Review

Biopolymers in Aerobic Granular Sludge—Their Role in Wastewater Treatment and Possibilities of Re-Use in Line with Circular Economy

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Abstract: Aerobic granular sludge (AGS) technology for wastewater treatment ensures better quality effluent and higher process sustainability than wastewater treatment systems based on activated sludge. One of the reasons for the better operational results of AGS systems is the high content of extracellular polymers (EPS) in the granule structures. EPS produced during granulation have a very complex composition with a predominance of polysaccharides and proteins, and one of the main components, alginate, may have a wide variety of practical applications. This review summarizes up-to-date information on the composition of EPS in AGS, the manner in which their production and composition are affected by the operational parameters of wastewater treatment, and the effects of EPS in biomass on wastewater treatment and sludge management. Additionally, the possibility of polymer recovery from AGS is presented together with information regarding potential applications based on the newest findings. Re-use of AGS-derived polymers will increase the sustainability of wastewater treatment processes by making them more economical and reducing the amount of sludge that requires management.

Citation: Cydzik-Kwiatkowska, A. Biopolymers in Aerobic Granular Sludge—Their Role in Wastewater Treatment and Possibilities of Re-Use in Line with Circular Economy. *Energies* **2021**, *14*, 7219. <https://doi.org/10.3390/en14217219>

Academic Editor: Dino Musmarra

Received: 8 October 2021

Accepted: 29 October 2021

Published: 2 November 2021

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Keywords: AGS; extracellular polymeric substances (EPS); alginate (ALE); EPS composition; polymer extraction

1. Introduction

One of the challenges in modern wastewater treatment is to make the processes sustainable by increasing the economic recovery and reducing the amount of sludge that needs further management.

Aerobic granular sludge (AGS) technology has been extensively studied and is used in wastewater treatment plants to remove biodegradable organic matter and to overcome difficulties with conventional activated sludge systems. It has been successfully implemented in over 40 full-scale plants in locations around the world [1,2]. AGS technology, in which biomass has a form of compact and dense granules, offers advantages over conventional activated sludge such as a high settling capacity, more effective sludge-effluent separation, higher biomass retention, tolerance to high organic loads and toxicity, the possibility of simultaneously removing nutrients, and adsorption of heavy metals. AGS technology allows wastewater treatment plants to be operated with 30% less energy input and with significantly lower investment costs [3].

To cultivate granular sludge, the operational parameters must be properly selected to stimulate bacterial aggregation, which results mainly from secretion of self-produced extracellular polymeric substances (EPS), which form a matrix that encapsulates and protects the bacteria. EPS not only contributes to the formation and preservation of biofilm architecture, but also protects bacterial cells against desiccation, antibiotics and host immune defenses. The content of EPS is related to the size of the granules in the biomass. In AGS, granules with a diameter of 0.5–1 mm had the highest content of stable EPS while the smallest granules had the highest content of bound EPS [4]. EPS can be

excreted by microorganisms, released during cell lysis and also adsorbed as organic matter from wastewater. Recovery of biopolymers from excess AGS provides a valuable source of biomaterial.

This review summarizes up-to-date information on the composition of EPS in AGS, the manner in which their production and composition are affected by the operational parameters of wastewater treatment, and the effects of EPS in biomass on wastewater treatment and sludge management. Additionally, the possibility of polymer recovery from AGS is presented together with information regarding potential applications based on the newest findings.

2. Composition of Polymers in Biomass

The composition of EPS in biomass varies depending on the microbial composition of the biofilm and the environmental conditions [5]. In EPS, polysaccharides (PS) and proteins (PN) predominate, but EPS may also contain other macro-molecules, such as nucleic acids, lipids, and humic substances [6,7]. Wang et al. [8] proposed that large amounts of PS and PN conjugate in the EPS matrix, with PS as the skeleton, deciding the granular stability by affecting hydrophobicity interactions and hydrogen bonds, which are two important parameters of gel properties. The chemical structure of EPS varies considerably, not only between different bacterial species but even with the same species. Generally, macromolecular compounds predominate in EPS although the distribution of molecular weights may vary considerably, from <3 kDa to >235 kDa [9]. The formation of EPS PN with a high molecular mass (≥ 116 kDa) was stimulated by the presence of Ca^{2+} in the reactors [10]. The presence of Ca^{2+} significantly reduced the electrostatic repulsion between EPS and enabled a dense EPS matrix to be formed [11]. PN are usually more abundant in biomass than PS, and PN/PS ratios of 1–8 have been reported [4,12,13]. In AGS from a full-scale wastewater treatment plant (WWTP) treating urban wastewater, PN and humic substances were the main EPS components (329–494 and 259–316 mg/g VSS of AGS, respectively), while PS and DNA represented minor EPS fractions [14]. EPS can contain hetero- or homopolymers, simple linear sugars, or sugars with branched side chains. These molecules can be dimers and trimers, can be composed of thousands of saccharides arranged in long repeatable units, or can be knitted together forming fibers [15]. Jahn et al. [16] reported that the tryptophan content in EPS isolated from AGS was almost twice as high compared to EPS extracted from activated sludge. Tyrosine- and tryptophan-like substances were identified in EPS in high amounts after cell exposure to toxic compounds such as nonylphenol or nitrobenzene [17,18].

