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# Extraction of extracellular polymeric substances of activated sludge and their application for wastewater treatment

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**Abstract.** Extracellular polymeric substances (EPS) are efficient and environmentally friendly biofloculants in wastewater pollution that are free from the shortcomings typical of traditional coagulants and flocculants which can pose direct threat to human health and life as well as to the environment. In this paper, the authors have investigated the possibilities of extracting EPS from excess activated sludge and subsequent use of the obtained fractions as reagents for wastewater treatment. The study analyzes the existing methods for extracting EPS, with further selection and implementation of four biopolymer extraction methods. As part of the work, soluble, loosely bound and tightly bound activated sludge EPS fractions were generated using each method. The study of EPS chemical composition demonstrated a significant difference in the obtained fractions from each other; moreover, all the fractions showed the prevailing content of EPS protein components over polysaccharides and humic acids. The most efficient method for extracting proteins from the excess activated sludge biomass is the  $\text{NH}_4\text{OH}/\text{EDTA}$  method; the one for extracting polysaccharides – the  $\text{HCHO}/\text{NaOH}$  method, and the one for extraction of humic acids – the  $\text{CH}_3\text{NO}/\text{EDTA}$  method. The study of efficiency of wastewater treatment with the use of obtained fractions has shown that EPS extraction method has an impact not only on the extraction performance, but also on the feasibility of using extracted biopolymers for wastewater treatment. The efficiency of wastewater treatment ranged from 0.2 to 62.6%, depending on the EPS fraction used as a reagent.

## 1. Introduction

EPS is a complex mixture of high-molecular polymers secreted by activated sludge microorganisms in the process of metabolism [1]. EPS represents a biopolymeric mucous substance in the form of viscous colloidal solution consisting of various types of organic macromolecules, such as polysaccharides, proteins, nucleic acids, phospholipids and other polymeric compounds [1-5].

The use of extracellular polymeric substances in various fields has been increasing lately. Owing to their unique properties, EPS are now used in food industry as food supplements with gelling and stabilizing properties and also for cooking dietary products [6, 7]. These biopolymers are also used in pharmaceutical industry as probiotics, oral agents (capsules, powders) and local-action preparations (ointments, gels, liniments). In cosmetology, biopolymers are included in the compositions of sunscreen creams, lotions, gels, shampoos. In agriculture, they are used in fertilizers, supplementary feeds. In oil-producing industry, EPS can be used to make drill fluids and to increase the performance of shelf oil extraction; in other industries - as surfactants and emulsifiers [1, 2, 8]. It should be noted that such extensive prospects for the use of extracellular microbial polymers are conditioned not only



by the broad range of their physicochemical properties, but also by the variety of possible forms of their use: powder, fibre, film, granules, highly viscous solutions.

One of the most relevant areas for the use of extracellular polymeric substances is environmental protection, in particular, their use for treatment of drinking and waste water. The need to use biopolymers is caused by the search for safer ways of water purification. The traditionally used reagents, iron and aluminum salts, synthetic polymer flocculants have significant drawbacks. For instance, ions of heavy metals in the composition of water purification reagents are toxic and can pose direct threat to human health and life [9, 10]. Synthetic flocculants, in turn, produce toxic monomers that penetrate into purified water and the sediment, limiting their suitability for further use [11, 12]. At the same time, the main advantage of using EPS in wastewater treatment is, along with high purification efficiency, avoidance of secondary pollution of the treated water and ecological purity of sediments resulting from such treatment [1, 13, 14, 15].

Recently, research of EPS extraction methods is abundant; most of the papers focus on obtaining EPS from the biomass of specially cultivated strains of microorganisms [1, 6, 16]. However, the use of artificially cultivated strains is expensive, energy-consuming and generally not lucrative, while there exists an insufficiently used resource - excess activated sludge. Activated sludge is a community of microorganisms of various taxonomic groups, including those producing extracellular polymeric substance for own sustainment, used in aerobic wastewater treatment facilities [17]. In the process of wastewater biological treatment, a large amount of excess activated sludge is formed as a result of transformation of the original pollutants into active biomass. At the same time, millions of tons of excess activated sludge get accumulated annually in the form of waste; its processing and utilization is more laborious than the process of water purification [17, 18, 19]. Thus, excess activated sludge is a natural heavy-tonnage resource the production of which does not require additional costs and energy. Consequently, the research of potential of extracting EPS from excess activated sludge, the study of their characteristics and properties with regard to subsequent use in wastewater treatment is a promising area.

