A review on recovery of extracellular biopolymers from flocculent and granular activated sludges
Cognition, key influencing factors, applications, and challenges

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Review

A review on recovery of extracellular biopolymers from flocculent and granular activated sludges: Cognition, key influencing factors, applications, and challenges

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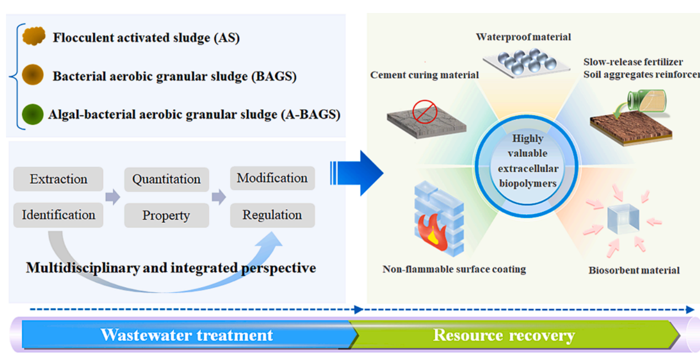
HIGHLIGHTS

- Excess sludge can be turned into wealth by producing EPS-based biomaterials.
- Recovery of extracellular biopolymers from AS, BAGS, and A-BAGS is reviewed.
- Existing ALE extraction and identification methods are updated but with limitations.
- Combining the properties and functions of biopolymers benefits further applications.
- Future perspectives of sludge-based extracellular biopolymers recovery are outlined.

GRAPHICAL ABSTRACT



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ABSTRACT

A reasonable recovery of excess sludge may shift the waste into wealth. Recently an increasing attention has been paid to the recycling of extracellular biopolymers from conventional and advanced biological wastewater treatment systems such as flocculent activated sludge (AS), bacterial aerobic granular sludge (AGS), and algal-bacterial AGS processes. This review provides the first overview of current research developments and future directions in the recovery and utilization of high value-added biopolymers from the three types of sludge. It details the discussion on the recent evolvement of cognition or updated knowledge on functional extracellular biopolymers, as well as a comprehensive summary of the operating conditions and wastewater parameters influencing the yield, quality, and functionality of alginate-like exopolymer (ALE). In addition, recent attempts for potential practical applications of extracellular biopolymers are discussed, suggesting research priorities for overcoming identification challenges and future prospects.

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1. Introduction

In addition to pollutant removal and water purification, resource and energy recovery from wastewater is currently one of the most important options for achieving a recyclable economy (Kehrein et al., 2020). The Energy & Raw Materials Factory (ERMF) of the Dutch Water Authorities has demonstrated the feasibility of recovering cellulose, bioplastics, phosphate, alginate-like exopolymer (ALE), biomass, clean water, and other resources from municipal wastewater (van Leeuwen et al., 2018). In conventional wastewater treatment plants (WWTPs), the disposal expense of waste activated sludge generally accounts for approximately 50 % of the overall operating costs (Cho et al., 2014). However, there is a turnaround when the considerable amounts of extracellular polymeric substances (EPS) in the excess sludge could be recycled, which can serve medical, horticulture, construction, paper, agriculture, and food industries (Xiao and Zheng, 2016).

EPS secreted by microorganisms themselves can aggregate microbial cells into flocs, biofilms, sludge, and granules (Xiao and Zheng, 2016). A variety of physical and biochemical interactions such as electrostatic force, hydrogen bonding, and ionic bonds contribute to the formation and stability of microbial aggregates (Basuvaraj et al., 2015). EPS are a complex mixture of high molecular weight biopolymers, including polysaccharides, proteins, lipids, nucleic acids, and humic substances (Felz et al., 2016). As a natural biopolymer with a wide range of uses, EPS can not only protect cells from dehydration, external heavy metals, and toxic compounds, but also contribute to the maintenance of matrix stability, and provide hinderance to environmental stress and energy storage for carbon sources supply (Schambeck et al., 2020b).

ALE, which somewhat resembles the commercial sodium alginate, is one of the major functional polymers associated with gel-forming capabilities embedded in the biomass-based EPS (Lin et al., 2010). Alginate macromolecules are unbranched polysaccharides consisting of D-mannuronic acid (β-D-Mannuronic acid, M) and L-guluronic acid (α-L-guluronicacid, G) (Pawar and Edgar, 2012). As illustrated in Fig. 1**a**, the whole chain of macromolecules is randomly distributed in three ways, namely, the homopolymeric blocks (guluronic/guluronic acid or GG blocks, and mannuronic/mannuronic acid or MM blocks) and the heteropolymeric blocks (mannuronic/guluronic acid or MG blocks) of two kinds of uronic acid residue (Lin et al., 2010). Although the commercial alginates are traditionally produced by brown seaweeds, *Pseudomonas* and *Azotobacter* have also been reported to synthesize bacterial alginates (Hay et al., 2010; Remminghorst and Rehm, 2006) as shown in Fig. 1**b**. There is also a great potential for recycling ALE from conventional flocculent activated sludge (AS), bacterial aerobic granular sludge (AGS), and algal-bacterial AGS biomass as sustainable biomaterials (Fig. 1**c**) (Chen et al., 2022a; Li et al., 2021; Lin et al., 2010).

In addition to ALE with gel-forming abilities, other functional extracellular biopolymers are gradually being isolated, characterized, and applied. However, the complexity and specificity of the structures and functions of extracellular biopolymers are considerably beyond the current recognition, and a comprehensive understanding of EPS remains insufficient, which may hinder the application potentials for further upgrading the recovery of excess sludge into specific functional biomaterials with high added values.

This review attempts to update the research efforts on the recovery of extracellular biopolymers from flocculent AS, bacterial AGS, and algal-bacterial AGS. Specifically, the current development of cognition on the extracellular biopolymers is summarized. The key factors that may influence ALE production are introduced as comprehensively as possible. The challenges in the extraction, identification, and characterization of extracellular biopolymers are also discussed. In addition to future application perspectives, research directions for stable and effective production of extracellular biopolymers from excess biomass and wastewater are proposed. Overall, this review aims to deepen the understanding of bioresource recovery from advanced wastewater biosystems, providing scientific guidance for future practical applications.

2. Updating the cognition on extracellular biopolymers

2.1. Alginate-like exopolysaccharides

By isolating and identifying ALE from bacterial AGS, Lin et al. (2010) found that ALE possessed the similar spectral properties to seaweed alginate and contained a high percentage of GG blocks (69.07 ± 8.95 %), so "alginate-like exopolysaccharides" was first termed and proposed. In the decades thereafter, an increasing number of researchers have performed more in-depth studies on the properties, characterizations, applications, and influencing factors of ALE (Lin et al., 2013; Rollemberg et al., 2020a; Schambeck, et al., 2020a; Yang et al., 2014). The chemical and mechanical properties, as well as the functional groups and hydrogel properties of ALE from flocculent AS and bacterial AGS were compared and discussed (Lin et al., 2013; Sam and Dulekgurgen, 2015; Schambeck et al., 2020b). Most recently, the extraction of ALE from another promising type of AGS, algal-bacterial AGS, also came into the limelight (Chen et al., 2022a, 2022b; Meng et al., 2020). In addition, ALE extracted from anaerobic granular sludge had similar