Regarding PS, two crucial constituents, alginate-like exopolysaccharide (ALE) and granulan, have been identified as the functional gel-forming constituents of AGS. Granulan is a complex heteropolysaccharide [19]. ALE is a mixture of both neutral and uronic polysaccharides [20]. ALE is an important constituent of biopolymers extracted from both floccular and granular sludge. In AGS, an increase in the ALE content was a signal of granule maturation [21]. In a study on sludge granulation, the ALE content in biomass increased from 32 mg/g VSS in the sludge to 100 mg/g VSS in mature AGS [12]. Another study found ALE only in mature granules [22].

Recent studies have revealed that, in EPS, complex glycoconjugates are also present such as glycosaminoglycans (GAGs). GAGs are linear heteropolysaccharides and were identified not only in the EPS matrix but also in the spaces between the cells inside the microcolonies. Felz et al. [7] speculated that, in granules, GAGs-like polymers are involved in attracting cations, forming hydrogels, binding proteins, and signaling in biological processes. Moreover, sialic acids were found in EPS from seawater-adapted AGS. These compounds were located in the outer layer of the EPS matrix, functioning as a shield that protected the underlying sugars from degradation [23]. Glycosylated amyloid-like proteins have been extracted from granules with a predominance of ammonium-oxidizing bacteria [24].

The role of EPS for biomass formation is related with the presence of multivalent cations that bind to it [25,26]. Those ions support bacterial adhesion by neutralizing negative charges on cell surfaces [27] and also act as a bridging agents in the EPS matrix. In the presence of multivalent cations, the granule matrix is predominantly composed of large and complex proteins. Ca^{2+} has been shown to have a considerable regulatory influence on production of EPS during aerobic granulation [10]. Via addition of divalent cations into saline wastewater with a low organic load, halotolerant AGS with an average diameter of $910 \pm 10 \mu\text{m}$ was cultivated. The positive correlation between the concentration of divalent cations in wastewater and particle size indicated that Ca^{2+} played an important role in granulation [28]. The presence of multivalent ions speeds up AGS formation. Wang [29] observed that addition of Fe^{2+} (3–5 mg/L) to the reactor caused granules with an average size higher than 0.35 mm to be cultivated within seven days, and that addition of iron ions did not negatively affect removal of COD and ammonium from wastewater. However, the presence of ions in the AGS structure affects the biodegradability of AGS. Wang et al. [30] observed that the EPS from the outer granule layer, in which multivalent ions were mostly present, was not biodegradable, which was in contrast to EPS found in the inner layers. Also, Leenen [31] showed that the Ba-Ca-EPS complex was not biodegradable.

Quorum sensing (QS) is an important mechanism involved in aerobic granulation. QS regulation systems support biofilm development via production of hydrophobic gel-forming EPS, which increases the aggregation and stability of granules [32]. During granulation, an increase in the concentration of acyl-homoserine lactones (AHL) was observed [33]. Granulation can also be affected by the amount of cyclic diguanylate (c-di-GMP) in bacterial cells, which is commonly used by bacteria to regulate the production of exopolysaccharides. A decrease in the intracellular concentration of c-di-GMP after addition of Mn^{2+} ions decreased the concentration of EPS, resulting in granule disintegration and wash out from the reactors of PS producers belonging to *Acinetobacter* sp., *Bdellovibrio* sp., *Thauera* sp., and *Paracoccus* sp. [34]. Metabolomics analysis combined with microbiological analyses were used to explore granulation mechanisms and the EPS structure; these analyses showed that the amino acid biosynthesis pathway was stimulated by a low COD/N ratio, which increased the hydrophobicity of EPS. The operation of an AGS reactor also affected the QS in biomass. Partially denitrified granular sludge had stronger AHLs-based QS than denitrified granular sludge [35].

In the literature, EPS in biomass are most often classified as bound and soluble EPS (Sol-EPS). Among bound EPS, loosely-bound (LB-EPS) and tightly-bound EPS (TB-EPS) are distinguished. In a laboratory-scale study of sludge from a meat utilization plant, the TB-EPS contained nearly two times more PNs than sugars, while the LB-EPS contained more sugars than PNs [36]. The concept of “structural EPS” has been proposed and discussed in the literature [24,37]. The characteristics of this structural EPS, which was extracted from both aerobic and anaerobic AGS, and investigation of the behavior of the hydrogel that was formed, indicated that the structural EPS was a part of the TB-EPS in the AGS. A study by Zhang et al. [38] indicated that tyrosine, tryptophan, and aromatic protein-like substances present in TB-EPS played critical roles in the granulation process, as reflected by their strong correlation with particle size variation.