A number of studies have substantiated the feasibility and benefits of using activated sludge biopolymers as a perspective biodegradable flocculent additive for wastewater treatment which does not lead to secondary pollution of the treated water and the environment, unlike synthetic flocculants and reagents [1, 20].

A number of experiments were conducted within the framework of the presented research, which confirmed the prospects of extracting EPS from the micro-organic biomass of excess activated sludge and their subsequent use as wastewater pollution bioflocculants.

## 2. Materials and methods

A number of excess activated sludge samples were selected from the secondary settling tanks at the biological treatment facilities of the State Regional Unitary Enterprise Murmanskvodokanal in the urban-type settlement of Murmashi, Murmansk Region, Russia. The collected samples were transported to the laboratory within 2 hours after sampling at the temperature 4°C. The hydrochemical parameterization of the activated sludge was made in accordance with [21]. The main properties of sludge water are given in Table 1.

Table 1. Characteristics of activated sludge

Sample	Suspended Solids, g/l	Sludge Volume Index	Settled Sludge Volume, ml/l	pH	Temperature, °C
Activated sludge	1,6	107	150	7,0	6,0

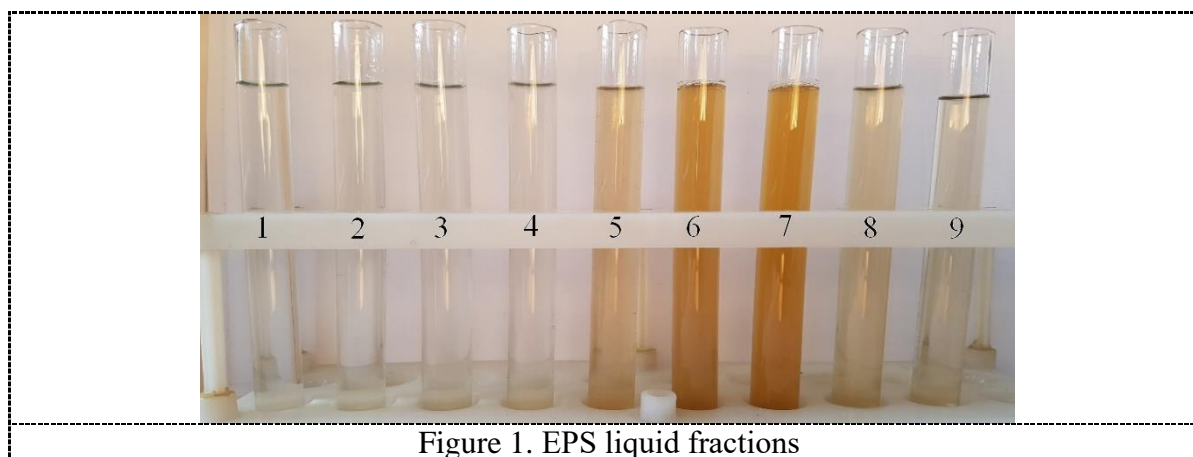
The determination of suspended matter content was made in accordance with [22]. The determination of dry residue content was made in accordance with [23]. The determination of protein content was made according to the Lowry method, with bovine albumin serum as a reference [24]. The determination of carbohydrate content in the obtained fractions was made using the phenol-sulfuric method, with glucose as a reference [25]. The determination of humic acids in the obtained fractions was made using the modified Tyurin method [26]. In addition to the indicators common to the Russian sources, the characteristics accepted in the English-language literature and research were determined and evaluated in accordance with «Standard Methods for the Examination of Water and Wastewater» [27]. The analysis of efficiency of using EPS for wastewater treatment was made using the Couran's method [28]. The analysis of chemical consumption of oxygen was made in accordance with [29]. The analysis of biological consumption of oxygen was carried out in accordance with [30].

Currently, the relevant literature and patent documents contain disparate information about the methods for extracting EPS from micro-organic biomass. In the course of the study, the existing technologies were analyzed, with further selection and implementation of four methods for extracting biopolymers, which were designated in accordance with the main reagents used in chemical processing [4, 31, 32]. EPS extraction from the excess activated sludge biomass was made using the following methods: the HCHO/NaOH method [4], the CH<sub>3</sub>NO/NaOH and CH<sub>3</sub>NO/EDTA methods [31], the NH<sub>4</sub>OH/ EDTA method [32].

