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Review

Extracellular polymeric substances (EPS) in sewage sludge management: A call for methodological standardization

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ABSTRACT

Extracellular polymeric substances (EPS) are crucial in sewage sludge management, influencing key processes such as sedimentation, dewatering, and drying. Despite their importance, the lack of standardized methods for EPS extraction and analysis has led to inconsistent research findings, hindering a thorough understanding of EPS's role in sludge treatment. This review paper addresses this issue by critically comparing various EPS extraction and analysis methods, emphasizing the urgent need for standardization in the field. Standardized methodologies will enable researchers to compare studies more accurately and derive meaningful insights into EPS's role across different stages of sludge treatment, ultimately advancing EPS knowledge and application in sludge management. Additionally, this paper summarizes findings from numerous studies on EPS impact in sedimentation, dewatering, and drying, offering a holistic view of their significance in sludge management. Moreover, it explores the potential EPS applications, highlighting both the future directions and the challenges associated with their production.

1. Introduction

Sewage Sludge (SS) is an unavoidable by-product of all biological wastewater treatment plants. Depending on the treated wastewater quality, it may contain a variety of toxic substances, including pathogens, heavy metals, and organic contaminants, which may pose serious environmental problems, if not appropriately managed and disposed of (Wu et al., 2020). In Europe (EU-27), SS production is estimated to be approximately 9.25 million tons of dry solids per year (Feng et al., 2023), with Germany, Spain, France, Italy, and Poland standing out as the top 5 sludge producer countries (Eurostat, 2019; Collivignarelli et al., 2019). Regarding sludge disposal, around 35.14% of sludge was applied to the land for agricultural purposes, while about 18.83% underwent composting, 34.15% was subjected to incineration, and 3.90% was disposed of in landfills, according to Eurostat (2024) (Table S1). However, as depicted in Fig. 1, there is a significant disparity among member states, with countries like Spain, Italy (data not shown in Fig. 1), and Sweden favoring agricultural use, while Germany and the Netherlands prioritize thermochemical methods like incineration. In Belgium, the Wallonia regions are known for their focus on agriculture, while Flanders is oriented towards incineration. These differences reflect how, in each state, regional policies can shape the direction of SS management.

Managing SS is a pressing, costly, and multifaceted task, particularly since the European Council Urban Wastewater Treatment Directive (UWWTD) 97/271/EC came into effect. The UWWTD plays a crucial role in regulating SS management and disposal within the EU. Over time, the Directive has become more rigorous, imposing stringent guidelines for sludge pre-processing and post-processing to guarantee appropriate treatment and mitigate the public health and environmental effects. The UWWTD Directive focuses on appropriate waste management and is consistent with Europe's 3R concept (Reduce, Reuse, Recycle). The 3R approach emphasizes reducing waste, promoting materials or products reuse whenever possible, and implementing recycling practices to maximize resource recovery.

SS, often termed as mixed sludge (i.e., the combination of primary sludge, originating from raw wastewater settling, and secondary sludge, derived from excess biomass growth in biological processes), typically contains about 93–99% water (Tezel et al., 2011; Yang et al., 2015; Rajput et al., 2022). Sedimentation, dewatering, and drying are three

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crucial stages in sewage sludge management to reduce its water content significantly, thereby facilitating its handling, enabling safe utilization, and minimizing transportation expenses. Numerous research studies have highlighted the relevance of Extracellular Polymeric Substances (EPS) in affecting the efficiency of these processes, as they adsorb a significant amount of water (Li et al., 2022).

EPS is a complex, high molecular-weight polymer mixture mainly secreted by bacteria involved in the degradation of organic matter during the activated sludge wastewater treatment process. They are dispersed outside the cells and within microbial aggregates. EPS constitutes a considerable portion of SS, corresponding to 50–80% of organic components (Liu and Fang, 2003a). Polysaccharides and proteins are the major EPS components (70–80%) (Dignac et al., 1998; Nouha et al., 2018). Other components, including nucleic acids, humic acids, and lipids, constitute up to 20–30% of EPS (Neyens, 2004). Researchers have employed various methods to extract and analyze EPS from sludge. Notwithstanding, using heterogeneous methods has made it arduous to compare results and interpret findings across different studies. Several review papers have discussed and compared EPS extraction methods, highlighting their impact on yield and composition. However, a significant gap remains in comparing EPS analysis methods. EPS influence on sludge sedimentation, dewatering, and drying processes is an area requiring further investigation. This review paper aims to address these gaps by highlighting the urgent need for standardized EPS extraction and analysis methods to facilitate the comparison of findings, as well as exploring EPS impact on the sludge treatment process. Additionally, the paper discusses the potential EPS applications, the challenges faced in their production, and strategies to improve their production process.

2. Extracellular polymeric substances (EPS): An overview

EPS are intricate high-molecular-weight biopolymers produced by microorganisms, forming a gel-like matrix that provides shelter and structural stability to sludge flocs (Flemming and Wingender, 2010). EPS plays a crucial role in microbial attachment, aggregation, and immobilization, as well as in the removal of contaminants from wastewater (Sheng et al., 2010). These biopolymers consist mainly of polysaccharides and proteins, with minor fractions of nucleic acids, lipids, and humic substances, forming a protective layer around microbial cells. Proteins constitute the major fraction of EPS in sludge and contribute significantly to its stability and integration within flocs (Liu and Fang, 2002; Hou et al., 2015; More et al., 2014). EPS also serves as a nutrient reserve, providing a carbon and energy source for microbial growth under nutrient-limiting conditions (Flemming et al., 2007).

EPS composition can be greatly influenced by several factors such as microbial species (Nouha et al., 2018), carbon sources (Ye et al., 2011), nitrogen and phosphorus ratio (Hoa et al., 2004), sludge type (Liu et al., 2021a), treatment process, environmental conditions (Sheng et al., 2010), and extraction and analytical methods (Comte et al., 2006a; Sheng et al., 2010). For instance, aerobic sludge typically contains higher amounts of proteins and polysaccharides than anaerobic sludge, which may have a higher lipid content (Sheng et al., 2010). Moreover, Liu and Fang, (2002) demonstrated that sludge from municipal wastewater treatment plants has a different EPS composition compared to sludge from industrial plants, reflecting the influence of the influent characteristics on EPS production.

EPS biosynthesis is a complex process involving intracellular synthesis followed by export outside the cell. Exopolysaccharides are synthesized from simple sugar substrates primarily derived from glucose, and biosynthesis can be generally divided into three main steps: precursor synthesis, polymerization, and export. Protein biosynthesis involves DNA transcription to mRNA, followed by mRNA translation to form polypeptides, which are then folded and modified to form functional proteins that integrate into the EPS matrix (Wingender et al., 1999). These processes are tightly regulated and influenced by environmental conditions and the nutrient availability.