A study focused on targeting potential EPS producers in municipal wastewater sludge showed that, out of the EPS-producing bacterial strains that were isolated, eleven were *Bacillus* sp., three were *Serratia* sp., two were *Pseudomonas* sp., two were *Enterobacter* sp., two were *Yersinia* sp., two were *Microbacterium* sp., and one strain was from each of the genera *Pantoea*, *Photobacterium*, and *Pectobacterium*. The concentration of slime and capsular EPS produced by those strains ranged from 5.0 to 36.4 g/L [39]. Nohua et al. [40] conducted similar research and isolated eight EPS-producing bacterial strains from sludge from municipal WWTP including *Chryseobacterium gregarium*, *Staphylococcus epidermidis*, *Stenotrophomonas acidaminiphila*, *Brevibacillus parabrevis*, *Cloacibacterium normanense*, *Pseudomonas veronii*, *Acinetobacter soli*, and *Acinetobacter parvus*. EPS production by the different strains varied greatly, and *Cloacibacterium normanense* produced the largest amounts of

EPS (11.8 ± 1.2 g/L). The authors attributed the differences in EPS production to the genetic organization of the gene cluster involved in the EPS biosynthesis in the individual strains, and the differences in gene structure, regulatory elements, and sugar precursor biosynthesis. With regard to AGS, significant changes in the microbial structure were associated with granulation [41]. Cydzik-Kwiatkowska [42] reported that *Thauera* sp. and other Rhodocyclales-related bacteria, such as Xanthomonadaceae, Sphingomonadales, and Rhizobiales, mainly contributed to the production of EPS in mature AGS in a full-scale municipal wastewater treatment system. In a glycerol-driven partial-denitrification reactor, Saccharibacteria were enriched to over 60% and were believed to be responsible for granulation [38]. Observations of Paulo et al. [43] during the treatment of fish canning wastewater with variable levels of organic, nutrients, and salts showed that most of the bacteria in the AGS core microbiome were EPS producers (e.g., *Thauera* and *Paracoccus*) and their abundance increased at higher OLR loadings. A high microbial diversity of those producers ensured preservation of bacterial groups responsible for nutrients' removal.

Alginate (ALE) is one the most important components of EPS. This polymer has a high molecular weight and is composed of linear polysaccharides comprising β -D-mannuronic acid (M-block) and α -L-guluronic acid (G-block) blocks linked by 1–4 glycosidic bonds. The presence of G-blocks favors formation of gels in the presence of multivalent cations and affects such gel properties as swelling, stiffness, and porosity [44]. G blocks support interchain ion binding and formation of hydrogels; the multivalent cation creates interchain linkages by connecting two neighboring G-blocks or G-blocks in a second ALE chain. In contrast, MM and MG blocks in the ALE chain support chain elasticity and connect GG blocks, supporting gelation [45]. Schambeck et al. [21] observed that the hydrogel properties of ALE depended on the type of bioaggregate that was used. ALE extracted from AGS was more elastic and had stronger gelling properties than ALE isolated from flocs because the latter had a higher content of GG blocks.

Azotobacter vinelandii is a well-recognized bacteria that can produce ALE under environmental stress conditions. For example, during nitrogen starvation, *A. vinelandii* forms a dormant cyst and deposits a protective extracellular material composed primarily of ALE. The composition of ALE produced by *Azotobacter* sp. depends on the activity of a set of extracellularly produced and Ca^{2+} -dependent mannuronan C-5-epimerases. Each of these introduces a specific pattern and ratio of M and G residues [46]. Another bacteria capable of efficient ALE synthesis is *Pseudomonas aeruginosa*. Comparison of the properties of ALE produced by these two genera indicates that *P. aeruginosa* strains can secrete large amounts of ALE and form thick biofilms that lack GG-blocks [47], whereas *Azotobacter* sp. produce stiff ALE containing many GG-blocks, which is closely associated with the cell [48].

The quantity of EPS excreted by AGS (including ALE) is higher than that excreted by conventional activated sludge. Extractable ALE can comprise up to 25% of AGS [37]; extraction of ALE can, therefore, decrease the solids content of AGS and contribute to sludge management [49]. In a study by Cydzik-Kwiatkowska et al. [50], the content of ALE was about 184 mg/L of excess activated sludge, whereas the amount of ALE in granular sludge was over three times higher. The amount of ALE per g unit of biomass was over two-fold higher in AGS (86.0 ± 11.2 mg/g MLSS) than in activated sludge (49.0 ± 9.0 mg/g MLSS).

3. Methods of EPS Isolation

To draw conclusions about the role of the EPS matrix in the formation and action of biofilms, including AGS, credible methods of EPS extraction and characterization must be applied. Isolating, identifying, and characterizing biofilm EPS faces a number of obstacles, due to the complexity of the biofilm matrix and the need for EPS purification procedures. During EPS isolation from cell surfaces, the isolate may be contaminated as a result of cell lysis [51]. Isolation of EPS from culture supernatants does not solve the problem because the extracted polymers may have different properties than their cell-associated counterparts. Removal of contaminants from the isolated EPS can be accomplished using

chemical precipitation of contaminants, enzymatic digestion, precipitation of EPS, or chromatographic techniques [51]. High yields of gel-forming EPS can be extracted using the sodium carbonate method [52], but it is almost impossible to extract all components of EPS with a single procedure, so different isolation methods are often combined. Adav and Lee [53] compared several physical and chemical extraction methods and observed that the highest yield of EPS was obtained if ultrasound-formamide-NaOH treatment was applied. Felz et al. [37] extracted EPS using centrifugation, sonication, extraction in EDTA, formamide-sodium hydroxide extraction (NaOH), formaldehyde-NaOH extraction, and high-temperature sodium carbonate extraction (Na_2CO_3). Only EPS extracted from aerobic granules with Na_2CO_3 formed a drop-like shape during ALE gelation in a 2.5% (w/v) aqueous solution of CaCl_2 and formed stable hydrogel beads. Wang et al. [54] stress the role of EPS extraction methods on the secondary structure of extracellular proteins. They tested ten EPS extraction methods and concluded that the treatment of 0.5% Tween-20 for 4 h preserved the protein secondary structure, ensured a high EPS yield ($44.4 \pm 1.4 \text{ mg/g VSS}$), and limited the lysis of cells' anammox granules.