EPS extraction using each of the above methods supposed, inter alia, distinguishing three fractions differing in the extent of binding with the cells of activated sludge microorganisms, according to [33]: soluble EPS, loosely bound EPS and tightly bound EPS.

### 3. Results and discussion

Following the experiment results, 9 samples of EPS were generated (in liquid form and after lyophilisation), representing different fractions of EPS obtained by different methods (Figures 1 and 2).



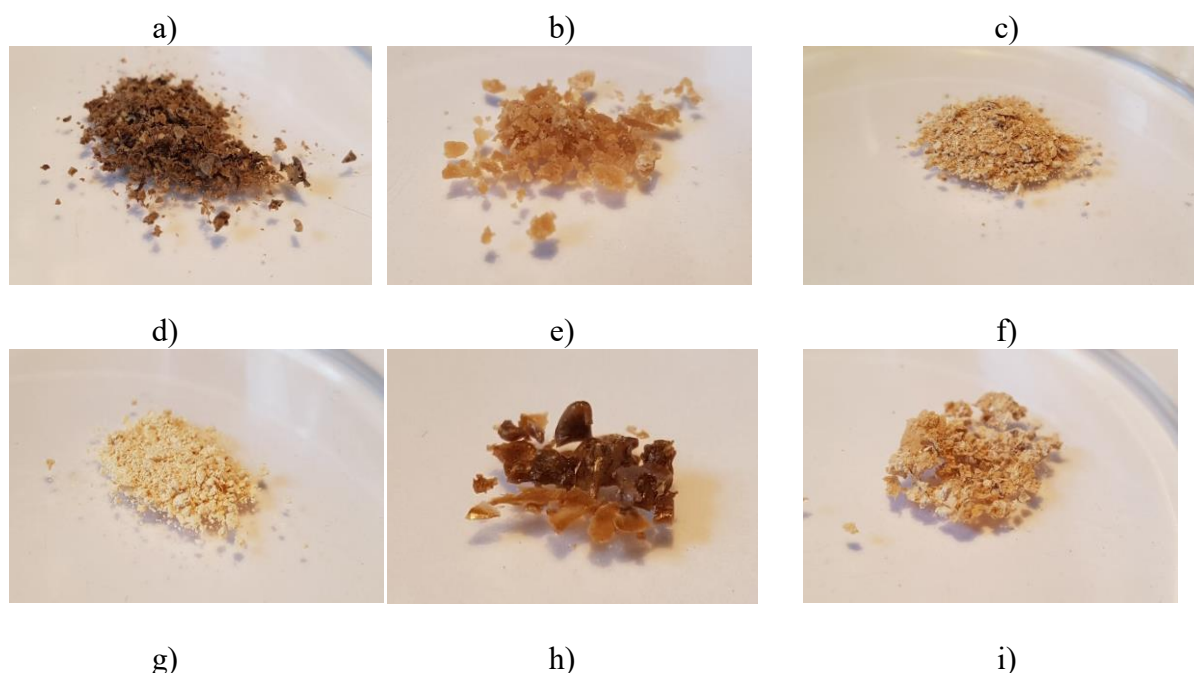


Figure 2. EPS Lyophilized Fractions: (a) the HCHO/NaOH and  $\text{NH}_4\text{OH}$ /EDTA methods, unbound ESP fraction; (b) the  $\text{CH}_3\text{NO}$ /NaOH and  $\text{CH}_3\text{NO}$ /EDTA methods, unbound ESP fraction; (c) the HCHO/NaOH method, loosely bound EPS fraction; (d) the  $\text{CH}_3\text{NO}$ /NaOH and  $\text{CH}_3\text{NO}$ /EDTA methods, loosely bound EPS fraction; (e) the  $\text{NH}_4\text{OH}$ /EDTA method, loosely bound EPS fraction; (f) the HCHO/NaOH method, tightly bound EPS fraction; (g) the  $\text{CH}_3\text{NO}$ /NaOH method, tightly bound EPS fraction; (i) the  $\text{NH}_4\text{OH}$ /EDTA method, tightly bound EPS fraction

The study of the chemical composition of the generated EPS (Table 2) showed significant difference between the obtained fractions. The soluble EPS differed insignificantly, mostly by comparable content of polysaccharides and humic acids the amount of which varied depending on the extraction method from 13.21 to 19.22 mg/l and from 16.03 to 20.68 mg/l respectively. The protein substances content was in the range from 38.26 to 45.49 mg/l. The protein content in the loosely bound EPS fractions varied significantly depending on the used extraction methods and ranged from 63.45 to 125.58 mg/l. The content of humic acids and polysaccharides slightly varied from method to method and was in the range from 35.41 to 43.17 mg/l and from 21.33 to 36.52 mg/l respectively.