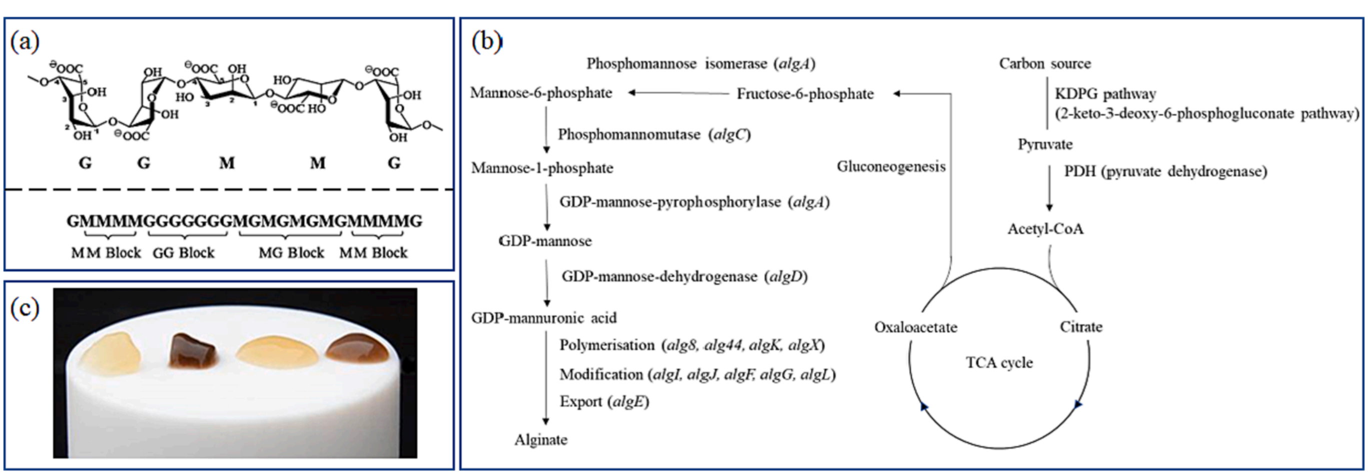


Fig. 1. (a) Macromolecular conformation of alginate polymer and the chain sequences of alginate (Lin et al., 2010); (b) Proposed bacterial alginate biosynthesis pathway (Remminghorst and Rehm, 2006); (c) Image of the ALE samples after cross-linking with Ca^{2+} (from left to right: ALE extracted from bacterial AGS fed with simple synthetic WW, real WW, complex synthetic WW, and flocculent AS fed with real WW) (Schambeck et al., 2020a). ALE, alginate-like exopolymer; GG, guluronic acid/guluronic acid; MM, mannuronic acid/mannuronic acid; MG, mannuronic acid/guluronic acid; TCA, tricarboxylic acid; WW, wastewater.

characteristics to polysaccharide alginate according to the results from nuclear magnetic resonance (NMR) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis (Gonzalez-Gil et al., 2015).

2.2. Alginate-like exopolymer

Further research has found that the composition of ALE is far more complex than currently understood (not just polysaccharides), including neutral saccharides, uronic acids, proteins, nucleic acids, lipids, and phenolic compounds, etc. (Felz et al., 2019; Schambeck et al., 2020a). Therefore, "alginate-like exopolymer" or "alginate-like extracellular polymer" is more appropriate than "alginate-like exopolysaccharides" to distinguish this kind of extract from others. The resource recovery potential and application prospects of ALE are gradually being recognized due to its composition complexity and wide organic bioproducts availability. This awareness is instrumental for exploring more accurate methods for the extraction and characterization of extracellular biopolymers, which is particularly promising for the recycling of biomaterials and then the development of circular economy.

2.3. Kaumera

The operation of WWTPs is gradually transforming from simple water purification units to production/recycling sites for high value-added biomaterials (Ferreira et al., 2021). Kaumera is a new biopolymer material (containing ALE) extracted from the Nereda® bacterial AGS (van Leeuwen et al., 2018). The first and second large-scale Kaumera production plants have been established in Zutphen and Epe, the Netherlands, in 2017 and 2020, respectively (Royal HaskoningDHV, 2022). The word "Kaumera" comes from the Maori language of New Zealand, which means "chameleon". Studies have found that alginate extracted from bacterial AGS can exhibit versatile properties when combined with various raw materials, just like a chameleon. It can be further developed into a hydrophilic (used as a coating material for slow-release fertilizer and paper) or hydrophobic (used as a curing agent for concrete) material, amplifying and extending the specific properties of the original material (Kaumera, 2022). The new path of recycling wastewater resources by employing Kaumera as a primary product and expanding wastewater treatment to the field of fine chemicals production for mass consumers appears to be highly promising.

2.4. Structural EPS and other specific definitions of extracellular biopolymers

Currently, it is worth noting that the common ALE extraction method only extracts the structural EPS from biomass, rather than all the EPS that exist (Felz et al., 2016). This kind of extract exhibits hydrogel properties, which is believed to provide mechanical strength and structural stability; its composition is more complex than alginate, so it is recommended to refer to this extracted substance as structural EPS (Felz et al., 2020a). Due to the complexity of its composition, the unique EPS extracted according to different properties, functions, and requirements are also specifically defined. For example, from the perspective of polysaccharide properties, glycosaminoglycans (GAGs) have been found in bacterial AGS, including hyaluronic acid-like and sulfate GAGs-like polymers (Felz et al., 2020b). According to the amphiphilicity of the biomaterial isolated from bacterial AGS, Lin et al. (2015) named it as "sustainable polysaccharide-based biomaterial". A similar situation happened to "EPS-based flame-retardant materials" due to the self-extinguishing property of the recycled biological materials (Kim et al., 2020).

3. ALE derived from three types of sludge

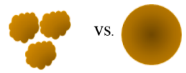
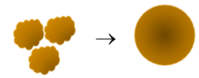
3.1. ALE derived from flocculent AS

Conventional flocculent AS process has been widely used in the traditional WWTPs for more than 100 years (Zhang et al., 2021). Although granular sludge technology is being gradually improved and extended, flocculent AS is still the mainstream sewage treatment process in the world. The disposal of a large amount of excess flocculent AS in WWTPs can also be turned into a treasure (Li et al., 2021). Currently, most of the extraction and purification of ALE are performed on bacterial AGS, and fewer studies have been conducted on the recovery of ALE from flocculent AS (Table 1). Initially, Lin et al. (2013) conducted a comparative analysis of ALE isolated from flocculent AS and bacterial AGS. Results indicated that the bacterial AGS (160 mg/g-volatile suspended solids (VSS)) yielded more than twice as much ALE as the flocculent AS (72 mg/g-VSS). Subsequently, the dynamic changes in ALE content during granulation from flocculent AS to bacterial AGS were also traced. Yang et al. (2014) and Meng et al. (2019) extracted 32 and 13 mg/g-VSS of ALE from flocculent AS at the beginning of the experiment by gravimetry and colorimetry, respectively, which were less than half of the amount of ALE extracted from bacterial AGS after flocculent AS granulation. Distinct rheological properties were also noticed in the different types of sludge. ALE hydrogels extracted from flocculent AS were less elastic than those from bacterial AGS (Schambeck et al., 2020a). However, in pilot- and full-scale studies, the ALE content (90–190 mg/g-VSS) isolated from flocculent AS fed with real municipal wastewater is significantly higher than those data obtained from the lab-scale tests (Li et al., 2021; Schambeck et al., 2020b). Furthermore, the capacity to recover ALE from flocculent AS (120–300 mg/g-VSS) may be fully comparable to that from bacterial AGS by optimizing operating conditions and feeding patterns (Li et al., 2022; Schambeck et al., 2020a). Therefore, given the ubiquitous usage of flocculent AS, technical advancements aimed at increasing the yield of ALE from flocculent AS (without granulation) should not be disregarded.