In the field of sludge treatment, the interest in EPS is significantly increasing. Studies have shown that EPS determines sludge flocculation, sedimentation, and dewatering which are crucial steps for sludge

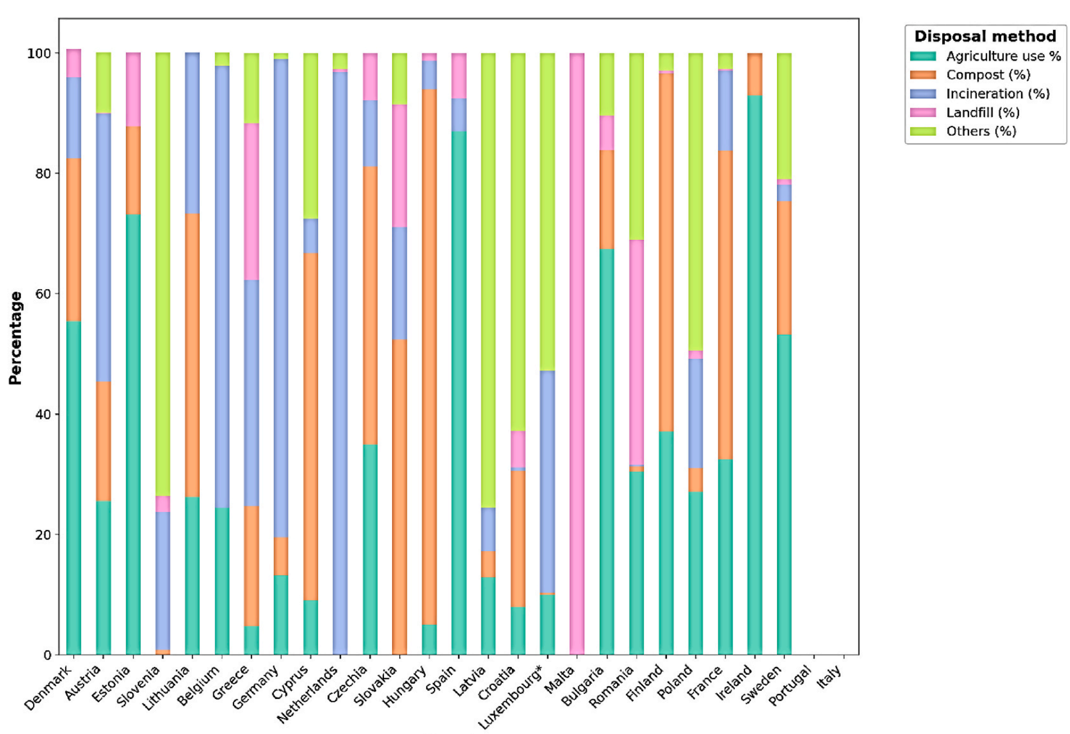

Fig. 1. Percentage distribution of sewage sludge disposal methods in EU-27 countries according to Eurostat data 2024. Note: Data for Portugal and Italy are unavailable. *Data for Luxembourg are estimated.

treatment processes (Sheng et al., 2010; Sam et al., 2022; Ebrahimi et al., 2022; Zhang et al., 2016).

Given the pivotal role of EPS in these processes, understanding their composition is essential. This necessitates robust methods for EPS extraction and analysis.

3. EPS extraction and analysis

3.1. EPS extraction

Nouha et al. (2018) and Atmakuri et al. (2024) reviewed the different EPS extraction methods, underlined their mechanisms, and evaluated the advantages and disadvantages of each. Typically, EPS extraction methods are classified into physical and chemical methods. Physical methods involve the application of external forces to facilitate EPS detachment from cells. These methods include sonication, vibration with glass beads, high-speed centrifugation, and heating (Feng et al., 2019; Huang et al., 2022a; Shen et al., 2022). Chemical methods are employed to disrupt the interaction between EPS and cells, and include alkaline/acid treatment, cation exchange resin (CER), ethylenediaminetetraacetic acid (EDTA), enzymatic treatment, NaCl, NCHO/NaOH, and glutaraldehyde (Comte et al., 2007; D'Abzac et al., 2010; Huang et al., 2022a,b; Sheng et al., 2010). Notably, the combination of these methods is typically more effective (Comte et al., 2006a; D'Abzac et al., 2010; Domínguez et al., 2010).

Depending on how strongly they are linked to microbial cells, EPS are classified into three fractions: Soluble-EPS (S-EPS), Loosely-Bound EPS (LB-EPS), and Tightly-Bound EPS (TB-EPS) (Ramesh et al., 2006). S-EPS is simply extracted by centrifugation, while bound EPS (LB and TB) requires chemical/physical methods to break the binding force between EPS and cells.

Various studies have compared EPS extraction methods to determine yield and composition, as well as their effects on the properties of the extracted EPS. For instance, Comte et al., (2006a) found that chemical methods are more effective in extracting EPS but potentially contaminate the solution, which is further supported by (D'Abzac et al., 2010). In another study, Comte et al. (2007) showed that chemical extraction methods significantly affected the high-pressure size exclusion chromatography fingerprints of EPS, whereas physical methods only influenced the molecular weight distribution.

Likewise, in a study conducted by Hong et al. (2017), it was shown that the combination of formaldehyde and sodium hydroxide resulted in high extraction efficiency of EPS from activated sludge without causing significant cell lysis. Excitation-emission matrix (EEM) analysis revealed that alkaline extraction is ideal for extracting large EPS molecules with diverse molecular weight (MW), but changes in MW distribution may occur, making method selection crucial for effective extraction.

Lu et al. (2015) compared three commonly used methods for extracting EPS from activated sludge: centrifugation had the lowest extraction yield of 3.8% (based on total solids in sludge), while formaldehyde + NaOH and EDTA had comparable yields of 10.3% and 10.9%, respectively. Furthermore, EPS extracted using EDTA exhibited reversible oxidation-reduction peaks and higher electron-accepting capacity, indicating superior redox properties due to the efficient extraction of humic substances compared to the other two methods.

EPS extraction from waste-activated sludge using the CER method resulted in 1.1–2.2 times greater EPS amounts than formaldehyde + NaOH, and 1.1–1.5 times greater amounts than formaldehyde + NaOH + sonication (Domínguez et al., 2010). In a recent study by Wong et al. (2022), it was found that longer extraction CER times of 0.75 h, 4 h, and 24 h were associated with higher EPS yields, producing 90, 158, and 316 mg/VSS, respectively. Finally, Vasilieva et al. (2019) demonstrated that the NH_4OH/EDTA method is efficient for extracting proteins, whereas the HCHO/NaOH method is more effective for polysaccharides.

Table 1 presents a comprehensive overview of various comparative studies on EPS extraction methods, highlighting the efficiency of each

Table 1
Comparison of extraction efficiency and EPS components using various methods. Protein (PN), Polysaccharides (PS) and Humic Substances (HS).