AGS may have diameters exceeding 1 mm, which limits the surface area for extraction. Therefore, sometimes, EPS isolation can be preceded by mechanical treatment. McSwain et al. [55] applied homogenization to support the release of EPS from the internal parts of granules and observed that the PN content in EPS increased from about 20 g/L in a non-homogenized sample to over 70 g/L in a homogenized sample. The first minute of homogenization is the most important because, during this time, EPS is released as a result of breaking down large particles and destruction of the three-dimensional EPS matrix [56]. It was also shown that homogenization intensity matters: the highest yields of EPS were obtained at the highest homogenization intensity applied [57].

For extraction of ALE-like exopolysaccharides from AGS in lab-scale reactors, Lin et al. [58] used a protocol used for ALE extraction from seaweed, which was based on the alkaline lysis method. In this method, sodium carbonate is added to the biomass to increase the pH, to solubilize exopolysaccharides/EPS. The mixture is homogenized and incubated at a high temperature. The centrifuged supernatant is adjusted to a low pH (2.2 to 2.0) to precipitate alginic acid. The collected alginic acid is dissolved in a NaOH or potassium hydroxide solution and finally precipitated in alcohol media (ethanol or isopropanol).

Figure 1 shows ALE extracted from AGS taken from the aeration tanks of full-scale WWTP in Poland. This WWTP operates at a low organic load (OLR) and now uses AGS technology to treat wastewater corresponding to a population equivalent of 15,000. The wastewater flow is about 3200 m^3/day and about 30–40% of the influent is wastewater from the dairy industry. The OLR of the WWTP is $0.77 \text{ m}^3/(\text{m}^3 \cdot \text{day})$ [59]. The sodium carbonate method was modified and optimized, and the average content of ALE in the AGS was 10% [57]. The extracted ALE had the same dark brown color as granular sludge. The ALE demonstrated the unique property of gelation with divalent cations over a wide range of temperatures and pH values, and ALE beads were successfully obtained (Figure 1). The excellent gelling ability of ALE indicates that it is important in the formation and structural stability of AGS.

Currently, it is stressed in the literature that, to better understand the role of EPS in biofilm formation, research should focus more on in-depth analysis of particular EPS components, not just the overall amount of EPS in biomass. Therefore, analytical methods like Fourier transformed infrared spectroscopy, high-pressure liquid chromatography, gas chromatography, or nuclear magnetic resonance are now commonly used for studying the material that comprises biofilm EPS [20]. Felz et al. [7] used isotope dilution mass spectrometry to study amino acids in EPS from AGS. Although a total of 14 amino acids were identified, including glycine, alanine, leucine, isoleucine, etc., the authors indicated that total amino acids comprised merely 1.5% of the structural EPS by weight. High throughput identification of EPS composition can also be achieved with quantitative proteomics. A study by Chen et al. [26] used this technique to indicate that, in anammox biofilm, the extracellular PN are mainly associated with the binding of multivalent cations.

Figure 1. ALE extracted from AGS from a full-scale WWTP.

4. Effect of Operational Parameters on Polymer Production and the Effect of Polymer Content on Wastewater Treatment and Sludge Management

EPS production and composition in the biomass in a wastewater treatment system depend on the substrate type and operational conditions, such as dissolved oxygen (DO), shear forces, OLR, hydraulic retention time (HRT), sludge retention time, growth stage, solution chemistry (ionic strength, pH, concentration of divalent cation) and the presence of toxic substances such as drugs and heavy metals [4,60].

The OLR is one of the most important of the parameters that affect the production and composition of EPS in AGS. Higher OLRs in the early granulation period promote an increase in EPS production and affect granule stability, which is related to the PN/PS ratio [61]. Kang and Yuan [62] cultivated granules at an OLR of 2.7 kg COD/(m³·day), then gradually reduced the OLR to 1.4 kg COD/(m³·day). At the lower OLR, the EPS content was about 150 mg/g VSS and the PN/PS ratio was about 25. Further lowering the OLR to 0.8 kg COD/(m³·day) decreased both the EPS content in biomass and the PN/PS ratio in EPS, which resulted in granule break-up. After acclimatization of the sludge to the lower OLR, the authors observed re-granulation, and both EPS production and the PN/PS ratio increased. The increase in EPS production was stimulated by a longer period of hunger in the reactor cycle, as the impact of all OLRs was tested at the same HRT. Similarly, Rusanowska et al. [4] reported that the highest EPS content occurred in the granules operated at the lowest OLR. The OLR also affects the distribution of EPS in AGS. Xu et al. [63] cultivated AGS using synthetic wastewater and increased the OLR over time by reducing the HRT. Those authors observed that, with an increased OLR, the LB-EPS content in the sludge increased, while the TB-EPS content remained almost unchanged. In a study on the treatment of landfill leachate in MBR reactors, an increase in OLR, resulting from raising the share of leachate in the influent from 75% to 100%, stimulated the production of PNs and Sol-EPS [64].