3.2. ALE derived from bacterial AGS

Bacterial AGS is a biological wastewater treatment process developed in the last decades, featuring layered structure, excellent sludge settleability, small footprint, and efficient wastewater treatment (Morgenroth et al., 1997; Pronk et al., 2015; Tay et al., 2002). As an extension and evolution of flocculent AS, the resource recovery potential of bacterial AGS has drawn considerable interest. The numerous advantages of bacterial AGS (such as stronger mechanical property, more compact structure, and better settleability) have been reported to be associated with the greater gel formation ability of its ALE synthesized (Lin et al., 2013). Since the G-blocks are able to chelate with Ca^{2+} and other divalent cations to form tightly held junctions (egg-box structure), GG blocks are mainly responsible for hydrogel formation (Pawar and Edgar, 2012). A greater proportion of GG blocks was observed in the ALE extracted from bacterial AGS, thus leading to better gel formation and stronger mechanical properties (Lin et al., 2013). As shown in Table 1, Lin et al. (2013) found that the monomer ratio of G/M in bacterial AGS was about 4 times higher than that of flocculent AS, reflecting that the fine chemical structures of the two types of sludge are significantly different. However, there is no substantial distinction in the element composition and functional groups of ALE derived from the flocculent AS and bacterial AGS (Sam and Dulekgurgen, 2015; Schambeck et al., 2020b). In addition to the comparative analysis with flocculent AS in the previous section, more in-depth studies on ALE recycling from bacterial AGS are progressively emerging. Rollemberg et al. (2020b) fed synthetic wastewater to bacterial AGS in a lab-scale sequencing batch reactor (SBR) with a working volume of 7.6 L, and explored the effects of different operational strategies on the recovery of ALE, tryptophan (TRY), and phosphorus (P). Results show that the ALE yield could reach

Table 1
Operating conditions, wastewater features, and ALE yields from different types of sludge modified from Chen et al. (2022b).

	Sludge type	Flocculent AS		Comparison between AS and BAGS		Dynamic change from AS to BAGS			BAGS			Comparison between BAGS and A-BAGS		
	Diagram													
Operating conditions	Scale	WWTPs	Lab	Lab	Pilot	Lab	Lab	Pilot	Lab	Pilot	Pilot	Lab	Lab	Lab
	SBR working volume (L)	–	5	12.9	–	4.78	2	110	7.6	140	980	16	0.9	20
	H/D ratio	–	–	8.4	H × W= 6 m × 0.6 *m*	18.75	16.67	8.96	10	6.67	–	2.5	6.67	L × W × H = 30 × 16 × 50 cm^3
	DO (mg/L)	–	3–4	2	2–3	–	7–9	–	1–3	Stage I: > 4; Stages II-IV: 2–3	–	–	–	7–9
	Upflow air velocity (cm/s)	–	–	–	–	2.65	1.77	–	1.2	1	0.55	0.3	0.6	0.6
	Volumetric exchange rate (%)	–	–	30	57.87	57.9	50	65	50	40–60	50	50	50	50
	HRT (h)	–	–	18.67	10.37	6.91	8	9.23	12	12	12	12	12	9.5
	SBR cycle (h)	–	8	5.6	6	4	4	6	6	6	6	6	6	4
	Volume of WW treated (L/day)	–	–	16.64	5000	16.61	6	286	15.2	224–336	1960	32	1.8	60
	SRT (days)	–	10, 15, 20	–	–	–	20	15	10, 15, 20	8–25	19 ± 9	32	20	20
	Temp. (°C)	–	12, 18, 24	–	–	28–30	25 ± 2	–	–	31 ± 2	–	25 ± 2	25 ± 2	23 ± 2
WW features	Types of WW	Real municipal/ industrial WW	Synthetic WW	Real/ synthetic municipal WW	Real municipal WW, consisted of 25 % of slaughterhouse WW	Synthetic WW	Synthetic saline WW	Real municipal WW	Synthetic WW	Real municipal WW	Real municipal WW	Synthetic WW	Synthetic WW	Synthetic saline WW
	Carbon source	–	Glucose, starch and sodium acetate	Acetate, propionate, amino acids, glucose, starch, peptone	–	Propionate	Glucose, sodium acetate	–	Sodium acetate	–	–	Sodium acetate	Sodium acetate	Glucose, sodium acetate
	Sludge type	Flocculent AS		Comparison between AS and BAGS		Dynamic change from AS to BAGS			BAGS			Comparison between BAGS and A-BAGS		
WW features	OLR (gCOD/ L·day)	–	0.20*, 0.36*, 0.50*	0.75	1.35	RS: 4.4–17.4; RC: 15	1.8	1.33	1, 2, 3	> 0.5	–	0.6	0.6, 0.9, 1.2	1.8
	Influent COD (mg/L)	78.1–345.3	150–1000	582 ± 65	585	RS: 1250–5000, RC: 2500	600	513±283	500, 1000, 1500	461	–	300	300, 450, 600	600
	Influent NH_4^+-N (mg/L)	15.8–49.5	20–50	40 ± 8	55	334	50	46.4±15	50	36.9	–	30	30	50
	Influent PO_4^{3-}-P (mg/L)	1.72–4.06	4–10	5.0 ± 1.1	6.3	150	10	7.1±1.8	5	4.8	–	5	5	10
	COD/N	–	3, 5, 7	14.55	10.64	3.74–14.97	12	11	10, 20, 30	12.5	–	10	10, 15, 20	12

(*continued on next page*)

Table 1 (*continued*)

	Sludge type	Flocculent AS		Comparison between AS and BAGS		Dynamic change from AS to BAGS			BAGS			Comparison between BAGS and A-BAGS		
	ALE quantification methods	Gravimetry	Gravimetry	Gravimetry	Gravimetry	Gravimetry	Colorimetry	Gravimetry	Gravimetry	Gravimetry	Gravimetry	Colorimetry	Colorimetry	Colorimetry
Results	PS content (mg/g-VSS)	–	–	Total EPS: 472–598	–	34–110	30–90	Total EPS: 540 ± 150 (Phase 1); 529 ± 90 (Phase 2)	113–155	136–144	–	BAGS: 48.24; A-BAGS: 55.49	BAGS: 62.36; A-BAGS: 73.65	BAGS: 34.2; A-BAGS: 39.8
	PN content (mg/g-VSS)	–	–		–	210–815	150–305		139–167	431–514	–	BAGS: 267.31; A-BAGS: 220.79	BAGS: 287.77; A-BAGS: 216.54	Both BAGS and A-BAGS: 275.1
	ALE content (mg/g-VSS)	90–190	120–300	AS: 176; BAGS: 165–261	AS: 72; BAGS: 160	AS: 32; BAGS: > 100	Original: 13; 1% salinity: 50	AS: 187; BAGS: 236	204–244	219	213	BAGS:3.2; A-BAGS: 8.8	BAGS: 8.16; A-BAGS: 13.37	BAGS: 17.3; A-BAGS: 28.5
	Remarks	8 AS-WWTPs in China	Optimal condition: starch, 12°C, OLR, C/N, SRT	Mixed VFA feeding.	Granules sampled from the Nereda® pilot plant	Alternating OLR; granulation; cyclic diguanylate (c-di-GMP)	Salinity: 0%, 1%, 3%.	Phase 1: no or low granulation; Phase 2: full granulation.	Optimal condition: process, SRT, COD:N.	Optimal condition: aeration rate, cycle distribution, SRT	ALE for P removal	Simultaneous recovery of ALE/P	Optimal condition: OLR.	Salinity: 1%, 2%, 3%, 4%.
Reference		Li et al. (2021)	Li et al. (2022)	Schambeck et al. 2020a	Lin et al. (2013)	Yang et al. (2014)	Meng et al. (2019)	Schambeck et al. (2020b)	Rollemberg et al. (2020b)	Rollemberg et al. (2020a)	Dall' Agnol *et al.* (2020)	Chen et al. (2022b)	Chen et al. (2022a)	Meng et al. (2020)