Sample	Extraction method	Unit	PN	PS	DNA	HS	EPS	Remarks	Reference
Algae-bacteria biofilm	Centrifugation	mg/g EPS for all data except EPS yields in mg/g dry biofilm	–	57	–	–	7.2	• EDTA or formaldehyde-NaOH increased yield by about 1 order of magnitude compared with other methods. • PN and PS content in EPS prepared with EDTA or formaldehyde + NaOH were low.	Pan et al. (2010)
	Ultrasonication		22.3	56.6	–	–	12.7		
	EDTA		1.7	3.2	–	–	164		
	Formaldehyde		8	25.2	–	–	14.2		
	Formaldehyde + NaOH		17.07	13.3	–	–	114.7		
Activated sludge	Controle	mg/g DW of EPS for all data Quantities of EPS (milligrams of EPS DW per gram of sludge VSS)	317	170	39	63	16	• Chemical methods yield significantly higher quantities of EPS extracted compared to physical methods. • The EPS extracted by physical methods can show a different qualitative composition with PN and PS	Comte et al. (2006b)
	EDTA		–	24	8	–	96		
	Formaldehyde + NaOH		107	53	9	83	284		
	Sonication		343	140	46	61	27		
	Resin		301	132	24	107	21		
	Sonication + Resin		252	103	25	126	30		
	Heat		378	166	17	126	62		
Activated sludge	HCHO/NaOH	mg/g VS-sludge	91	26	1.7	26	–	• NaOH/HCHO had high efficiency with reduced cell lysis.	Hong et al. (2017)
	CER		30	7	1.1	10	–		
	NaOH/Heat		186	46	4.8	56	–		
	Sonication		98	31	1.5	32	–		
Activated sludge	Control	mg/g VSS	13.49	0.96	0.83	3.2	18.4	• Sonication-based, NaOH, and triton extractions showed higher extraction efficiencies. • Base and triton extractions resulted in higher biomass lysis, while CER, triton, and sonication treatments showed higher cell viabilities.	Pellicer-Nàcher et al. (2013)
	Base		258.5	33.23	1.12	52.1	345		
	Acid		58.74	10.98	0.02	11.8	81.5		
	CER		71	10.42	6.09	7.31	94.8		
	Triton		215.5	23.05	5.77	30.2	274		
	Sonication		200.4	24.87	4.22	31.5	261		
Activated sludge	Control	mg	7.9	7.7	0.06	6.4	–	• Formaldehye-NaOH is most effective in EPS extraction.	Liu and Fang (2002)
	Formaldehyde–NaOH		54.6	40.5	0.35	50.4	–		
	EDTA		22.9	12.4	0.47	59.2	–		
	Formaldehyde-ultrasound		20.4	28.9	0.13	18.9	–		
	CER		17.6	12.7	0.14	16.4	–		
	Formaldehyde		12.3	15.9	0.07	10.9	–		

method in extracting EPS and their components. The DNA content within the extracted samples serves as a crucial indicator of cell lysis during the extraction process.

In conclusion, while determining the most suitable method for EPS extraction remains a challenge due to the lack of a universally standardized approach, in recent research certain methods have emerged as more effective and widely adopted. The combined CER method with mild sonication has emerged as the preferred technique for total EPS extraction, while component-specific isolation requires tailored approaches: CER method for proteins, formaldehyde + NaOH for polysaccharides, and EDTA for humic-like substances. The selection of the extraction methodology demonstrates strong dependence on sludge characteristics, with the CER method showing optimal performance for activated sludge, while formaldehyde + NaOH proves superior for aerobic granular sludge. However, researchers should conduct their own comparative EPS extraction studies, focusing preferably on combined methods before selecting the most appropriate technique for their specific sludge type and research objectives. This comparison should consider various parameters such as extraction time, chemical dosage, sonication intensity, centrifugation speed, heating temperature, and evaluation of cell lysis.

3.2. EPS analysis

Understanding EPS content in sludge treatment is essential for understanding floc properties. EPS composition and properties are more important than EPS quantity in governing sludge behavior (Liao et al., 2001; Cetin and Erdincler, 2004; Shao et al., 2009). Proteins and polysaccharides are key EPS components, with their combined amount used to measure the overall EPS yield.

3.2.1. Polysaccharides analysis

Colorimetric methods are commonly used to measure the content of polysaccharides in EPS. The phenol-sulfuric acid (or Dubois method) is a widely employed method developed by DuBois et al. (1956). It involves the use of concentrated sulfuric acid to break down polysaccharides and dehydrate them to form furfural derivatives, which form yellow-gold complexes, absorbing light in the visible range with a maximum of absorption at 490 nm (Kurzyna-Szklarek et al., 2022).

The anthrone method, which shares the same principle as the phenol-sulfuric acid method, is considered safer due to anthrone use instead of phenol, eliminating its potential hazards (Kejla et al., 2023). The furfural reaction with anthrone reagent results in the formation of blue-green complexes with absorption maxima at 625 nm (Kurzyna-Szklarek et al., 2022). However, color development is limited when analyzing polysaccharides containing mixtures of uronic acids and hexosamines, especially in complex mixtures of monomeric and polymeric sugars (Haldar et al., 2017).

Various studies have extensively compared these methods, resulting in inconsistent or even contradictory conclusions. Frølund et al. (1996) and Eduardo Ramirez Asquieri (2019) reported that the two methods showed almost identical yield and accuracy. In contrast, Fiset et al. (2017) revealed that the Dubois method yielded almost double the amount of polysaccharides compared to the anthrone method. Moreover, Jimenez et al. (2013) suggested that the Dubois method is the most accurate for determining polysaccharides in SS samples, showing better results in terms of rightness and specificity than the anthrone method, although the anthrone method is more sensitive. Furthermore, Piccolo et al. (1996) found that the Dubois method was more accurate than the anthrone method by a factor ranging from 7 to 81, and Brown and Lester (1980) demonstrated that the Dubois method had a 24% higher recovery compared to anthrone method. However, Raunkjær et al. (1994), as cited by Jimenez et al. (2013), stated that the Dubois method showed poor precision with a standard deviation of 50%, while the anthrone method exhibited high precision without interferences.

3.2.2. Proteins analysis

Proteins are the dominant organic constituent of the EPS matrix (Frølund et al., 1996). Proteins may influence both surface and bulk properties of sludge flocs, thereby modulating sludge hydrophobicity, and hence impacting its flocculation, dewaterability, and digestibility (Park et al., 2008). Therefore, it is crucial to carefully analyze the protein content in EPS samples.

Colorimetric methods are frequently employed to determine protein concentration. The Lowry method (Lowry et al., 1951), the modified or corrected Lowry method (Frølund et al., 1996), the Bradford method (Bradford, 1976), and the Bicinchonic Acid (BCA) method (Smith et al., 1985) are the most widely used methods.

Several studies have diligently compared these analytical methods to shed light on their effectiveness and sensitivity to interferences. Avella et al. (2010) compared three protein quantification methods in the presence of humic acid, an interfering substance: the classic Lowry method and commercial assay kits may overestimate protein content, while the modified Lowry method can lead to underestimation, particularly in diluted solutions. To minimize errors, they recommended using the commercial kits for low protein concentrations and the modified Lowry method for samples with a protein content higher than 50 mg/L. In another study by Jimenez et al. (2013), the classical Lowry method showed high sensitivity and accuracy. However, the modified Lowry method showed slightly lower sensitivity and tended to underestimate values, as observed by (Frølund et al., 1996). The BCA method showed good sensitivity but may be susceptible to some matrix interference. Comparing the modified Lowry method and the BCA method, Ras et al. (2008), concluded that the BCA method was more suitable as it showed less interference with the extractant used for EPS dosage.

However, a recent study by Felz et al. (2019) contested the reliability of both the Lowry and BCA methods due to interference from compounds (uronic acid, phenolic compounds, and glucosamines) present in the EPS matrix. Furthermore, the study illustrated that the protein standards significantly influence measurements, and emphasized the importance of using an appropriate protein standard that closely resembles the composition of proteins in the EPS sample to ensure accurate results.

Commercial kits are frequently used for their ease of use and time-saving advantages. Le et al. (2016) studied the reliability of five commercial kit assays in the presence of various interfering substances commonly found in wastewater, indicating that diverse substances can significantly interfere with these assays, leading to false positive results. Additionally, they highlighted a poor correlation between standard colorimetric assays and the real protein content.

Beyond considering sludge origin and composition, the method used for EPS extraction may introduce potential variations in protein quantification. Consequently, it is important to choose a proper quantification/extraction method for efficient EPS determination (Comte et al., 2006b, 2007; Ras et al., 2008).