The ALE content in the biomass was positively correlated with the concentration of organics, especially volatile fatty acids, in wastewater [12]. AGS fed with acetate and propionate yielded significantly more ALE (up to 261 ± 33 mg VS_{ALE}/g VS_{sludge}) than sludge fed with wastewater containing no volatile fatty acids [21]. In reactors fed with wastewater containing volatile fatty acids in form of acetate and propionate, uronic sugars predominated among polysaccharides found in the ALE extracts.

Changes in COD/N ratios result in considerable variations in the functional groups and composition of EPS. In a study by Shi and Liu [65], two reactors were fed with wastewater with COD/N ratios of 100:5 and 100:10. At the lower COD/N ratio, granulation was faster and granules were stable. The characteristics of the EPS indicated that a larger content of polysaccharides, a greater content of β -sheets in the proteins, and reduced mineral surface adhesion and viscoelasticity benefited aerobic sludge granulation and granule maintenance. Decreasing the COD/N ratio from six to three during treatment of synthetic mariculture wastewater in a sequencing batch biofilm reactor decreased the PN and PS contents in LB-EPS and TB-EPS [66]. Decreasing the COD/N ratio from two to zero during the treatment of high-strength ammonia wastewater did not significantly affect the EPS contents in nitrifying sludge; however, the PS content was significantly larger than that of PN when the COD/N ratio was increased from two to ten. Tryptophan-containing PN predominated in the EPS, and humic-acid-like substances were abundant at both extremely low and high COD/N ratios [67].

EPS production is also associated with wastewater salinity [68,69]. At a high salinity (>20 mg NaCl/L), 90% of the EPS consisted of TB-EPS and the PN/PS ratio was high [69]. A sudden increase in salinity in the reactor and uncontrolled sludge retention time increased the content of LB-EPS [70,71], thus deteriorating the quality of the EPS matrix. In contrast, a lower SRT and gradual increases in salinity promoted formation of sludge with good physical properties and a stable PN/PS ratio. High salinity may affect the content of ALE in the biomass. For instance, in AGS fed with synthetic saline wastewater, ALE comprised only about 5% of VSS [72,73]. Treatment of low-strength organic saline wastewater by granular sludge can be supported by addition of Ca^{2+} , which accumulates in the halophilic biomass. Halophilic bacteria belonging to the family Flavobacteriaceae that predominated in the biomass secreted a lot of EPS that was mostly composed of proteins (about 70%) [28].

EPS secretion by biomass is affected by both DO concentration and aeration regimes in biological reactors. Liu et al. [74] observed that high DO leads to longer famine periods in batch reactors, and more EPS, especially PS, is consumed, decreasing the overall EPS content in the biomass. Kinyua et al. [75] reported that EPS production was stimulated by decreasing the length of the aeration period in the reactor cycle, which was associated with the release of Sol- and LB-EPS from TB-EPS. Zhang et al. [76] have shown that, in reactors with air recirculation, the production of ALE was higher in a reactor with DO over 8 mg/L than in reactors with DO at 5.13–6.52 or 3.48–4.53 mg/L. In the study of Wang et al. [77], moving bed biofilm reactors were operated with continuous aeration or intermittent aeration (20 min on/15 min off). In the reactor with continuous aeration, the greater microbial activity resulted in a larger sludge discharge and a higher biofilm detachment rate than in the one with intermittent aeration. In the reactor with intermittent aeration, the larger number of dead cells resulted in a higher ratio of humic substances to PS in EPS than in the reactor with continuous aeration.

During filamentous bulking, which is one of the most problematic phenomena in wastewater treatment, EPS may deteriorate sludge floc stability and structure. During sludge bulking, EPS content and the content of PN in EPS gradually decreased, and simultaneously, an increase in PS was observed. The number of PN associated with synthesis of hydrophobic amino acids decreased and the number associated with synthesis of hydrophilic amino acids increased [78].

Production of EPS can be impacted by the presence of pollutants, including micropollutants, in wastewater. It was reported that polystyrene nanoplastics inhibited the total production of EPS and PNs after 12 h exposure [79]. The presence of polystyrene nanoplastics at a concentration of 100 mg/L caused cellular oxidative stress and cell membrane damage. Accumulation of polypropylene microplastics in the range of 0.14 to 0.30 g/L in a membrane bioreactor did not affect the efficiency of removal of COD and ammonium but inhibited the growth of microorganisms, enhanced the secretion of EPS, and reduced microbial richness and diversity [80]. On the other hand, it was observed that nanoplastics released into an aquatic environment interacted with EPS. EPS biomolecules robustly

encapsulated nanoplastics to develop an eco-corona layer. This layer altered both the physicochemical properties of nanoplastics and also their bioreactivity, fate, and ecological impacts [81].