*kg BOD_5/kg-MLSS·d. AS, activated sludge; AGS, aerobic granular sludge; BAGS, bacterial AGS; A-BAGS, algal-bacterial AGS; ALE, alginate-like exopolymer; BOD_5, 5-day biological oxygen demand; COD, chemical oxygen demand; DO, dissolved oxygen; EPS, extracellular polymeric substances; H/D, height/diameter ratio; HRT, hydraulic retention time; N, nitrogen; OLR, organic loading rate; P, phosphorus; PS, polysaccharides; PN, proteins; SBR, sequencing batch reactor; SRT, sludge retention time; WW, wastewater; WWTPs, wastewater treatment plants.

204–244 mg/g-VSS (Rollemberg et al., 2020b). In addition, bacterial AGS was observed to possess higher P bioavailability than algal-bacterial AGS (Chen et al., 2022a). Upon the simultaneous recovery of P and ALE from waste sludge, P-rich biomass and/or P-rich bioproducts can be further recycled and reused for multiple purposes (Chen et al., 2022b). ALE can also be used as a biosorbent to remove P, which outperforms the commercial alginate. As pointed out, P-rich ALE has the potential to be used as a sustained-release P fertilizer (Dall' Agnol et al., 2020).

3.3. ALE derived from algal-bacterial AGS

Owing to the pressing demand for energy transformation, carbon neutrality is the current development goal of sewage treatment technology (Mo and Zhang, 2012). Such unique features like low energy consumption, less greenhouse gases (GHGs) emission, and high energy/resource recovery potential make the algal-bacterial AGS process more attractive (Lee and Lei, 2019, 2022; Zhang et al., 2021). The strong mutualistic symbiosis between microalgae and bacteria holds the promise of transforming the sewage treatment process from a carbon source to a carbon sink (Chen et al., 2019; Zhang et al., 2022). As shown in Table 1, some comparative studies on resource recovery from algal-bacterial AGS and bacterial AGS have also been carried out, with the former (8.8 mg/g-VSS) having a much higher content of recoverable ALE than the latter (3.2 mg/g-VSS), despite the generally low amount quantified by colorimetric methods (please refer to Section 5.1 for the detailed reasons) (Chen et al., 2022b). A modest increase in volumetric organic loading rate (OLR, 0.6–1.2 g chemical oxygen demand (COD)/(L·d)) facilitated the yield of extractable ALE (13.37 mg/g-VSS from algal-bacterial AGS and 8.16 mg/g-VSS from bacterial AGS) (Chen et al., 2022a). Furthermore, the ALE contents recovered from algal-bacterial AGS and bacterial AGS were 28.5 and 17.3 mg/g-VSS at a 4 % salinity condition, respectively (Meng et al., 2020). However, since the current research on algal-bacterial AGS is still in its infancy (primarily on lab scale), the information on ALE recovery at pilot or full scale and its economic feasibility are still insufficient.

4. Key factors influencing ALE production

4.1. Operating conditions

4.1.1. Cycle distribution

The operating phases in the repeated cycle of SBRs commonly used for AGS process include filling, anaerobic, aerobic, (anoxic), settling, discharging, and idling, among which anaerobic, aerobic, (anoxic), and settling phases are important ones for EPS and ALE accumulation.

(1) Anaerobic phase

The operation of patented Nereda® bacterial AGS and another pilot-scale bacterial AGS for the treatment of municipal wastewater included anaerobic periods of 150 min and 120 min, respectively, accounting for 25 % and 33 % of the total operation cycle (Rollemberg et al., 2020a; van Haandel and van der Lubbe, 2012). The duration of the anaerobic phase is closely related to the growth and metabolic activities of polyphosphate-accumulating organisms (PAOs), during which the hydrolysis of organic carbon sources into organic acids (such as acetate) may affect the accumulation of EPS and ALE (Rollemberg et al., 2018).

(2) Aerobic phase

A longer aeration duration may lead to endogenous denitrification as EPS may be used as electron donor when COD is depleted. In contrast, a shorter aerobic phase favors higher EPS production, better biomass settleability and retention, and less energy consumption (He et al., 2018).

(3) Anoxic phase

Results show that a short anoxic period is conducive to the growth of denitrifying phosphate-accumulating organisms (DPAOs), and an optimized cycle distribution may promote the recovery of ALE and TRY (He et al., 2018; Rollemberg et al., 2020b).

(4) Settling phase

By controlling the settling time, microbes with better settleability (such as polymer-accumulating bacteria) can be further enriched and screened out, thus obtaining different ALE yields (Wang et al., 2018).

4.1.2. Loading rate

Volumetric OLR and food to microorganisms (F/M) ratio or sludge loading rate based on unit reactor volume and unit biomass can be expressed as g COD/L·d and g COD/g-VSS·d, respectively (Ghangrekar et al., 2005). According to Yang et al. (2014), a sudden increase in OLR could stimulate the release of extracellular cyclic diguanylate (c-di-GMP) by functioning microorganisms, resulting in increased ALE production. The strategy of appropriately increasing OLR from 0.6 to 1.2 g COD/L·d also improved the recovery of ALE from both bacterial AGS and algal-bacterial AGS (Chen et al., 2022a).

4.1.3. Sludge discharge

The factors influencing the long-term operation of AGS such as the selection of sludge discharge method and the control of sludge retention time (SRT) may impact the recovery of ALE. Whether it is discharged from the middle or bottom of the reactor, timely discharging sludge can improve P removal, reduce SRT, and promote the renewal and regeneration of granular sludge, resulting in a more stable state of the biosystem (Rollemberg et al., 2020a; Winkler et al., 2011). Rollemberg et al. (2020b) found that the ALE content recovered from bacterial AGS was significantly lower at SRT of 20 days than the results under shorter SRT conditions. A shorter SRT is generally considered more suitable for ALE recovery, which may be attributed to a faster renewal of the dominant microbial community, higher bacterial metabolic activity, and lower demand for endogenous respiration (Li and Wu, 2014). In addition, since the sludge discharge process or the reduction of SRT can effectively remove the bacterial AGS saturated with P from the reactor during the aerobic phase, the yield of ALE is also reported to be relevant to the growth of PAOs (Rollemberg et al., 2020b). By contrast, Li et al. (2022) found that different SRTs (10, 15, and 20 days) had little effect on ALE formation in flocculent AS. More in-depth research needs to be conducted on the relationship between SRT and ALE production in the flocculent and granular sludges.

4.1.4. Shear stress

Shear stress has a great influence on the rapid formation of AGS, granular structure stability, EPS production, and inhibition to excessive growth of filamentous bacteria and/or the overgrowth of granules as well (Liu and Tay, 2002; Rollemberg et al., 2018). The hydrodynamic shear stress imposed on the SBR is closely associated with the aeration regime, upflow air velocity, and height/diameter (H/D) ratio of the reactor (Adav et al., 2008; Wang et al., 2021). As shown in Table 1, the upflow air velocity applied in the previous works ranged from 0.3 to 2.65 cm/s. Neither too high nor too low upflow air velocity is optimal for extracting substantial amounts of ALE, which needs to be determined in combination with specific characteristics of various influents. On the other hand, the smaller the reactor H/D ratio designed, representing a shorter annular flow trajectory of aerated bubbles in the reactor, the higher airflow demand is needed to provide sufficient hydraulic friction and turbulence for microbial aggregation (Liu and Tay, 2002). The main studies involving different H/D ratios and extractable ALE contents are summarized in Table 1. As shown, a higher H/D ratio with an appropriate upflow air velocity is recommended for the stable granulation and resource recovery.