In conclusion, quantifying proteins and polysaccharides can be challenging and intricate. The various methods can often yield conflicting results, making it akin to navigating a complex maze. This underscores the imperative for a standardized colorimetric method, or the use of advanced methods, such as Fourier-Transform Infrared Spectroscopy (FT-IR), Gas Chromatography (GC), High-Performance Liquid Chromatography (HPLC), Excitation-Emission Matrix Spectroscopy (EEM) and Tandem Mass Spectrometry (MS/MS) to ensure reliable results and facilitate effective cross-referencing and comparison.

4. EPS and sludge settleability

Sludge settleability is a critical factor for the efficiency of wastewater treatment plants, as poor settleability can cause operational problems and discharge of suspended solids to the effluent. EPS's role in SS settling is a complex topic, as evidenced by the diverse (somewhat contradictory) literature findings. For instance, some researchers have observed that lower EPS concentrations lead to faster sludge settling (Li and Yang, 2007; Liu et al., 2021b; Wang et al., 2022), whereas others have reported improved settleability with higher EPS levels (Shen et al., 2020; Wang et al., 2019).

The specific EPS components also play distinct roles in sludge settleability. Li and Yang (2007) have shown that sludge settling strongly correlated with LB-EPS content rather than TB-EPS content, which was also supported by Melo et al. (2024), indicating that excessive LB-EPS can weaken the floc structure and impair sludge-water separation. However, other studies have demonstrated that TB-EPS, and more precisely the ionic fraction of TB-EPS (An et al., 2023), is the "active fraction" that governs sludge aggregation and settling (D. Q. Wu et al., 2022; R. X. Wu et al., 2022).

EPS composition has been deemed more crucial than its quantity in determining sludge settleability (Li and Yang, 2007). Zhang et al. (2016) showed that protein and polysaccharides contents in EPS positively correlated with SVI, with correlation coefficients of $R^2 = 0.88$ ($P = 0.00$) for proteins and $R^2 = 0.93$ ($P = 0.00$) for polysaccharides. Sun et al. (2022) supported these findings by revealing a positive correlation between protein content and SVI, while no correlation was observed between polysaccharides and SVI. Conversely, Gao et al. (2020) showed an obvious positive correlation between SVI and polysaccharides ($R^2 = 0.954$) and a poor correlation with proteins ($R^2 = 0.156$). The variations may arise from differences in operational conditions or the specific analytical methodologies used in each study. Logically, proteins are more important than polysaccharides in sludge settling due to their unique charge properties. The amino groups in proteins carry a positive charge, which can neutralize the negative charges from carboxyl and phosphate groups, reducing the net negative surface charge of the sludge, thereby promoting sludge flocculation and settling (Pan et al., 2004; Dong et al., 2017; Tian et al., 2019).

The protein to polysaccharide (PN/PS) ratio in EPS is also an important factor, with a higher PN/PS ratio generally associated with better sludge settling characteristics. Considering that polysaccharides are hydrophilic substances and proteins are hydrophobic molecules (Zhang et al., 2017), a high PN/PS ratio ensures dominant hydrophobic characteristics of EPS, and helps promote the accumulation and flocculation of microbial cells (X. Liu et al., 2022).

Table 2 reports the main literature findings related to EPS's role in SS settleability. The EPS effect on SS settleability highlighted several recurring outcomes: i) higher EPS contents were often measured in the presence of sludge granules, which are gaining momentum in aerobic configurations (e.g., granular sequencing batch reactors) as an alternative to conventional flocculent processes (activated sludge); ii) ionic and hydrophobic EPS promote the formation of flocs, while negative effects are detected due to electrostatic repulsion; iii) biochar addition enhances the EPS secretion, leading to improved settling properties; iv) glucose feedings, atrazine exposure, and Ca and Mg presence stimulate EPS concentration increase; v) the presence of polysaccharides and proteins, increasing with time, alters sludge sedimentability capacities; vi) the protein/polysaccharide ratio raises during granulation processes, favoring improved settleability.

5. EPS and sludge dewatering

EPS play a crucial role in SS dewatering as well. EPS, in fact, which make up a significant portion of the total sludge biomass (around 80%), have a high water-holding capacity due to their hydrophilic nature (Mickaël Raynaud, 2010; Zhou et al., 2014; Yu et al., 2017). Their predominance within sludge affects dewatering processes (Liu and Fang, 2003b).

EPS influence on sludge dewatering has been extensively investigated. Some studies have found that increasing the EPS content can initially improve sludge dewaterability by enhancing sludge flocculation and reducing the number of small particles (Kang et al., 1989; Sanin and Vesilind, 1994; Houghton et al., 2001). Once an optimal EPS level is

Table 2
Summary of literature studies related to EPS effect on sludge settling.

Sludge type	Main results	Reference
Activated sludge	• Sludge settling negatively correlated with LB-EPS (no correlation with TB-EPS) • Flocculation and settling positively correlated with sludge retention time • Sludge fed on glucose showed higher EPS concentration than sludge grown on sodium acetate	Li and Yang (2007)
Anammox and aerobic granular sludge, flocculent sludge	• Granules show higher EPS content (mostly proteins) • Anaerobic environment and aerobic conditions with easily degradable carbon sources stimulate protein and EPS formation • EPS hydrophobicity improves microbial aggregation	Liu et al. (2021b)
Hollow anammox granular sludge	• EPS content increased as settling properties deteriorated • EPS-hydrolyzing bacteria weaken granule structural strength	Wang et al. (2022)
Activated sludge	• EPS and protein content decreased during sludge bulking, reducing sludge hydrophobicity and increasing surface negative charge • These changes ultimately deteriorated flocculation	Shen et al. (2020)
Activated sludge	• Higher EPS content was measured in mature granules, contributing to their fast-settling characteristics • EPS played a vital role in sludge granulation	Wang et al. (2019)
Activated sludge	• Under atrazine exposure, both TB-EPS (strongly related to the rise of large aggregates) and LB-EPS increase • EPS release is a defence mechanism	Melo et al. (2024)
P. stutzeri strain XL-2	• TB-EPS include C-containing functional groups deriving from hydrophobic R groups of amino acids, significantly affecting protein surface hydrophobicity and promoting self-aggregation	(D. Q. Wu et al., 2022)
P. stutzeri strain XL-2	• Ca^{2+} and Mg^{2+} stimulate EPS production, respectively through proteins and polysaccharides, enhancing biofilm formation • The interaction between Ca^{2+}/Mg^{2+} and specific functional groups in EPS made the structure more compact	(R. X. Wu et al., 2022)
Activated sludge	• Ionic and hydrophobic TB-EPS positively contribute to flocs formation, while S-EPS and LB-EPS show negative effects due to electrostatic repulsion • Ionic EPS promote sludge aggregation through ion bridging	An et al. (2023)
Waste activated sludge	• Proteins and polysaccharides in EPS negatively affected sludge sedimentability and effluent total suspended solids (TSS); they were positively correlated with digestion time. • Short-term digestion produced sludge with better dewaterability and flocculability	Zhang et al. (2016)
Activated sludge	• Nitrogen removal and EPS production (including polysaccharides, proteins, and nucleic acids) increased at free ammonia concentrations from 0.5 to 10 mg/L • This led to improved sludge settleability and dewaterability	Sun et al. (2022)

(*continued on next page*)

Table 2 (*continued*)