The presence of EPS and the fact that microorganisms grow in the form of a biofilm affect treatment efficiency. A high content of EPS in AGS protects microbial communities from stressful conditions. The presence of large amounts of EPS increased the bioavailability of anthracene, acenaphthene, fluorene, naphthalene, and a mixture of polycyclic aromatic hydrocarbons, as well as their biodegradation by *Klebsiella pneumoniae* [82]. Oliveira et al. [83] observed that increased production of EPS contributed to the robustness of AGS during a long-term exposure to 2-fluorophenol in saline wastewater. The EPS concentration in AGS decreased just after addition of 2-fluorophenol but then gradually recovered. The moderate salinity and presence of 2-fluorophenol in wastewater had a more detrimental effect on nutrients' removal than on production of EPS. Similarly, exposition of *Dictyosphaerium* sp. to nonylphenol caused overproduction of PS in Sol-EPS and of PNs in bound EPS, which reduced the toxicity of the pollutant. Tyrosine- and tryptophan-like substances were the main functional components in the PNs in EPS from nonylphenol-exposed cells [17].

EPS are critical for the resistance of biofilms to heavy metals and to the migration and transformation of heavy metals. In Sol-EPS, TB-EPS, cell walls, and membranes in *Pseudomonas putida* biofilms, 60–67% of the copper was located in the extracellular fraction of the biofilms, mostly in TB-EPS, while only 17.2–21.2% of copper was found in the intracellular fraction. The copper was primarily bound by carboxyl-, hydrosulfide-, and phosphate-like ligands within the EPS matrix, intracellular fraction, and cell walls and membranes, respectively [84].

EPS in AGS serve as the first barrier that prevents the direct contact of antibiotics with the cells, thus increasing the stability of the AGS. In experiments with tetracycline, the antibiotic interacted with PNs and humic acids in AGS-EPS by forming complexes via hydrogen bonds and van der Waals forces, which trapped the antibiotics. Nonetheless, concentrations of tetracycline that are too high may destabilize AGS due to the limited interaction sites that are available in the AGS-EPS [85]. The presence of antibiotics generally stimulates EPS production. In a sequencing batch reactor operated under norfloxacin stress, the PN and PS contents in LB-EPS and TB-EPS increased as the norfloxacin concentration in the wastewater increased [86]. Ciprofloxacin present in wastewater was mostly bound by tryptophan-like and tyrosine-like proteins in EPS, but addition of Ca^{2+} into EPS and ciprofloxacin binding systems caused release of ciprofloxacin from the sludge as a result of cation competition for CO groups in amide I [87]. EPS-associated antibiotic resistance genes (ARGs) are a source of extracellular ARGs, and they may play an important role in horizontal gene transfer in WWTPs. In activated sludge sampled from four WWTPs, typical ARGs (*sulI*, *sulII*, *blaTEM-1*, *tetO*, *tetA*, *tetQ*, *tetW*) and class I integron (*intI1*) were quantified in EPS-associated, intracellular, and cell-free DNA. The absolute abundances of EPS-associated ARGs were 0.2–4.6 orders of magnitude higher than the abundances of corresponding ARGs in the cell-free DNA [88].

The reduced biodegradability of AGS in comparison to that of activated sludge, resulting from the high EPS content of the AGS, severely affects the management of excess sludge. It was shown that the biochemical methane potential of AGS from laboratory and full-scale municipal wastewater treatment systems was lower than that of waste activated sludge [89]. EPS in AGS possess anion-repelling and cation-binding properties [90]. This impacts downstream processing of waste sludge because ions that are preferentially transported by EPS are also more toxic for methanogenic cells, which may inhibit biogas production. Some minerals, such as magnetite, are known to improve the anaerobic digestion of organic wastes. The latest findings indicate that interspecies electron transfer promoted by magnetite was a result of magnetite-stimulated secretion of EPS containing redox-active organic functional groups [91].

The dewaterability of waste sludge strongly depends on EPS content and composition. Decomposition of sludge EPS during a five-day denitrification process triggered by nitrate

supply released the bound water and improved the filterability of the sludge [92]. Application of an ultrasound-activated persulfate oxidation efficiently degraded the gel-like EPS matrix and attacked cells. As a result, the moisture that was trapped in cells and EPS was released [93]. A mechanism for improving dewaterability of AGS by Fe(II) activated peroxydisulfate conditioning was proposed by Ding et al. [94]. $\text{SO}_4^-/\text{OH}^-$ radicals destroyed the structure of EPS and cells, and the bound water was released from the AGS. The Fe(III) that was generated decreased the electrostatic repulsion and facilitated the re-flocculation of sludge. Regarding EPS composition, it was reported that PNs were the primary component in the AGS, and that changes in PNs in TB-EPS during conditioning and granulation were associated with changes in sludge dewaterability [94]. It was observed that sludge dewaterability was related to the composition of the amino acids in EPS. The presence of glycine, serine, and threonine in EPS resulted in highly repulsive hydrophilic interactions, which reduced sludge dewaterability. In sludge that contained these amino acids, hydrophilic CO and C–OH functional groups were found to be more prevalent [95]. The dewatering performance of sludge can be increased by bioleaching. As shown by Li [96], the application of different DO concentrations decreased the content of PN in TB-EPS, thus improving sludge dewatering. Increased DO favored the growth of the genera *Acidithiobacillus*, *Metallibacterium*, *Alicyclobacillus*, *Acidibacter*, *Acidocella*, and *Luteococcus*, which played important roles in EPS biodegradation.