4.1.5. Temperature

Temperature has a direct effect on microbial metabolism and growth, making it a key factor in the yield and composition of biopolymers (Feng et al., 2021). For energy savings and practical application considerations, the majority of investigations were conducted at

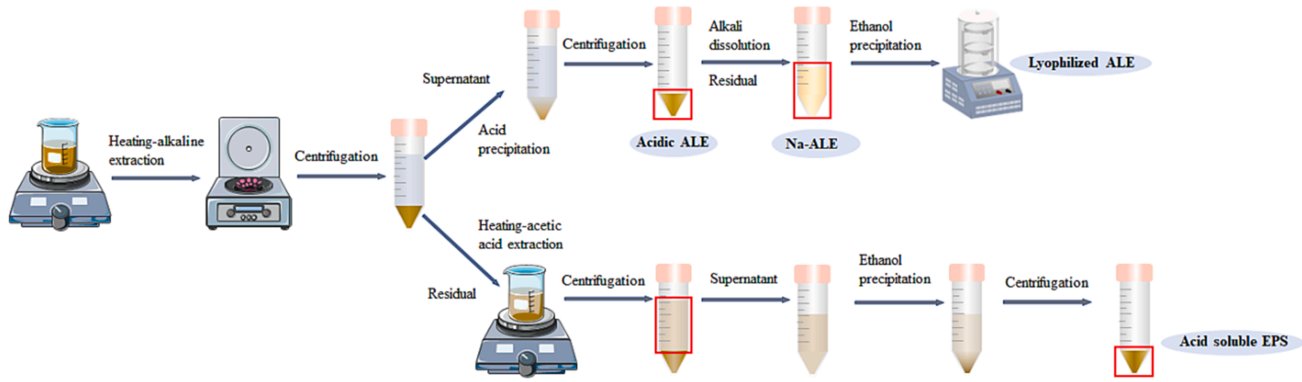


Fig. 2. Extraction process of ALE/EPS in different forms summarized from Meng et al. (2020), Pronk et al. (2017), and Rollemberg et al. (2020b). ALE, alginate-like exopolymer; EPS, extracellular polymeric substances.

Table 2
Summary of recent attempts on the extraction, characterization and identification of valuable extracellular biopolymers.

EPS sources	Extraction methods	Characterization methods	Recycled biomaterials	Potential applications/Descriptions	Reference
Nereda® bacterial AGS	Commonly used structural EPS extraction method (Lin et al. (2010))	Atomic force microscopy, size exclusion chromatography, pyrolysis–gas chromatography–mass spectrometry, material adhesion to hydrocarbons (MATH) test, contact angle measurement.	Polysaccharide-based biomaterial	The recycled biomaterial was expected to be used as a coating material due to its amphiphilic properties.	Lin et al. (2015)
Acetate fed bacterial AGS (dominated by *Defluviicoccus* Cluster II organisms)	Acetic acid extraction method (focusing on the residue after centrifugation)	^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR), Fourier Transform Infrared Spectroscopy (FTIR), Raman spectroscopy, fluorescence lectin-binding analysis, Transmission and scanning electron microscopy (TEM, SEM), confocal laser scanning microscopy (CLSM)	Acid soluble EPS	14 mg/g-VSS of ALE and 162 mg/g-VSS of acid soluble EPS were recovered. The latter was characterized as α-(1 → 4) linked polysaccharide with glucose and galactose as monomers.	Pronk et al. (2017)
Bacterial AGS enriched with ammonium-oxidizing bacteria	Sodium dodecyl sulfate-heating-alkaline method	Raman, FTIR spectroscopy, protein analysis by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) , Rheological test	Glycosylated amyloid-like proteins in the structural extracellular polymers	The extract exhibited rich cross β-sheet secondary structure and included glycosylated protein/polypeptide and tryptophan.	Lin et al. (2018)
Flocculent AS obtained from sulfate-laden wastewater	Sulfated polysaccharides (SPs) extraction procedure (remove alginate, nucleic acid, proteins)	Ultra-performance liquid chromatography (UPLC) analysis, high-performance liquid chromatography (HPLC), inductively coupled plasma optical emission spectrometry (ICP-OES), bio-activities assay	SPs-containing EPS	The extracted SPs (fucoidan, carrageenan and heparin) were highly valuable recovered products with remarkable bioactivity and purity.	Xue et al. (2019)
Seawater-adapted bacterial AGS	Direct staining	Fluorescence in situ hybridization (FISH), lectin staining, sialic acid quantitation kit, genome analysis, high-performance anion-exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD), FTIR analysis	Sialic acids in EPS	In the form of sialic acid glycoprotein, sialic acids were abundantly distributed in EPS.	de Graaff et al. (2019)
Nereda® bacterial AGS	Commonly used structural EPS extraction method (Lin et al. (2010))	SDS-PAGE analysis, FTIR, Hyaluronic acid and sulfated GAG analysis kits, ion exchange chromatography (IEC), HPAEC-PAD, gas chromatography-mass spectrometry (GC–MS), enzymatic digestion and specific in situ visualization	Glycosaminoglycans (GAGs), including hyaluronic acid-like and sulfated GAGs-like polymers	Similar to biopolymers secreted by vertebrates, GAGs isolated from bacterial AGS were linear heteropolysaccharides with amino sugar derivatives.	Felz et al. (2020b)

AS, activated sludge; AGS, aerobic granular sludge; ALE, alginate-like exopolymer; EPS, extracellular polymeric substances; VSS, volatile suspended solids.

room temperature (20–30 °C). However, the ideal temperature for microbial growth and biopolymer synthesis varies among different microbial communities in the SBR systems (More et al., 2014). Li et al. (2022) noted a higher ALE content (303.3 mg/g-VSS) extracted from flocculent AS at a lower temperature (12℃), while its ionic hydrogel properties deteriorated due to the reduction on bacterial metabolism. This observation may be attributed to the slower growth rate and lower nutrient/energy transfer capacity of microorganisms under low temperature conditions, providing more precursors for ALE production and biopolymer synthesis (Li et al., 2022). In addition, the composition and purity of the biopolymers recovered from different sludges still need further investigation as temperature may also greatly affect the enzymatic activity. Notably, the ideal temperature for the growth of a particular microorganism may not necessarily be the same as the optimal temperature for biopolymer synthesis.