Sludge type	Main results	Reference
Activated sludge	• Polysaccharides and proteins increased in the LB-EPS and TB-EPS with time progression • LB-EPS were positively correlated with sludge volume index mainly due to polysaccharide content	Gao et al. (2020)
Aerobic granular sludge	• HRT of 1 h led to biomass washout, while HRT of 24 h showed bioflocs instead of granules. • At intermediate HRTs aerobic granules were stabilized and showed improved retention and settling properties	Pan et al. (2004)
Aerobic granular sludge	• Proteins increased more than polysaccharides during granulation, contributing to sludge formation • Polysaccharides increased under shock-loading conditions	Dong et al. (2017)
Aerobic granular sludge	• Biochar addition stimulated EPS secretion, especially proteins • This led to excellent settling properties, enhanced degradation ability, and improved biomass retention	Zhang et al. (2017)
Aerobic granular sludge	• EPS content significantly increased during granulation (>333 mg/g mixed liquor volatile suspended solids- MLVSS) and successively stabilized around 240 mg/g MLVSS • Increased EPS contents were negatively correlated with SVI during granule formation	(X. Liu et al., 2022)

reached, further increases in EPS may further enhance sludge dewaterability (Houghton et al., 2001). In contrast, Cydzik-Kwiatkowska (2021) demonstrates that a high EPS content effectively reduces sludge dewaterability by entrapping water. Consequently, Cai et al. (2023) suggest that EPS degradation could be an effective strategy to improve sludge dewatering performance. In the same context, it has been demonstrated that an acidic pH (2.5–3.6) facilitates EPS dissolution from physically bound water, improving sludge dewaterability (Julian Tosoni, 2015). Li et al. (2016) demonstrated that a hybrid process combining electrolysis/electrocoagulation and zero-valent iron-activated persulfate oxidation significantly improved sludge dewatering, reducing SRF (Specific Restistance to Filtration) and CST (Capillary Suction Time) by 87.4% and 49.1%, respectively. The process disrupted EPS, broke cells, and released bound water, enhancing dewaterability. Similarly, Chen et al. (2021) explored the use of an ionic liquid as a conditioner, achieving a significant reduction in moisture content (64.99 $\pm$ 0.92%) by effectively destroying EPS structures. In contrast, Cao et al. (2018) found that controlled EPS dissolution through alkalization and oxidation improved dewatering, but excessive dissolution could lead to pore clogging and increased filtration resistance. Neyens (2004) emphasized that advanced sludge treatment processes, such as thermal hydrolysis and peroxidation, could degrade EPS and enhance flocculation at pH levels near the isoelectric point. However, over-dissolution can destabilize cells and release intracellular materials, hindering filtration. Additionally, Ben Hamed et al. (2024) observed that EPS dissolution during SS stabilization worsened filterability over time. So, optimal EPS management involves finding a balance between sufficient dissolution to release bound water and maintaining enough EPS structure for effective solid/liquid separation.

The protein secondary structure significantly influences the hydrophilicity and hydrophobicity of EPS, which, in turn, impacts sludge dewatering performance (Badireddy et al., 2010). Recently, several studies have explored the influence of protein secondary structure. According to You et al. (2017) α-helix, β-sheet, and random coil are the key secondary structures in proteins that affect sludge dewaterability. So, an alteration in the relative proportions of these structures can modify sludge functional properties, including its binding affinity, hydrophobicity, and network stability.

According to Wang et al., (2024a,b), the dewaterability improvement of sludge conditioned by tannic acid and polymeric quaternary ammonium salt is a result of the enhancement of protein hydrophobicity. This was achieved by an increase in the α-helix from 18.59 % to 24.03 % and a decrease in the β-turn from 32.41 % to 25.38 %. This finding was supported by Hou et al. (2015); Pei et al. (2020), as both highlight a strong correlation between hydrophobicity and the secondary structure of proteins, suggesting that a higher prevalence of α-helix and a reduced presence of β-sheet could contribute to enhanced sludge dewaterability. This is because, in the three-dimensional structure, α-helix has a more consistent arrangement of water-repelling (hydrophobic) residues along its structure, unlike β-sheet, which shows a less regular pattern (Serrano et al., 1991).

Additionally, the composition of amino acids within the proteins plays a pivotal role. Proteins with a higher content of hydrophobic amino acids (e.g., alanine, valine, leucine) and less hydrophilic amino acids (e.g., serine, lysine) exhibit enhanced hydrophobicity, further promoting aggregation and dewaterability (Hou et al., 2015). Zhu et al. (2020) revealed that both CST and SRF have a strong negative correlation with hydrophobic amino acids and a strong positive correlation with hydrophilic amino acids. This interplay between protein secondary structure and amino acid composition underscores the importance of EPS protein properties in determining sludge dewatering performance.

The composition and distribution of EPS fractions, such as LB-EPS, TB-EPS, proteins, and polysaccharides, significantly impact sludge dewaterability. The LB-EPS play a crucial role in hindering sludge dewatering compared to the TB-EPS (Neyens, 2004; Yang and Li, 2009). In fact, the high hydrophilicity of LB-EPS, which contain a large amount of bound water, can lead to the deterioration of sludge dewaterability (Li and Yang, 2007). In contrast, Zhou et al. (2015) reported that the decomposition of LB-EPS during oxidative treatments, such as Fenton's peroxidation, improves sludge dewaterability, which was confirmed by Kurade et al. (2016). Concerning TB-EPS fraction, Xu et al. (2011) reported no correlation between TB-EPS and sludge dewaterability. However, Zhen et al. (2013) found that the decomposition of TB-EPS and cells can significantly improve sludge dewaterability through electrolysis and Fe (II)-activated persulfate oxidation. They suggested that the decomposition of TB-EPS and cells leads to bound water release, which is the primary mechanism for enhanced dewaterability.

Regarding composition, Faye et al. (2019) found that increasing the polysaccharides content or PS/PN (polysaccharides/proteins) ratio could enhance sludge dewatering performance. In contrast, Bai et al. (2019) observed that sludge with low polysaccharide content or a high PN/PS ratio exhibited superior dewatering capability. Furthermore, Houghton and Stephenson (2002) demonstrated that an increase in protein content reduced sludge dewatering efficiency. Dai et al. (2018) indicated that a higher organic content in S-EPS or a lower organic content in LB-EPS could improve dewaterability.

The molecular weight and secondary structure of EPS components, particularly proteins, have been identified as key factors influencing sludge dewaterability. Xiao et al. (2017a) and Wu et al. (2017) showed that the removal of low molecular weight proteins (<20,000 Da) and the unfolding of protein secondary structures were crucial for better sludge dewatering performance. Similarly, Zhang et al. (2019) reported that the unfolding of slime proteins and the moderate weakening of TB-EPS rigidity enhanced sludge dewaterability. Additionally, Xiao et al. (2017b) found that polysaccharides with a molecular weight of 10^6–5×10^7 Da in LB-EPS were the key organic compound that deteriorated sludge dewaterability.

Finally, the hydrophilic and hydrophobic properties of EPS components significantly influence sludge dewatering performance. As reported by He et al. (2023), the increase in the content of hydrophobic tyrosine-like proteins and the decrease in the content of hydrophilic humic-like substances were associated with improved sludge dewaterability. As can be seen, EPS's role in SS dewatering is crucial and intricate. Specific EPS properties, including composition, structure, and distribution, play a significant role in sludge dewaterability. The analysis of available literature studies (Table 3) revealed the following key aspects: i) process conditions in biological wastewater treatment (e.g., solid retention time- SRT) can be tailored to limit LB-EPS content in the produced sludge, improving downstream dewatering; ii) sludge chemical pretreatment, eventually combining more than one chemical reagent, and pH modification help to solubilize EPS, degrading large molecules and releasing internal water, with remarkable benefits on dewatering performance; iii) environmental-friendly additives are being studied in alternative to conventional flocculants to "green" the overall sludge treatment chain.

