Optimization of alginate recovery from activated sludge for waste sludge valorization

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Introduction Microorganisms responsible for aerobic wastewater treatment tend to aggregate by forming flocs, biofilms, and granules thanks to extracellular polymeric substances (EPS), which may comprise up to 80% of sludge mass (Tian et al. 2006). EPS consist of proteins, polysaccharides, glycoproteins, lipids, and humic and nucleic acids. The content of EPS determines the physical and chemical properties of biomass, including hydrophobicity, and the polysaccharide/protein ratio influences the settling properties of the sludge (Tu et al. 2012). Organic substances that are stored in EPS can be hydrolyzed into monomers that are easily transported in the structure of the biomass and provide a source of nutrients during starvation. The excessive sludge produced in wastewater treatment processes is considered to be waste, and the cost of handling/disposal of the waste sludge represents up to 50% of the wastewater treatment costs (Kroiss 2004). If valuable chemicals can be recovered from the waste activated sludge (WAS), the sustainability, and economics of wastewater treatment can be substantially increased. WAS can be a source of polysaccharides which are part of EPS, and can constitute more than 10% of the biomass (Adav et al. 2008). Isolated polysaccharides resemble commercially available alginate (Lin et al. 2015), which has unique gel-forming properties and is commonly secreted by bacteria belonging e.g. to the genera *Pseudomonas* and *Acetobacter*. Information about alginate-like polymers (ALP) synthesis in activated sludge in full-scale wastewater treatment plants (WWTPs) is limited. The hydrodynamic conditions in a full-scale reactor will be different than in a laboratory-scale reactor, which will translate into a different production and content of ALP in the biomass. In this study, the recovery of ALP from WAS was optimized and the obtained ALP were characterized. The proposed approach is in line with the trends of using waste as a source of bioproducts that is the basis of the modern bioeconomy.

Materials and methods The activated sludge was collected from a full-scale WWTP in Poznań, Polska (52.4493_N, 16.9826_ E) treating municipal wastewater (averaged 3 million m^3/month). The composition of wastewater averaged: 1322.2 ± 132.3 mg COD/L, 607.4 ± 79.1 mg BOD_5/L, 632.6 ± 117.2 mg TSS/L, 112.6 ± 10.5 mg N_{tot}/L, 63.6 ± 7.4 mg N-NH_4/L, 16.3 ± 2.5 mg P_{tot}/L. The technical system of the sewage treatment plant consists of a mechanical and a biological part and ensures efficient C, P, and N removal. In the biological part, 6 biological treatment lines are operated in parallel. In each treatment line, wastewater is first fed into the anaerobic chamber and then into the aerobic chamber. In the aerobic chambers, the oxygen content is kept between 0.8 and 2.2 mg/L depending on the ammonium concentration. The organic loading rate in biological reactors was at a level of 0.11-0.13 kg COD/(kg MLSS·d). Activated sludge samples (4 L) were collected in about 2-month intervals from three identically operated aerobic chambers in the period from June 2021 to March 2022. After sampling, activated sludge was immediately transported to the laboratory and ALP isolation was conducted.

The modified protocol proposed by Lin et. al (2008) was used for ALP isolation. To optimize the extraction of ALE, a series of extraction experiments were conducted changing the time of sludge homogenization (2 min, 4 min, 10 min), time of extraction with sodium carbonate (15 min, 30 min, 1 h, 2 h, 3 h), the temperature of extraction (60°C, 70°C, 80°C) and the amount of sludge (1 g WAS, 2 g WAS, 3 g WAS). The best protocol in terms of the ALE yield was selected. The UV-visible spectroscopy (UV-visible Spectrophotometer, Varian) was applied to compare pure sodium alginate (Sigma-Aldrich) with ALP extracted from WAS. Staining and microscope imaging of activated sludge was performed based on Chen et al. (2007) to probe α-polysaccharides and β-polysaccharides, respectively. After staining, sludge samples were cryosectioned (Shandon Cryomatix, Thermo Scientific) into 20 µm sections and examined with a Leica TCS SP5 confocal scanning laser microscope.

Results and discussion After optimizing the procedure, the highest yield of ALP was obtained with the following protocol: 2 g of WAS, 4 min of homogenization at 9.500 rpm, 1 h extraction at 70°C using 100 mL of the 0.2 M Na_2CO_3. The average recovery of ALP in the investigated period varied from about 41 mg ALP/g MLSS in July 2021 to about 63 mg ALP/g MLSS in February 2022 (Fig. 1). For comparison, in aerobic granular sludge, which is by definition richer in polymers than activated sludge, the concentration of ALP monitored for over 1 year of stable performance of full-scale batch reactors at a municipal WWTP mostly varied between 60 and 100 mg/g MLSS. Similar to this study, the highest values for granules were reported in the transition periods from winter to spring (Cydzik-Kwiatkowska et al. 2022). ALP was mainly located in the internal parts of activated sludge flocs (Fig. 1).