4.1.6. Microbial diversity

Although commercial alginates are currently predominantly

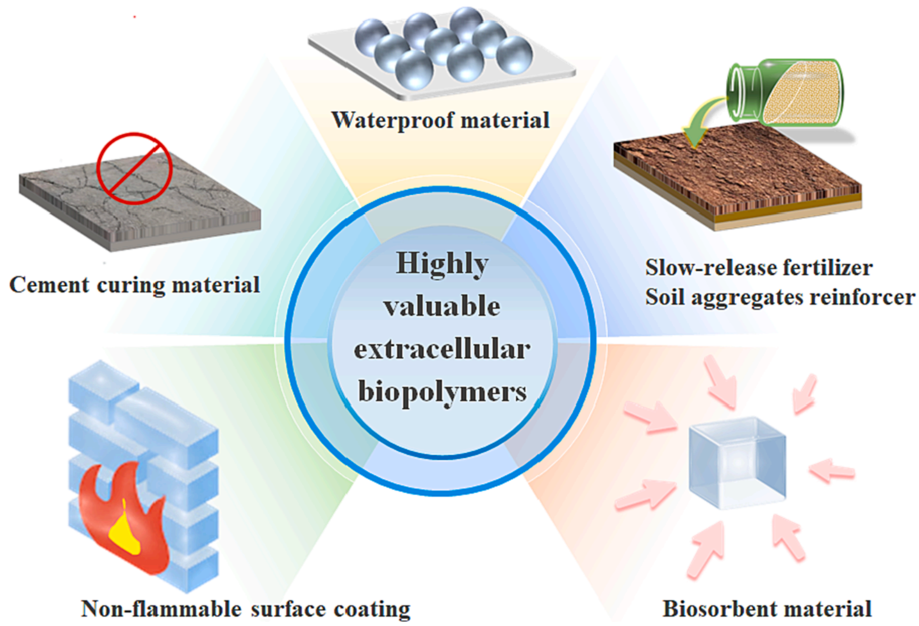


Fig. 3. Various applications of the highly valuable extracellular biopolymers.

originated from algae, particularly brown seaweeds, it is increasingly possible to obtain alginates from bacterial sources (Pawar and Edgar, 2012). For instance, the two bacterial genera, *Pseudomonas* and *Azotobacter* have been reported to produce alginate (Remminghorst and Rehm, 2006). At the family level, Rollemberg et al. (2020b) found that Rhodobacteraceae and Competibacteraceae relating to PAOs and glycogen-accumulating organisms (GAOs) may be associated with granular stability and ALE production. Another study also demonstrated that a stable microbial community composition favors the accumulation of large amounts of ALE into the bacterial AGS (Schambeck et al., 2020b). Besides, quorum sensing (QS) and its antagonistic process, namely quorum quenching (QQ), are widespread chemical communication mechanisms between cells in microbial communities (Li et al., 2020). QS/QQ-related activity changes and community evolution may play an important role in regulating extracellular polymer production, floc formation, and aerobic granulation (Li and Zhu, 2014; Salehiziri et al., 2018). The main factors addressed in this review may have impacts on the microbial metabolic activities, QS signals production and reception, and the symbiotic relationships among microorganisms, hence influencing the dominant microbial community. Therefore, microbial diversity and abundance of key functional groups play an important role in EPS secretion, granule formation, and granular stability (He et al., 2019). As a part of EPS, the accumulation of ALE is also certainly influenced by microbial diversity.

According to Schambeck et al. (2020b), a higher ALE content is correlated with a more stable microbial community composition. The dynamic changes of EPS and ALE during the granulation process from flocculent AS to bacterial AGS are also summarized in Table 1. As noted, the processes of aerobic granulation and granule maturation may also be accompanied by the changes in content, composition, and properties of extracellular biopolymers (Schambeck et al., 2020b). The increased ALE content appears to be a prerequisite for granule maturation, but it's not the only necessity (Yang et al., 2014).

4.2. *Wastewater characteristics*

4.2.1. *Synthetic or real wastewater*

Even though it is highly variable with the seasons and other factors, accompanied by a low reproducibility, the real wastewater is rich in trace elements, minerals, vitamins, and some special organic substances that can be used as carbon sources (O'Flaherty and Gray, 2013). The practical application of bioaggregates is hampered by the fact that the simple synthetic wastewater used in most lab works can only cover limited constituents. The optimized synthetic wastewater with specific components can be targeted to achieve the corresponding research objectives. For example, the bacterial AGS fed with the simple synthetic wastewater produced a relatively low yield of ALE (100 mg/g-VSS) (Yang et al., 2014). When fed with the real municipal wastewater, about 236 and 213 mg/g-VSS of ALE were recovered from bacterial AGS by Schambeck et al. (2020b) and Dall' Agnol et al., 2020, respectively. However, Schambeck et al. (2020a) also attempted the bacterial AGS with the optimized synthetic wastewater (*i.e.*, the mixture of acetate and propionate as carbon source), which yielded a higher amount of ALE (261 mg/g-VSS). In addition, the rheological properties of ALE extracts are influenced by the type and composition of wastewater; and a higher elasticity could be obtained in the ALE hydrogels derived from bacterial AGS fed with the real wastewater, particularly the wastewater rich in volatile fatty acids (VFAs). Besides VFAs, Li et al. (2022) recommended starch (macromolecular organic matter) as one of the carbon sources that may promote ALE accumulation, as it aids the fast formation of a stable sludge aggregation matrix by directly lowering the energy barrier. Among the micromolecular organic compounds, sodium acetate has been reported to be more biodegradable and readily converted to acetyl Co-A, thereby engaging in the tricarboxylic acid (TCA) cycle and promoting higher levels of extracellular enzymes involved in electron transfer and energy metabolism (Li and Yang, 2007). In addition, the differences between organic and inorganic carbon sources have been also reported to impact the synergistic carbon metabolism and enzyme regulation, thus influencing the growth and biochemical composition of microalgal-bacterial biomass (Gao et al., 2021).

4.2.2. *Influent C/N ratio*

The yields of EPS and ALE to some extent depend on the properties of organic substrate, such as influent carbon/nitrogen (C/N) ratio. The allocation of C and N sources may have some direct effect on the accumulation of polysaccharides and proteins. As shown in Table 1, Li et al. (2022) noticed significant differences ($p < 0.05$) in the extractable ALE content from flocculent AS when the C/N (calculated as the ratio of 5-

day biological oxygen demand (BOD_5) to total nitrogen (TN) in this study, or BOD_5/TN) ratio was set as 3, 5, and 7, respectively; and the highest ALE content was achieved at the C/N ratio of 5, reaching 137.8 mg/g-VSS. The effect of higher C/N (COD/N = 10, 20, 30) ratios on ALE production from bacterial AGS was also explored, and the ALE yield reached 28.97 % (VSS basis) when the C/N ratio was 20 (Rollemberg et al., 2020b). A low C/N ratio may result in the presence of more protein-like material in ALE and less accumulation of extracellular polysaccharides, as insufficient carbon sources may lead to cellular autolysis and endogenous respiration (Sheng et al., 2010). Furthermore, although an excessively high C/N ratio is more beneficial for microbial production of extracellular polysaccharides, insufficient N may drive the growth of filamentous organisms; and the overgrowth of aerobic granules may also inhibit carbon source diffusion and eventually lead to granule disintegration (Rollemberg et al., 2020b). Therefore, influent C/N ratio should also be paid attention and further optimized when the stable system operation with considerable resource recovery efficiency is targeted.

4.2.3. Salinity and essential cations

Changes in salinity may regulate the cellular osmotic pressure, and the composition and structure of EPS may also be altered during the protection of bacteria from salinity stress (Corsino et al., 2017).

In the case of simple saline matrices (NaCl only), Meng et al. (2020) found that bacterial AGS disintegrated when the salinity was increased from 1 % to 4 %, with 46.1 % and 86.5 % reduction respectively in its total ALE content (17.3 mg/g-VSS) and GG blocks providing gel formation ability. An appropriate salinity level has been shown to activate gene *alg*C expression to synthesize more phosphomannomutase (PMM), which may catalyze the interconversion between the key precursors (mannose-6-phosphate (M6P) and mannose-1-phosphate (M1P)) of alginate synthesis, and ultimately might contribute to the promoted ALE production (Jain and Ohman, 1998; Meng et al., 2019; Yu et al., 2015).