6. EPS and sludge drying

EPS plays a crucial role in determining sludge moisture-holding capacity and drying performance. Few studies have investigated so far the impact of EPS characteristics on sludge drying. EPS hydrophilic and hydrophobic properties, particularly influenced by tryptophan protein-like substances, significantly affect the sludge's moisture-holding capacity during hot-pressing drying (Ma et al., 2024b). The reduction in sludge moisture-holding capacity occurs in three stages: i) below 67 °C, moisture desorption is dominated by intracellular moisture release; ii) between 67 °C and 85 °C, increased protein content and exposure of hydrophobic functional groups enhance EPS hydrophobicity, facilitating moisture desorption; iii) above 85 °C, protein degradation due to thermal decomposition further aids in moisture release. Additionally, analysis showed a decrease in moisture-holding capacity with increased drying time and mechanical compression. In another study, Ma et al. (2024a) demonstrated that sludge drying using the hot-pressing approach was enhanced by using sawdust as a conditioning agent; the observed constant drying rate was due to the destruction of EPS and microbial cells, which increased the sludge temperature. This temperature rise accelerated moisture desorption and intracellular moisture release, promoting bound moisture conversion into free moisture. Cai et al. (2023), instead, investigated the effect of photocatalysis-mediated EPS degradation on sludge dewatering performance during biodrying: using TiO_2 as a catalyst efficiently degraded carbohydrates and proteins in EPS within 60 min. After 20 days of biodrying, photocatalysis significantly reduced moisture content and EPS by 17.64% and 6.88%, respectively, with a substantial decrease in hydrophilic amino acids, indicating improved EPS hydrophobicity. Overall, the findings suggest that photocatalysis-coupled sludge biodrying enhances EPS degradation and sludge dewaterability, improving biodrying process efficiency. Similarly, Cai et al. (2016) investigated EPS's role in sludge biodrying using EEM spectroscopy: EPS were significantly degraded during the thermophilic phase, leading to the decomposition of floc structures and reducing water retention capacity. This transformation of bound water into free water improved sludge dewaterability, enhancing the drying efficiency.

Finally, Fraikin Laurent (2012), focuses on predicting emissions of ammonia and volatile organic compounds during sludge drying by analyzing key compounds within the EPS: treatment methods like thermolysis and digestion can result in EPS accumulation in the leachable layer, causing an earlier release of these compounds. Furthermore, organic matter distribution within the EPS significantly affects emission levels, underscoring the importance of understanding the chemistry involved in sludge drying.

In summary, EPS plays a crucial role in determining sludge moisture-holding capacity and drying performance. Understanding and manipulating the EPS properties can lead to improved sludge drying efficiency, which is fundamental to reducing sludge transportation and disposal costs. In addition, improved moisture content in dried sludge can allow to apply energy recovery processes such as incineration or pyrolysis. The

Table 3
Summary of literature studies related to EPS's role in sludge dewatering.

Sludge type	Main results	Reference
Activated sludge	• Modified corn-core powder and sludge-based biochar combination significantly enhanced sludge dewaterability • Pearson correlation analysis revealed that humic acid in TB-EPS had a strong negative correlation with specific resistance to filtration (SRF) • Aggregated strands in S-EPS, β-sheet in LB-EPS, and α-Helix in TB-EPS all showed strong positive correlations with SRF	(Guo et al., 2020)
Activated sludge	• Changes in sludge conditions influenced EPS content (especially the LB-EPS) • Sludge dewaterability was positively correlated to LB-EPS • Excessive content of LB-EPS weakens cell attachment and deteriorates floc structure, showing poor solid-liquid separation	(Yang and Li, 2009)
Activated sludge	• Sludge flocculation and separation improved at longer solid retention times (SRTs) thanks to the reduced LB-EPS content	(Li and Yang, 2007)
Activated sludge	• The peroxidation treatment improved sludge dewaterability and modified EPS structure through solubilization • Fenton's conditioning broke both TB-EPS, LB-EPS and cells, increasing dewaterability	(Zhou et al., 2015)
Anaerobically digested sludge	• The bioflocculant decreased capillary suction time by 74% and specific resistance to filtration by 89% • The bioflocculant performed better than commercial flocculant • Sludge moisture content can be decreased to 70% after dewatering	(Kurade et al., 2016)
Activated sludge	• Ultrasonic pretreatment followed by anaerobic digestion improved digestion performance and sludge dewaterability • Sonicated sludge dewaterability was correlated with LB-EPS	(Xu et al., 2011)
Activated sludge	• Dewaterability negatively correlated to LB-EPS, TB-EPS, polysaccharide, and protein content • TB-EPS entrapped bacterial cells, protecting them from electrolysis disruption • Enhanced dewaterability was linked to TB-EPS and cell destruction	(Zhen et al., 2013)
Sludge (not specified)	• Improved dewaterability after conditioning with *Moringa oleifera* and chitosan, thanks to their ability to neutralize and settle EPS • Chitosan reduced the LB-EPS and its protein content	(Faye et al., 2019)
Municipal sludge	• Polysaccharides play a more important role in dewatering than proteins • EPS reduction allows for improved watering efficiency	(Bai et al., 2019)
Activated sludge	• Phosphogypsum addition causes EPS disintegration, leading to larger floc formation and bound water release	(Dai et al., 2018)

(continued on next page)

Table 3 (*continued*)

Sludge type	Main results	Reference
Mixed sludge, waste-activated sludge, anaerobically digested sludge	• Lower LB-EPS content and specific resistance to filtration improve dewatering performance • LB-EPS and TB-EPS decreased with Fe(II)-oxone conditioning, increasing sludge filterability due to LB-EPS removal • Cell membranes were damaged and intracellular water content was released	(Xiao et al., 2017a)
Waste activated sludge	• Advanced oxidation processes transformed extracellular proteins by membrane damage and release of intracellular substances, allowing interstitial water removal from sludge	(Wu et al., 2017)
Activated sludge	• Sludge filterability improved significantly (2.40 times) after Mn (VII) oxidation- in situ Fe(III) coagulation, with the disintegration of EPS wrapping on the cell surface • EPS migration may cause the formation of secondary protein structures affecting sludge dewaterability	(Zhang et al., 2019)
Activated sludge	• Salt addition and acid treatment increased LB-EPS content, releasing trapped water and reducing sludge volume • NaCl increased internal water content while decreasing EPS water content; under acidic pH, cell lysis released intracellular water, further improving sludge dewatering	(He et al., 2023)

reviewed studies demonstrate that reducing EPS, either through thermal decomposition, photocatalysis, or biodrying, significantly improves sludge dewaterability and drying performance. Targeted strategies to control and modify EPS properties could be a promising approach for optimizing sludge drying processes and enhancing the overall sustainability of sludge management. However, due to the limited studies on this subject, further research is needed, especially considering the rising popularity of solar-aided drying processes, such as solar greenhouses.

7. EPS applications

EPS have demonstrated their effectiveness in various environmental applications, particularly in water and wastewater treatment: EPS synthesized by different bacterial strains can serve as a flocculant to remove turbidity and natural organic matter from water (More et al., 2014; Siddharth et al., 2021). Buthelezi et al. (2009) reported turbidity removal efficiencies ranging from 84% to 93.5% with EPS concentration (isolated from bacteria in river water samples) of 10 mg/L. Additionally, Li et al. (2009) demonstrated that EPS produced by *Bacillus licheniformis* can effectively remove 61.2% of COD and 95.6% of turbidity from drinking water during potabilization. Actually, EPS applications in water treatment extend beyond these examples, as reviewed by Siddharth et al. (2021).

