightly alkaline conditions deprotonate the nucleophilic hydroxyl and amino groups and not overly protonates the leaving groups of P salts), and 4) the incubation time is 2 hours (in the same order of magnitude of phosphorylation incubation time in literature). The consequences of phosphorylation may vary depending on the polyphosphate concentrations in the sludge, EPS molecular conformation, the available phosphorylation sites, the extent of phosphorylation, operating pH, temperature, incubation time, and others. Future research should investigate all these different factors to assess the optimal conditions to promote better phosphorylation and better influence of EPS properties. EPS extraction conditions could be tweaked to induce a higher degree of phosphorylation by the following recommendations as shown in

1) Ensure higher initial polyphosphate and pyrophosphate concentrations in the sludge. This can be achieved by: Upstream: Optimizing the enhanced biological phosphorus removal in the WWTPs so a significant amount of phosphorus removed from wastewater is stored as polyphosphates. Upstream: Keeping the sludge retention time (SRT) in the sludge buffer tank before the EPS extraction process very short and always in aeration to avoid polyphosphate hydrolysis to phosphates. Along the EPS extraction process: Adding external polyphosphate or pyrophosphate salts into the alkaline solubilization step if a higher phosphorus salt concentration is needed to promote higher phosphorylation.

2) Promote phosphorylation in acidic conditions as well by changing the following: using H_3PO_4 instead of HCl + the application of condensing agents + using high temperatures (80 Celsius by removing the heat exchanger before acidification or higher). Condensing agents, e.g., urea, react with water to form new compounds, achieving low water activity as water is not a rare leaving group in the aqueous phase. Keeping the temperature high at the acidic step in that case is necessary as it will be the main source of
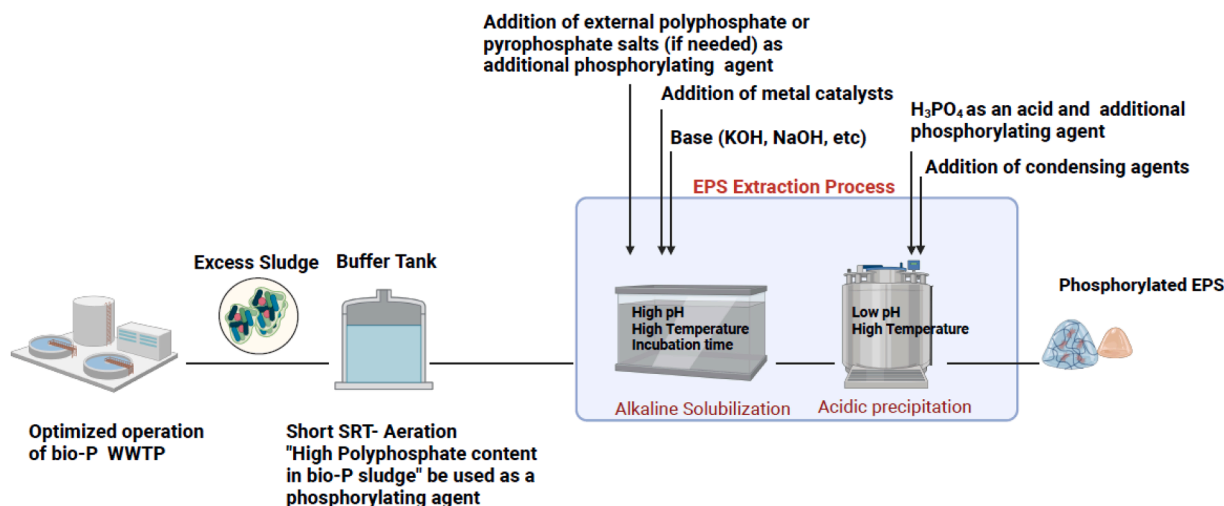
Fig. 4. Controlling the EPS extraction conditions to promote phosphorylation at the alkaline and acidic steps.

energy to drive the endothermic phosphorylation reaction as H_3PO_4 is used instead of polyphosphates as a source of phosphorus.

3) Addition of metals (e.g., Mg^{2+}) to the alkaline and acidic reactors to catalyze the phosphorylation reaction as they increase the electrophilicity of the phosphorus atom (Soderberg, 2016).

Further research should investigate the optimum point of all these different factors to achieve the highest degree of phosphorylation. The addition of external polyphosphates or pyrophosphate salts into the alkaline EPS extraction step could also be a way to engineer EPS properties if a higher P salt concentration is needed to promote phosphorylation. Other ways to engineer or select different P species in EPS could be adding Ca^{2+}, $Fe^{2+,3+,}$ or Mg^{2+} in the WWTP or during the EPS extraction process to have higher multivalent cation content (Mg, Ca, Fe), reach supersaturation levels and obtain higher content of precipitated P minerals in EPS at the optimal pH since metal content showed to enhance some properties as thermal stability (N.T. Bahgat et al., 2024). Future research on how to tweak the EPS extraction process and how it could change P speciation is necessary to assess if P is indeed influencing EPS properties. A further interesting approach could be to research how EPS P content and its properties could be influenced due to chemical differences in the influent stream, or operating conditions in WWTPs (upstream processing). Creating this knowledge could help to facilitate EPS engineering by leveraging the interplay with P based on the targeted property in relation to relevant industrial applications.

Fig. 4

5. Conclusions

EPS contains different P species e.g., polyphosphates, pyrophosphates, organic-P, orthophosphates minerals, etc. either formed microbially or induced chemically during the WWT or EPS extraction process. Phosphorus and EPS interplay has the potential to enhance EPS utilization in the industry and elevate the value and quality of EPS and phosphorus recovery from wastewater treatment plants (WWTPs). This interplay can potentially promote EPS utilization as a flame retardant, slow-release fertilizer, bio-stimulant, coating material, or others by enhancing its thermal, viscoelastic, emulsifying properties, water-holding capacity, or others. Consequently, this advancement could facilitate the substitution of fossil-based polymers, and P_4 derivatives, and make the recovery process from WWTPs more economically viable. This review represents a critical step toward realizing a WWTP model that acts as a production facility that produces high-value products that fit into industrial niches. We recommend doing more P speciation research over-extraction and seeing how P speciation modifications are changing the properties.

CRediT authorship contribution statement

Nouran T. Bahgat: Writing – review & editing, Writing – original draft, Investigation, Data curation, Conceptualization. **Philipp Wilfert:** Writing – review & editing, Investigation, Conceptualization. **Stephen J. Picken:** Writing – review & editing, Conceptualization. **Leo Sorin:** Investigation, Data curation. **Yuemei Lin:** Writing – review & editing, Conceptualization. **Leon Korving:** Writing – review & editing, Investigation, Conceptualization. **Mark C.M. van Loosdrecht:** Writing – review & editing, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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