As for the complex multi-ion seawater matrices, Li et al. (2017) observed that when the real seawater proportion was increased to 100 %, the total ALE and GG blocks contents in bacterial AGS were respectively 6 times and 2 times of those under 0 % proportion condition. This observation may be associated with the highly specific interactions of alginate with mineral cations over a wide range of temperatures and pH levels, such as cationic bridging and precipitate formation in the core of bacterial AGS (Li et al., 2017; Lin et al., 2013). In addition, extracellular biopolymers may influence the accumulation and transfer of K^+, Ca^{2+}, and Mg^{2+} in the bacterial AGS system (Wang et al., 2014). These authors claimed that the abundance of essential cations has a significant impact on the structure and composition of ALE. Most recently, it has been found that the type of metal ions (transition metal or alkaline earth metal) exerted a non-negligible effect on the hydrogel-forming capability and strength of structural EPS in addition to its structure and composition (Felz et al., 2020a).

In addition, the accumulation of intracellular compatible solutes is also a key approach for microorganisms to regulate external osmotic pressure under high salt stress (Hu et al., 2022). The results of metabolic analysis reveal that the synthesized intracellular compatible solutes such as glutamine and trehalose may help microorganisms bind water molecules and withstand high osmotic pressure (Hu et al., 2022). Hence, the generation of compatible solutes is also a noteworthy potential factor affecting the synthesis of extracellular biopolymers.

5. Challenges and developments of ALE identification

5.1. ALE identification and quantification with limitations

The potential of extracellular biopolymers in resource recovery is somewhat limited by the complexity of its composition and structure, the challenge of extracting specific components, and the non-specificity of quantitative approaches (Felz et al., 2019; Seviour et al., 2019). Prior to the further isolation of ALE, most studies on EPS extraction from wastewater biomass focused primarily on the contents of polysaccharides and proteins, the two dominate components in EPS (Wang et al., 2022). In practical wastewater treatment systems, however, the simple colorimetry for EPS quantification has gradually revealed its following limitations. (1) EPS are structurally and compositionally diverse biopolymers (including polysaccharides, proteins, humic substances, lipids, nucleic acid, uronic acid, and inorganic components), and an analytical method that quantifies only a few components can not accurately represent the true content of EPS (Lin et al., 2010; Sam and Dulekgurgen, 2015; Xiao and Zheng, 2016). (2) Colorimetry is highly dependent on the selection of standard compounds, and the quantification results by using different standard compounds vary substantially even for the same component (Felz et al., 2019). (3) There may be interference between the distinct components of the complicated EPS when a single compound is evaluated (Felz et al., 2019). The above defects are also reflected in the quantification of ALE with complex components (Chen et al., 2022a).

The quantitative approach for ALE is also worth noting. As shown in Table 1, the ALE extracted from flocculent AS, bacterial AGS, or algal-bacterial AGS has been reported to be quantified by gravimetry (100–300 mg/g-VSS) or colorimetry (<50 mg/g-VSS). The comparative analysis of the recovered ALE yield should be based on the premise of using the same quantitative approach to ensure its comparability. Gravimetry is currently the most widely used means for ALE determination, in which the extracted ALE samples are lyophilized and quantified according to the standard method (APHA, 2012). Since it is designed to determine the content of all components (proteins, neutral sugars, amino sugars, uronic acids, humic acid, etc.) in the extract, the results are inevitably higher (Chen et al., 2022a). The constraints are that the quantified results may be higher than alginate-like substances themselves, and it is difficult to distinguish the specific content of different unique components with various functions. The carbazole method using commercial sodium alginate as the standard is considered as a targeted colorimetry for ALE quantification (Meng et al., 2020), but it is still limited by selecting a single standard and the cross-interference of the actual presence of multiple compounds (Chen et al., 2022b; Felz et al., 2019). As a result, when determining the amount of ALE by applying gravimetry or colorimetry, comparing it using the phenol–sulfuric acid assay or carbazole assay, and utilizing glucose or alginate as the standard, the results will be markedly different. dos Santos et al. (2022) found that the content of ALE further extracted from EPS was higher than proteins, polysaccharides or even the sum of these two components. This is due to the fact that the quantitative methods and standards used for different constitutions are not consistently comparable. Therefore, the equivalent calculation also makes sense in some cases (Li et al., 2021, 2022).

Identification of ALE further separated from EPS is likewise challenging. During the extraction process of ALE, whether the granular structure has been destroyed or not (completely solubilized) would bring different results (Pronk et al., 2017). As shown in Fig. 2, it is crucial to distinguish whether the extracted ALE is in the sodium form (Meng et al., 2020), acid form (Rollemberg et al., 2020b), or acid soluble EPS (Pronk et al., 2017) according to the termination step during the extraction process, because only the same category of ALE is more comparable. One of the crucial considerations taken into account in order to guarantee the recovery effectiveness of extracellular biopolymers appears to be the strict management of consistent recycling procedures.

In addition, even if the extract is observed to have similar properties to sodium alginate, further analysis and confirmation of the characteristics of the extracellular biopolymers is required (Karakas et al., 2020). The characterization of extracted biopolymers with different special structures and functions in the existing literature is summarized in Table 2. More precise and comprehensive approaches for the identification, characterization, and quantification of extracellular biopolymers are demanding in order to expand their applications in circular economy and resource recovery.

5.2. Novel identification of valuable extracellular biopolymers

Instead of concentrating on the quantitative identification of extractable extracellular biopolymers (sometimes it is not necessarily to be so precise), a qualitative determination of their composition should be carried out to clarify their exact characteristics, linking their composition to the specific functions and properties (Boleij et al., 2019). A previous study has shown that it is not possible to obtain all types of structural EPS through a single extraction method (Pronk et al., 2017). Thus, it is necessary to develop novel extracellular biopolymer identification technologies for the specific types of sludge that have a greater potential for recovery of unique biomaterials with a wide variety of special uses. The notion of identifying the isolated EPS according to their functions, compositions, and characteristics is recommended, just as the extracellular biopolymers extracted from bacterial AGS are named alginate-like EPS because of their chemical and mechanical similarities to alginate (Feng et al., 2021; Lin et al., 2013). The emerging attempts at the extraction, characterization, and identification of valuable extracellular biopolymers are summarized in Table 2. A variety of criteria for extraction and clarification of extracellular biopolymers have been established based on the different types of sludge. Abundant recycled bio-products such as polysaccharide-based biomaterial (Lin et al., 2015), acid soluble EPS (Pronk et al., 2017), glycosylated amyloid-like proteins (Lin et al., 2018), sulfated polysaccharides (Xue et al., 2019), sialic acids (de Graaff et al., 2019), hyaluronic acid-like and sulfated GAGs-like polymers (Felz et al., 2020b) have been excavated and explored. Stimulated by the current challenges, there is an urgent need to improve the characterization of specific extracellular biopolymers from an interdisciplinary perspective. The combination of biology, biochemistry, glycomics, proteomics, and genomics with sophisticated analytical techniques (three-dimensional excitation emission matrix (3D-EEM), NMR, confocal laser scanning microscope (CLSM), high performance liquid chromatography (HPLC), inductively coupled plasma - optical emission spectrometry (ICP-OES), high performance anion exchange chromatography - pulsed amperometric detector (HPAEC-PAD), gas chromatography - mass spectrometry (GC–MS), fourier transform infrared spectroscopy (FT-IR), etc.) will provide more possibilities for practical applications of the EPS and ALE extracts (Seviour et al., 2019).

