# 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. EPS content determines biomass's physical and chemical properties, 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 cost of handling/disposal of the waste activated sludge (WAS) produced during wastewater treatment represents up to 50% of the wastewater treatment costs (Kroiss 2004).

If valuable chemicals can be recovered from WAS, the sustainability, and economics of wastewater treatment can be increased. WAS can be a source of polysaccharides which can constitute more than 10% of the biomass (Adav et al. 2008). Isolated polysaccharides resemble commercially available alginate, which has unique gel-forming properties and is commonly secreted by *Pseudomonas* sp. and *Acetobacter* sp. 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 installations, 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 & Methods

WAS was collected from a full-scale WWTP in Poznań (Polska) treating municipal wastewater (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 treatment line consists of a mechanical and a biological part. In the biological part, 6 biological reactors are operated in paralel at the organic loading rate of 0.11-0.13 kg COD/(kg MLSS·d). Activated sludge samples (4 L) were collected in 2-month intervals from three identically operated aerobic reactors from June 2021 to March 2022.

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 UV-visible spectroscopy (UV-visible Spectrophotometer, Varian) was applied to compare pure sodium alginate (Sigma-Aldrich) with ALP extracted from WAS. To probe polysaccharides, staining and microscope imaging of WAS was performed (Chen et al. 2007).

## Results & Discussion

After optimizing the procedure, the highest yield of ALP was obtained from activated sludge with the following protocol: 2 g of WAS, 4 min of homogenization at 9.500 rpm, and 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.

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. 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. Excess sludge-derived biopolymers could potentially be used in the chemical sector, and the paper and textile industries. However, their complex composition restricts their range of application. Therefore, it is more suitable to use them in processes that do not require a strictly defined chemical composition of biopolymers (Van der Roest et al. 2015).

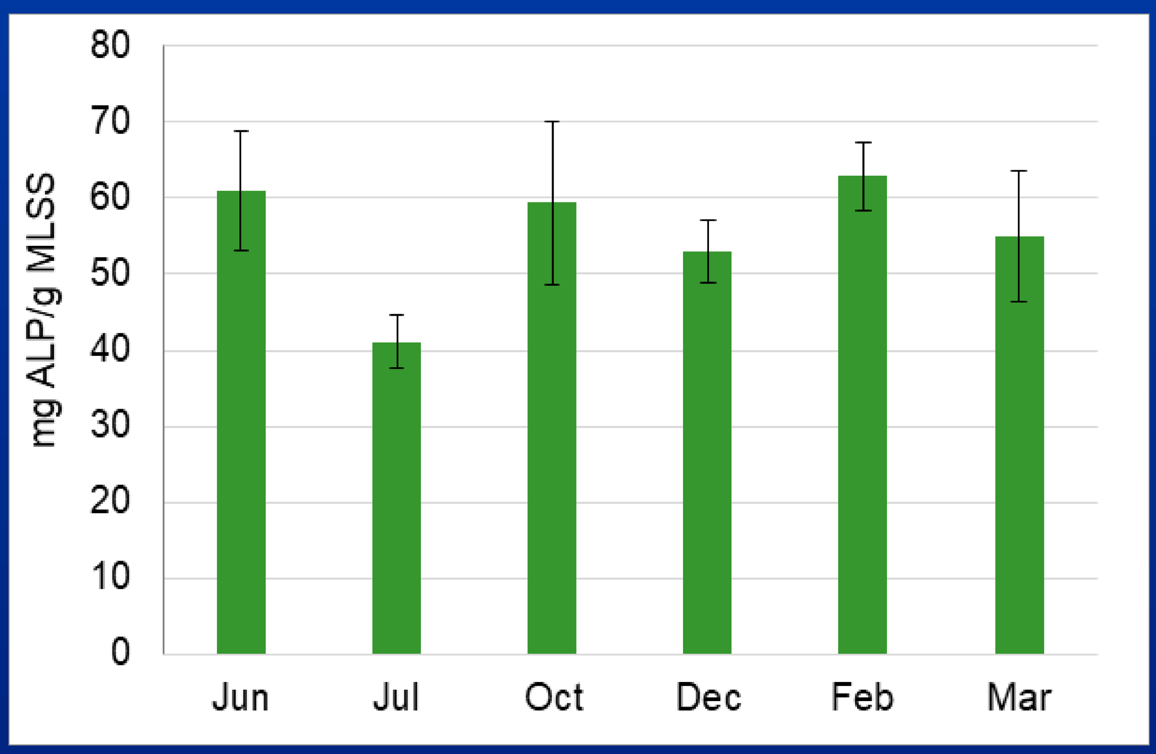


Figure 1: ALP recovery from activated sludge from Jun 2021 to March 2022 (bar graph, n = 3)

Figure 2: Lyophilized ALP isolated from activated sludge

## 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 from activated sludge during a year of WWTP operation 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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