EPS exhibit remarkable capabilities in heavy metals removal through various mechanisms, including adsorption, complexation, chelation, and ion exchange. The efficiency of these processes is influenced by pH, temperature, and the presence of other ions (Tian et al., 2019), so specific pilot or laboratory tests should be conducted case by case to assess their effectiveness. Nouha et al. (2016) studied heavy metals removal from primary effluents using EPS produced by *Cloacibacterium normanense*, reporting removal efficiencies of 73% for aluminium (Al), 36% for copper (Cu), 71% for iron (Fe), 85% for nickel (Ni), and 65% for zinc (Zn). Additionally, Zhao et al. (2016) showed that EPS could effectively remove arsenic species, achieving removal efficiencies of 84.6% for arsenite (As(III)) and 98.9% for arsenate (As(V)). However, the most relevant challenge lies in the downstream EPS separation from adsorbed heavy metals in aqueous solutions. In a recent study by Cao et al. (2020), EPS was concentrated into a cake using ultrafiltration, resulting in high removal efficiencies for heavy metals such as Pb^{2+} (94.8%), Cu^{2+} (88.9%), and Cd^{2+} (89.2%), while also achieving a high EPS recovery rate (up to 85.5%).

Still, in the realm of wastewater treatment, EPS has gained significant attention due to its ability to participate with microorganisms in the removal of various organic pollutants through adsorption and biotransformation (L. Wang et al., 2024). For instance, in a study by Zhang et al. (2018), the removal of ciprofloxacin (CIP) in an anaerobic sludge system was investigated: the EPS contributed to CIP removal, accounting for up to 23.7 ± 0.6% of the total removal efficiency.

EPS can successfully eliminate phenols (B.-B. Liu et al., 2022), antibiotics (Cao et al., 2019; Zhang et al., 2018; Zhao et al., 2023), and polycyclic aromatic hydrocarbons (Jia et al., 2017; Meng et al., 2024) from contaminated water sources. Additionally, EPS has shown promise in the decolorization and removal of dyes from industrial effluents, making it a versatile and sustainable solution for pollution control (Kamath Miyar et al., 2021; Mustafa et al., 2022; Nguyen et al., 2022). EPS derived from sludge, however, can sometimes complicate matters, as it may be responsible for a second contamination. To the best of our knowledge, there is no comparative research on the applications of EPS derived from sludge versus EPS from pure cultures. Therefore, it would be valuable for researchers to explore this path further.

EPS extracted from waste sludge have been found to contain a significant amount of phosphorus, as reported by Bahgat et al. (2024). Through extraction methods involving firstly high temperatures and alkaline conditions to solubilize EPS, and secondary acidic conditions to precipitate it, it is possible to recover EPS with high P content. Analyses have shown that EPS contain 25% free orthophosphate (O-P), 52% organic phosphorus, 16% non-apatite inorganic phosphorus (NAIP) and 7% pyrophosphate. Authors suggest that a high content of organic phosphorus makes EPS an effective flame retardant (Bahgat et al., 2024). Moreover, adding Fe or Al cations during wastewater treatment or EPS extraction could increase NAIP in EPS and reduce liquid O-P, making EPS useful for slow-release fertilizers to prevent rapid O-P leaching from the soil. Li et al. (2021) revealed a good performance of alginate-like extracellular polymers extracted from aerobic granular sludge with a similarity in chemical structure of up to 60% compared to a commercial alginate.

Finally, EPS have demonstrated significant potential in enhancing soil properties and agricultural productivity: they contribute to improving soil aggregation and structure, which in turn enhances water retention, nutrient availability, and resistance to erosion (Saha et al., 2020; Ali et al., 2024).

The possible applications of EPS and their specific role in environmental pollution control are summarized in Table 4, to give a thorough overview to the reader.

In conclusion, EPS versatility and effectiveness underscore their potential in environmental applications as a promising alternative to conventional treatment methods and chemicals, which are typically fossil-derived. However, to fully harness their potential in real-world applications, it is crucial to address and overcome the challenges associated with their production and usage, especially from complex matrices such as SS.

8. EPS production and future direction

8.1. EPS production

Unlike traditional EPS produced by bacteria, microalgae, and fungi, sludge EPS is distinct in its composition, function, and production

Table 4
EPS applications and their role in preventing environmental pollution; modified from More et al. (2014).

Application	EPS role	Main remarks
Water potabilization	Biosorbent, bioflocculant	EPS can be used to remove natural organic matter and suspended solids, eventually in combination with multivalent ions (e.g., Al^{3+}, Fe^{3+}, Ca^{2+}, Mg^{2+}), however, safety issues must be considered
Wastewater flocculation	Biosorbent, bioflocculant	EPS can reduce the dosage of chemical polymers (polyacrylamide, polyaluminium chloride) in the removal of organic matter and suspended solids
Pollutant removal from wastewater/soil	Bioabsorbent, bioflocculant, bioleaching, biodegradation	EPS can be used as an eco-friendly solution to remove heavy metals, dyes, and toxic organic compounds from wastewater or contaminated soils
Landfill leachate treatment	Bioleaching, biodegradation	EPS can help in bioleaching and biodegradation of heavy metals and toxicants from landfill leachate, preventing environmental contamination
Soil remediation	Biosorbent, bioaugmentation, biodegradation	EPS can reduce soil erosion and limit heavy metals transportation from soils into water bodies; also, they can improve soil aggregation, water retention, and nutrient availability

conditions. Sludge EPS, primarily produced by bacteria in wastewater treatment systems, is characterized by a high content of proteins, polysaccharides, and other organic compounds essential for the formation and stability of microbial aggregates such as sludge flocs, biofilms, and granules. These components significantly influence sludge bioflocculation, settleability, and dewaterability. In contrast, traditional EPS are produced in various environments and include a diverse range of polysaccharides, proteins, and other macromolecules, playing a role in general biofilm formation, protection, and nutrient capture (Costa et al., 2018).

The production of sludge EPS is influenced by factors such as substrate type, nutrient availability, growth phase of microorganisms, SRT, shear rate, pH, temperature, and the presence of toxic compounds (Garza-Rodríguez et al., 2022). On the other hand, traditional EPS production is more broadly affected by substrate type and environmental conditions. Various types of substrates can have a significant impact on microbial communities by influencing their metabolic processes and EPS production: for instance, glucose-fed sludge produces more EPS than acetate-fed sludge (Li and Yang, 2007). The difference in EPS production between glucose and sodium acetate-fed sludge is due to the metabolic pathways involved in their degradation. Glucose requires multiple steps to be converted into acetyl-CoA before entering the citric acid cycle, leading to a higher EPS abundance. In contrast, sodium acetate can enter the citric acid cycle directly, resulting in lower EPS production (Li and Yang, 2007). This research was supported by findings from Wilén et al. (2003).

Additionally, the concentration of substrates, particularly the organic loading rate (OLR) and nitrogen loading rate (NLR), plays a significant role in influencing EPS production and composition. The impact of OLR on EPS production is closely linked to the metabolic stress experienced by microorganisms. At high OLRs, microorganisms may produce more EPS as a protective mechanism, but this can also lead to an imbalance in sludge properties and overall system performance (Yang et al., 2018). Nitrogen availability, as indicated by the NLR, significantly influences EPS composition and production: under nitrogen-limiting conditions, EPS protein content can increase substantially, ranging from 1.25 to 8.56 mg of protein per gram of suspended solids (Hoa et al., 2004). Moreover, the nitrogen-to-phosphorus ratio also plays a crucial role, with phosphorus having a more pronounced effect on EPS carbohydrate than nitrogen content (Nouha et al., 2018).