6. Feasible applications and future perspectives

6.1. Feasible applications

Sludge-based (flocculent AS, bacterial AGS, or algal-bacterial AGS) extracellular biopolymers are incredibly diverse and specialized. With different extraction and recovery procedures, it is regarded as a treasure trove of valuable raw biomaterials with diverse properties that can be applied in a variety of industries (Fig. 3). As reported, the structural EPS extracted from the pilot-scale bacterial AGS systems showed gel-forming properties with divalent cations and hydrophilic features similar to those of commercial alginates (Karakas et al., 2020). Such bacterial AGS-based structural products have been utilized as industrial cement curing agents (NGCM, 2022). The recovered polysaccharide-based biomaterials have been reported to possess good film-forming and amphiphilic capabilities, making them suitable for use as waterproof and grease-resistant materials such as paper coatings (Lin et al., 2015). It has also been demonstrated that extracellular biopolymers recovered from both flocculent AS and bacterial AGS display self-extinguishing capabilities (Kim et al., 2020). The flax fabric coated with the latter bioproduct showed stronger fire-resistant qualities, which can be proposed as an alternative to halogenated flame retardants for non-flammable surface coatings (Kim et al., 2020). In addition, the recycled extracellular biopolymers are considered renewable, less expensive, and more environmentally friendly biosorbent materials, which well functioned in the adsorption of methylene blue (Ladnorg et al., 2019), heavy metals (Wang and Li, 2012), P (Dall' Agnol et al., 2020), etc. After further toxicological and nutrient element analysis of P-rich ALE beads, the extracellular biopolymers recovered from bacterial and algal-bacterial AGS are expected to be used as sustained-release P fertilizers, soil conditioners, and soil aggregates reinforcers (Chen et al., 2022a; Dall' Agnol et al., 2020). A broader range of practical applications of the extracellular biopolymers awaits being developed and promoted based on more sophisticated isolation, characterization, and analytical techniques.

6.2. Future perspectives

Despite the efforts and advances made in the optimization of production conditions and the description of the specific properties of extracellular biopolymers, there is currently insufficient data available to perform a comprehensive and integrated assessment of extracellular biopolymers derived from flocculent AS, bacterial AGS, and algal-bacterial AGS. In order to improve the applicability of the recovered high value-added biomaterials, the following key future research directions are proposed for the stable and effective synthesis of extracellular biopolymers in the biomass from wastewater treatment systems.

(1) Which type of sludge is used for extracellular biopolymers production?

Most studies on structural EPS to date have been carried out based on granular sludge systems, an approach that has enriched our understanding of the compact structure of bacterial AGS or algal-bacterial AGS as different from flocculent AS. However, considering the widely applied flocculent AS rather than bacterial AGS in the existing WWTPs, it would be more economically viable and realistic if the flocculent AS-based specific extracellular biopolymer could be produced at the same level as bacterial AGS by altering operating conditions. Furthermore, studies on the recovery of ALE from algal-bacterial AGS are tentative. Given that algae are currently the primary source of commercial alginate (Pawar and Edgar, 2012), the impact of microalgae co-existence in the granular symbiotic system of bacteria and microalgae on ALE production deserves further investigation. In addition, the unique potential of biological systems dominated by some particular microbes to synthesize functional extracellular biopolymers also needs to be further explored.

(2) What kind of extracellular biopolymers are produced? How to isolate, identify and characterize them?

Due to the high diversity of extracellular biopolymers, subtle variations in each step during the processes of cultivation, extraction, recovery, and identification can lead to completely different quantitative and qualitative results (Pronk et al., 2017). The consistent description of different extracted bioproducts has been challenging. At present, mild/single extraction methods and limited/biased analytical techniques are unable to meet the demands for further accurate recognition of extracellular biopolymers with complex structures and functions. Multidisciplinary and integrated knowledge is the prerequisite for a comprehensive understanding of the specific components and unique functions of EPS. Besides, a characterization strategy based on the same criteria provides greater comparability and allows for more rigorous assessment. Critical attention should also be paid to the particular phenomena or properties observed during the extraction and characterization of extracellular biopolymers. And the extraction strategy for sludge-based functional EPS can be explored and modified by referring to the extraction procedures of common substances with similar traits. For instance, the recovery of ALE was attempted using the extraction method of alginate after the discovery of its characteristics comparable to alginate (Lin et al., 2010).

(3) How to directionally regulate and control specific extracellular biopolymers with unique features?

Based on the capacity to obtain adequate yields, it is vital to work on enhancing the purity of functional extracellular biopolymers (Seviour et al., 2019). According to the gel-forming, film-forming, mechanical, amphiphilic, adsorption, viscoelastic, aggregation, and self-extinguishing properties of extracellular biopolymers, it is promising to improve the desirable qualities of the recovered biomaterials by optimizing the operating parameters and incubation conditions of the biosystem in a targeted manner. Advances in molecular biotechnology, particularly proteomics, glycomics and genomics, will provide useful insights into the targeted regulation of microbial metabolic pathways and responses to environmental conditions (Gao et al., 2022). For practical applications, in addition to lab scales, the feasibility of recovering highly valuable biomaterials from the long-term operation of biosystems treating real wastewaters needs to be verified at pilot or full scales. Moreover, the selection of conditions such as temperature is best combined with regional and climatic advantages in terms of economic feasibility.

(4) How about the economic feasibility of recycling specific extracellular biopolymers?

In the progress of considering excess sludge as a resource with recycling potential and market demand, it is vital to evaluate the economic feasibility of recovering specific extracellular biopolymers based on the concept of circular bioeconomy. The following concerns should be paid attention: 1) Whether the economic benefit of the recovered extracellular biopolymers can cover the costs of their recovery and purification? 2) Whether the extraction and purification of functional EPS-based bioproducts is more economical than the traditional routes? And 3) depending on different recycling practices, how can energy efficiency be improved to reduce economic, environmental, and energy expenditures as much as possible?

The above prospects are offered with a view to making the strategy of recovering high value-added bioproducts from waste sludge reasonable in process, feasible in technicality, and considerable in economic benefit.

7. Conclusions

Recent studies on the extraction and recovery of valuable extracellular biopolymers from flocculent AS, and bacterial/algal-bacterial AGS have been reviewed. The characteristics, influencing factors, limitations, and recent attempts to extract and identify specific extracellular biopolymers were discussed comprehensively. A deeper understanding of the composition, properties, and unique functions of different sludge-based extracellular biopolymers necessitates the advancement in the optimization of specific operating conditions, targeted extraction techniques, and multidisciplinary analytical perspectives. Many efforts are expected to improve the output and quality of extracellular biopolymer-based bioproducts while linking their properties with functions and expanding their application fields.

CRediT authorship contribution statement

Xingyu Chen: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Yu-Jen Lee:** Methodology, Writing – original draft, Writing – review & editing. **Tian Yuan:** Methodology, Writing – review & editing. **Zhongfang Lei:** Conceptualization, Formal analysis, Funding acquisition, Project administration, Supervision, Writing – review & editing. **Yasuhisa Adachi:** Formal analysis, Funding acquisition, Writing – review & editing. **Zhenya Zhang:** Formal analysis, Methodology, Supervision. **Yuemei Lin:** Formal analysis, Writing – review & editing. **Mark C.M. van Loosdrecht:** Formal analysis, Writing – review & editing.

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.

Data availability

Data will be made available on request.

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