5. Possibilities for Utilizing Granule-Derived Polymers

WWTPs produce a constant stream of excess sludge, and recovery of resources from this stream may significantly reduce the generation of waste, energy consumption, and greenhouse gases' emissions. WWTPs converted into facilities for recovery of resources will constitute a net source of valuable bioproducts, such as biopolymers, cellulose, phosphorus, or biogas [97]. Recovery of polymers from waste AGS would improve the economics of sewage treatment plants, especially taking into account the increasing number of WWTPs operated with AGS technology [98]. Moreover, even if activated sludge has a lower polymer content than AGS [21], recovery of biopolymers from activated sludge can still be beneficial, due to the popularity of these systems. Recovery of polymers may also improve sludge management. The cost of sewage sludge management accounts for about 50% of wastewater treatment cost [99]. Extraction of polymers from excess AGS reduces the amount of sludge that must be disposed of by 20–35% [100]. Removal of ALE prior anaerobic digestion can also act as a pre-treatment method to increase the rate of biodegradation of sludge [101]. Information on the possibility of reusing polymers obtained from waste sludge for different applications is presented in Figure 2.

5.1. Coatings, Agricultural Agents, Flame-Retardants

Polymers extracted from AGS appear in fibrous, gel, liquid, or foam forms. Polymer fibers can be used for the production of absorbing gels, tissues, or can be used as a gluing agent during fertilizer pellets' production. The liquid form can be used to thicken inks or improve paper quality, while the foam form of polymers is used to produce fire-resistant boards [102]. AGS-extracted biodegradable PS can be easily drawn into a homogeneous and flexible film that enhances the water resistance of the paper. As reported by Lin et al. [103], application of 5% (*w/v*) water solution of PS-based biomaterial provides the same water-resistant property to paper as the commercially used alkenyl succinic anhydride. AGS-derived polymers are used as a coating for concrete, which limits concrete from drying out during curing and prevents tearing.

Biopolymers derived from excessive AGS can be used in the agricultural sector as a coating for seeds, ensuring young plants can develop faster and are less vulnerable to diseases [100]. Moreover, they may be used to improve water retention in semi-arid areas. Go et al. [104] indicated that biopolymers isolated from excess sludge can be applied as corrosion inhibitors. EPS adsorbed on the metal surface act like a protective barrier to

isolate the metal from a corrosive environment. The main advantage of such an approach is that EPS-derived corrosion inhibitors are biodegradable and free from heavy metal.

Figure 2. Possibility of reusing polymers extracted from waste sludge from a WWTP.

There is a potential to use EPS for the development of bio-inspired high-performance flame-retardant materials. EPS extracted from activated sludge and AGS were tested as bio-based flame retardant materials. Biopolymers were used to coat flax fabric and its flammability was evaluated. Both types of EPS enabled the fabrics to achieve the self-extinguishment due to an effective formation of char, however, only samples coated with AGS-extracted EPS met the requirements for the aircraft interior [105]. EPS can also be used to decrease the flammability of synthetic polymers. Incorporation of EPS recovered from excess AGS into poly (vinyl alcohol) reduced the rate of heat release and CO emission of EPS/poly (vinyl alcohol) [106].

5.2. Sorption, Ion Exchange, Immobilization

Wastewater-produced EPS can be an attractive biosorbent for heavy metal removal and recovery. EPS isolated from the strain *Parapedobacter* sp. ISTM3 was used to remove heavy metals (Zn^{2+} , Cu^{2+} , Pb^{2+} , Cr^{6+} , Fe^{2+} , and Cd^{2+}). EPS showed the highest removal efficiency and metal adsorption capability for Cr^{6+} , and the Langmuir model was found to best fit Cr^{6+} adsorption by EPS, with a maximum adsorption capacity of 33.783 mg/g [107]. EPS extracted from nitrogen-limited glycerol/ethanol-rich wastewater were used to recover Pb^{2+} and Cu^{2+} from aqueous solutions. Flow-through tests carried out in columns packed with polyethyleneimine-coated silica gel, to which EPS were irreversibly attached, showed that immobilized EPS excellently adsorbed Cu^{2+} and Pb^{2+} . Desorption with 0.1M HCl ensured average recoveries 86% and 90% for Cu^{2+} and Pb^{2+} , respectively. For Cu^{2+} , five adsorption-desorption cycles were conducted with no reduction in the amount of adsorbed metal at the breakthrough point. The major mechanism responsible for ion removal by EPS was ion exchange [108].