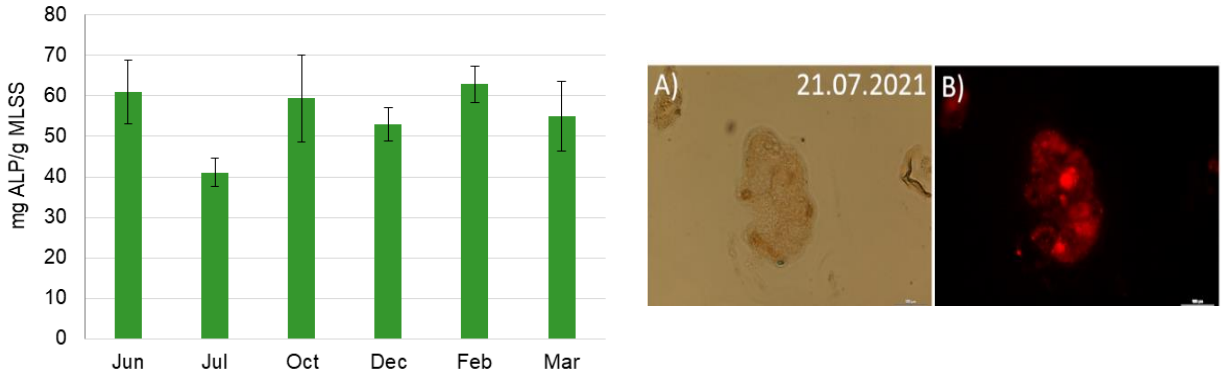

Fig. 1 ALP recovery from activated sludge from Jun 2021 to March 2022 (bar graph, n = 3) and visualization of the polysaccharides (B) in activated sludge flock in comparison to flock visualized with visible light (A)

UV-visible spectroscopy was applied to compare pure sodium alginate (Sigma-Aldrich) with ALP extracted from WAS. ALP showed an extra peak in absorbance at ~270 nm wavelength (Fig. 2). It indicated that ALP was not uniform i.e., contained a mixture of chemically variable ALE or contained impurities such as humic substances (HS) which can also be deduced from the brownish color of the isolated ALP (Fig. 2). Studies by Tang et al. (2020) indicate that HS were one the major active components of highly cross-linked structures in EPS isolated from dewatered sludge and aided in the energy metabolism of microorganisms involved in methanogenesis.

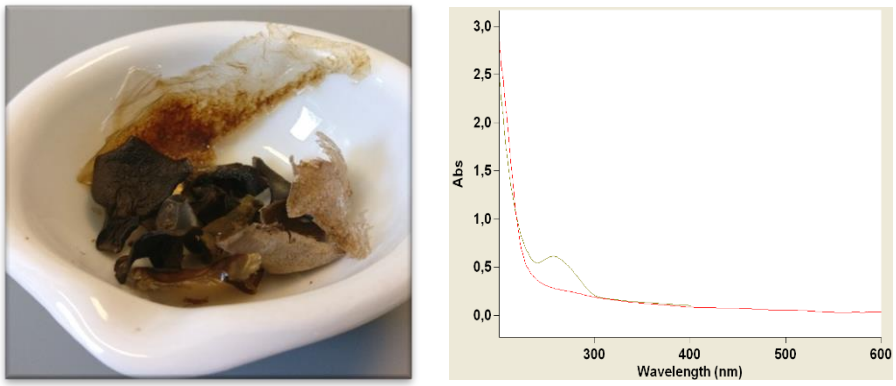

Fig. 2 Lyophilized ALP isolated from activated sludge (left) and UV-visible spectroscopy of pure sodium alginate (red line) and ALP extracted from WAS (green line) (right)

Conclusions The procedure of ALP isolation from activated sludge from a full-scale wastewater treatment plant was optimized and the characteristics of recovered ALP were investigated. The average ALP yield was 50 mg ALP/g MLSS. The recovered ALP was not chemically uniform therefore it is more suitable to use them in processes that do not require a strictly defined chemical composition of biopolymers, e.g. as soil amendments to improve water retention in semi-arid areas, fertilizer pellets, or seed or concrete coatings.

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