Biopolymers in Aerobic Granular Sludge

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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 many full-scale plants in locations around the world. 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 extracellular polymeric substances (EPS) alginate (ALE) EPS composition

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 entry 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].

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 [23]. During granulation, an increase in the concentration of acyl-homoserine lactones (AHL) was observed [24]. 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 Mn2+ 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. [25]. 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 [26].

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 [27]; extraction of ALE can, therefore, decrease the solids content of AGS and contribute to sludge management [28]. In a study by Cydzik-Kwiatkowska et al. [29], 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 [30]. 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 [30]. High yields of gel-forming EPS can be extracted using the sodium carbonate method [31], 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 [32] 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. [27] 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. [33] 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 ± 1.4 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. [34] 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 [35]. It was also shown that homogenization intensity matters: the highest yields of EPS were obtained at the highest homogenization intensity applied [36].

For extraction of ALE-like exopolysaccharides from AGS in lab-scale reactors, Lin et al. [37] 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).

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. [38] used this technique to indicate that, in anammox biofilm, the extracellular PN are mainly associated with the binding of multivalent cations.

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][39].

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 [40].

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 [41]. EPS in AGS possess anion-repelling and cation-binding properties [42]. 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 [43].

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 [44]. 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 [45]. A mechanism for improving dewaterability of AGS by Fe(II) activated peroxydisulfate conditioning was proposed by Ding et al. [46]. SO4 − /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 [46]. 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 [47]. The dewatering performance of sludge can be increased by bioleaching. As shown by Li [48], 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.

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