EPS can be used to reduce such metal ions as Ag(I) or Au(III) to corresponding metal nanoparticles (MNPs). Experiments with EPS from *Shewanella oneidensis* MR-1 showed that EPS with a low molecular weight (<3 kDa) was the major reducing agent but the fraction itself could not convert a high concentration (>25 mg/L) of Au(III) to stable AuNPs. On the other hand, EPS with high molecular weights (>50 kDa) acted as coating reagents, increasing the stability of the AuNPs with sizes of 20–50 nm, but were characterized by a low Au(III)-reducing activity [109]. EPS extracts can also be successfully used to immobilize

oxyanions. In the study of Wang [110], ClO_4^- oxyanions were trapped in 20 min by freely dissolved EPS extracted from *Bacillus subtilis*, but chemical reduction of ClO_4^- was not observed in 48 h.

The ion exchange properties of EPS were also investigated by Sudmalis et al. [90]. EPS were entangled with an inert binder (poly (vinylidene fluoride-co-hexafluoropropylene)) and a membrane was formed. This membrane was used in an electrodialysis cell. For a mixture of NaCl and KCl salts, the EPS membrane acted as a cation exchange membrane (CEM) with a current efficiency of ~80%. The membrane was characterized by a higher selectivity for transport of K^+ compared to Na^+ (separation factor SK^+/Na^+ of 1.3). These properties were compared to a membrane from a model EPS (ALE) and a commercial CEM. The ALE membrane had a similar current efficiency (~80%), but a higher SK^+/Na^+ of 1.9. The commercial CEM did not show selectivity towards Na^+ or K^+ . The selectivity of EPS and ALE towards K^+ transport can be applied for ion separation from water streams.

EPS recovered from AGS may also be used as a natural carrier to immobilize a specific microbial strain important for pollutant degradation. For example, *Rhodococcus* sp. FP1, able to degrade 2-fluorophenol, was immobilized in EPS and introduced to an AGS reactor fed with saline wastewater containing 2-fluorophenol. Nutrient removal was impaired by 2-fluorophenol load, but bioaugmentation improved the phosphate and ammonium removal efficiencies. Moreover, bioaugmentation resulted in horizontal gene transfer because many bacterial strains isolated from granules were able to degrade 2-fluorophenol [83].

5.3. Re-Use in WWTP

EPS isolated from waste sludge can be re-used directly in WWTP. Hu et al. [111] investigated the potential of EPS as natural redox mediators improving methanogenesis. Artificial redox mediators improve the electron transfer efficiency during sludge methanogenesis but they are expensive and often poorly biodegradable. Addition of 0.50 g EPS/L during methanogenesis increased the methane yield due to increased activity of acetotrophic and hydrogenotrophic methanogens, shortened the duration of the lag phase, and enhanced the sludge dewaterability. After EPS addition, the charge transfer resistance decreased and the efficiency of extracellular electron transfer was enhanced. EPS can also be used for preservation of granules to support the rapid start-up of reactors and improve the treatment stability. Zhang et al. [112] observed that EPS isolated from anammox sludge added to anammox granules stored at 4 °C enabled highly specific anammox activity and nitrogen removal efficiency to be preserved. Cunha et al. [113] indicated the potential of the microalgal-based EPS to replace hazardous synthetic flocculants used in wastewater treatment and to remove nano- and microplastic from wastewater.

5.4. ALE

Most commonly, ALE is derived from such genera of seaweed as *Laminaria*, *Macrocystis*, and *Ascophyllum*. This ALE is commonly used to thicken food products, for production of medicines, immobilization of biocatalysts such as enzymes, or as a gelling agent in cosmetics [98,114]. Since the price of seaweed-derived ALE is high, the recovery of ALE from waste sludge is an attractive option. AGS contains up to 10–20% of ALE; therefore, ALE is now recognized as one of the most promising bioproducts that can be recovered from WWTP [115,116]. In a field test on ALE recovery from waste AGS, it was demonstrated that 18 kg of ALE was produced from 80 kg of AGS [102]. In the Netherlands, it is planned that by 2030 about 85 ktons of ALE will be recovered from 10 different WWTP, generating 170 million euros [117].

The ALE extracted from AGS can form beads that can be used as sorbing agents. In the study of Ladnorg et al. [118], ALE, which accounted for about 58% of the total EPS, was used for sorption of methylene blue. The removal efficiency of dye increased with increasing initial concentrations of ALE, and the pseudo-first order model best described the kinetic behavior of the adsorption process. Increasing the temperature of the adsorption process to 35 °C increased the removal efficiency to 69%; however, at higher temperatures,

the removal efficiency decreased significantly. Similar research was conducted for sorption of cadmium from an aqueous solution. The use of AGS-extracted ALE ensured that the sorption efficiency was close to 90%, which was only slightly lower than the efficiency observed for a commercial ALE (data not published). Knowledge of the composition and hydrogel properties of ALE is necessary to develop ALE applications in the industry, for instance, in the chemical fields or as a rheology conditioner/emulsion stabilizer [13].

6. Conclusions

Biopolymer recovery from excess AGS provides a valuable source of biomaterial, increases the sustainability of wastewater treatment and reduces the amount of sludge that requires management. Challenges that must be faced include optimization of extraction methods to obtain biopolymers with well-defined characteristics, and operation of reactors in such a way that both the treatment efficiency and polymer content in excess sludge are high. The latest ideas for the use of biopolymers that are presented in this review article open a huge field for the development of new technologies based on waste-extracted EPS.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Conflicts of Interest: The author declares no conflict of interest.

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