EPS production is generally higher in aerobic processes compared to anaerobic ones (Morgan et al., 1990). suggested that this difference is due to the lack of peptidoglycan in methanogenic bacteria, which limits EPS release in anaerobic processes. Additionally, the lower yield coefficient of anaerobic processes ($Y = 0.05$) compared to aerobic processes ($Y = 0.5$) suggests that EPS in anaerobic systems may be degraded and used as an energy source (Hoa et al., 2004).

The cell growth phase influences EPS production, with differences observed between exponential and stationary phases. During the exponential growth phase, EPS content tends to increase, but it may decrease in the stationary phase due to nutrient limitations (Huang et al., 2022b). Additionally, pH is a critical parameter for EPS biosynthesis, with optimal production occurring between pH 5.0 and 7.0 (Shu and Lung, 2004). Extreme pH values can inhibit EPS biosynthesis and affect bacterial growth, leading to changes in the morphological characteristics of microbial aggregates. Maintaining a constant pH within the optimal range is essential for maximizing EPS production (Melo et al., 2022). Temperature is another significant factor, with most EPS-producing microorganisms performing optimally between 25 °C and 31 °C. A reduction of 10 °C from the optimal temperature can inhibit the EPS biosynthesis process (Liao et al., 2011; More et al., 2014). Exposure to high salt levels can cause changes in EPS composition and structure as well as in the surface properties of microbial granules (Feng et al., 2021). An increase in EPS content is believed to aid in protecting the bacteria from the harmful effects of high salt concentrations (Sheng and Yu, 2006; Wang et al., 2013) found that EPS produced by activated sludge increased concomitantly with salinity increase from 0.5% to 6%, supported by (Mishra et al., 2008; Zhang et al., 2011).

EPS production can be increased as a protective mechanism by bacteria in response to toxic compounds. For instance, the presence of pharmaceutical compounds and pesticides can trigger bacteria to secrete more EPS, which contains functional groups such as hydroxyl, carboxylic, sulfhydryl, and phosphate groups, that can help protect the bacteria by preventing the toxicants from reaching them (Melo et al., 2022).

The production and characteristics of EPS in sludge are significantly influenced by SRT and shear rate. Increasing SRT generally reduces EPS production as the microbial community shifts towards endogenous respiration, while shorter SRTs with higher food-to-microorganism ratios can lead to increased EPS production, potentially causing severe fouling issues in membrane bioreactors (Al-Halbouni et al., 2008).

High shear rates, particularly in reactors, can stress microorganisms, prompting them to produce more EPS as a protective mechanism. However, some studies suggest that increased shear conditions can decrease floc-associated EPS production, mainly through the disruption of microbial aggregates (Menniti et al., 2009).

Despite the extensive studies on EPS and sludge, optimizing EPS production from sludge remains a significant gap in current research. This may be due to the complexity of EPS composition, the variability in extraction methods, and the need for a comprehensive understanding of the interplay between environmental conditions, operational parameters, and microbial metabolism. Researchers have been actively working to isolate bacterial strains that produce higher EPS levels from sludge to address various issues. In a study by (Bala Subramanian et al., 2010), strains were selected from sludge, based on their mucoidal colonies and string-forming ability, and were cultured separately to yield EPS with specific characteristics. Recently, EPS recovery has been simplified through methods such as centrifugation, where the supernatant contains slime EPS and the bacterial pellet contains capsular EPS, as demonstrated by (Minari et al., 2024; Yan S and Rd, 2016).

8.2. Future direction

EPS production from sludge holds significant potential for enhancing wastewater treatment processes and beyond. However, several aspects need further investigation to fully harness this potential.

- *Optimization*

While EPS play a crucial role in biological wastewater treatment, there is a significant gap in research on optimizing their production from sludge. To address this, testing and adjusting various operational parameters is essential. A well-designed experimental approach, incorporating statistical analysis and modeling, can help identify the most influential parameters, their optimal ranges, and understand the complex interactions between them.

- *Molecular-level understanding*

The molecular-level understanding of the biochemical pathways and genetic mechanisms involved in EPS production is still limited. This lack of information on the genomics and proteomics of EPS-producing microorganisms is a major obstacle in developing more efficient production methods and harnessing the full potential of EPS in various applications.

- *Pilot-scale and real-world testing*

To move from laboratory studies to industrial applications, pilot-scale studies are essential. These studies should focus on assessing the scalability, cost-efficiency, and environmental impact of EPS production systems. This will help translate the laboratory findings into practical, large-scale applications and ensure the sustainability of EPS production processes.

- *Extraction methods*

The extraction process can significantly impact EPS composition, structure, and function. Chemical extraction methods may introduce contaminants that hinder the purification process and interfere with subsequent analyses. Therefore, it is crucial to select an appropriate extraction technique that minimizes alterations to the EPS properties while ensuring efficient extraction.

- *Use of sludge-derived EPS*

EPS produced from sludge, which may contain pollutants and toxic compounds, requires careful monitoring and analysis before any further utilization, as it can be responsible for a second contamination. By ensuring proper handling and assessment, EPS sludge can be repurposed in a safe and environmentally-friendly manner.

9. Conclusions and recommendations

This review paper provides a comprehensive analysis of the role of EPS in SS management, particularly focusing on their effect on sludge sedimentation, dewatering, and drying. The significant influence of EPS on these processes is highlighted, noting that the lack of standardized methods for EPS extraction and analysis has led to inconsistent and often uncomparable research findings, hindering a thorough understanding of EPS's role in sludge treatment.

Additionally, the potential EPS applications in environmental domains, such as water and wastewater treatment, heavy metal removal, and soil improvement, are explored, although significant challenges, such as high production costs and low yields, remain.

To advance the understanding and applications of EPS in SS management, the authors recommend the following key points to be further explored.

- Standardization of methods: "better late than never". The development of standardized methods for EPS extraction and analysis will ensure reliable and comparable results across studies, facilitating accurate comparisons.
- Further research: conduct additional studies to better elucidate the specific EPS roles and the influence of the protein-to-polysaccharide ratio on sludge settleability, dewatering, and drying. Understanding these factors will help optimize sludge treatment processes.
- Thorough exploration of applications: fully explore the potential EPS applications in environmental remediation, such as water and wastewater treatment, heavy metal removal, and soil improvement. Additionally, investigate strategies to enhance EPS production, such as metabolic engineering approaches, to overcome challenges related to high costs and low yields.
- Impact of emerging technologies: investigate the impact of emerging sludge treatment technologies, such as solar-aided drying processes, on the role and behavior of EPS during sludge drying. This will help understand how new technologies can be integrated with EPS management for improved efficiency.

CRediT authorship contribution statement

Hajer Ben Hamed: Writing – original draft, Funding acquisition, Conceptualization. **Matia Mainardis:** Writing – review & editing, Visualization, Supervision. **Alessandro Moretti:** Writing – review & editing, Visualization. **Dominique Toye:** Writing – review & editing, Validation, Supervision. **Angélique Léonard:** Writing – review & editing, Validation, Supervision, 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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Appendix A. Supplementary data

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Data availability

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

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