At the same time, the tightly bound EPS showed high protein content regardless of the extraction method – from 459.37 to 508.71 mg/l. The content of polysaccharides in this fraction fluctuated significantly depending on the extraction method – from 49.95 to 157.71 mg/l; the content of humic acid in fact was almost independent of the extraction method – 68.76 to 78.84 mg/l. The most efficient method for extracting the protein-containing EPS fraction from excess activated sludge biomass is the  $\text{NH}_4\text{OH}$ /EDTA method; for extracting the polysaccharides – the HCHO/NaOH method; for the humic acids – the  $\text{CH}_3\text{NO}$ /EDTA method.

Table 2. The results of the extraction of extracellular polymeric substances from excess activated sludge

Fractions	Method	Extraction efficiency, %	Protein content in EPS, mg/l	Polysaccharides content in EPS, mg/l	Humic substances content in EPS, mg/l
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Soluble EPS	HCHO/ NaOH	$5,95 \pm 0,65$	$38,26 \pm 5,77$	$13,21 \pm 4,59$	$16,03 \pm 1,84$
	CH <sub>3</sub> NO/ NaOH	$7,21 \pm 0,23$	$45,49 \pm 3,5$	$19,22 \pm 2,82$	$20,68 \pm 1,32$
	CH <sub>3</sub> NO/ EDTA	$7,21 \pm 0,23$	$45,49 \pm 3,5$	$19,22 \pm 2,82$	$20,68 \pm 1,32$
	NH <sub>4</sub> OH/ EDTA	$5,95 \pm 0,65$	$38,26 \pm 5,77$	$13,21 \pm 4,59$	$16,03 \pm 1,84$
Loosely bound EPS	HCHO/ NaOH	$11,59 \pm 0,86$	$72,53 \pm 4,95$	$21,33 \pm 1,41$	$35,57 \pm 3,96$
	CH <sub>3</sub> NO/ NaOH	$11,36 \pm 0,45$	$63,45 \pm 1,23$	$23,81 \pm 1,76$	$35,41 \pm 4,55$
	CH <sub>3</sub> NO/ EDTA	$11,36 \pm 0,45$	$63,45 \pm 1,23$	$23,81 \pm 1,76$	$35,41 \pm 4,55$
	NH <sub>4</sub> OH/ EDTA	$18,73 \pm 0,21$	$125,58 \pm 3,09$	$36,52 \pm 2,47$	$43,17 \pm 6,58$
Tightly bound EPS	HCHO/ NaOH	$62,14 \pm 0,34$	$472,79 \pm 4,33$	$157,71 \pm 4,94$	$72,63 \pm 2,31$
	CH <sub>3</sub> NO/ NaOH	$57,52 \pm 0,69$	$459,37 \pm 6,61$	$116,37 \pm 3,88$	$73,67 \pm 0,52$
	CH <sub>3</sub> NO/ EDTA	$56,34 \pm 0,52$	$508,71 \pm 0,62$	$49,95 \pm 3,18$	$78,84 \pm 0,00$
	NH <sub>4</sub> OH/ EDTA	$57,75 \pm 0,73$	$494,46 \pm 5,78$	$88,46 \pm 5,65$	$68,76 \pm 1,31$

The research of efficiency of using EPS for wastewater treatment, made by the Couran's method, showed different extent of purification for the considered EPS fractions (Table 3). In general, the purification result was in the range from 0.2% to 62.6% for the concentration of biofloculants from 12.6 to 793 mg/l. The best results in terms of efficiency of contamination flocculation for the soluble EPS fraction were demonstrated by the CH<sub>3</sub>NO/NaOH and CH<sub>3</sub>NO/EDTA methods. The NH<sub>4</sub>OH/EDTA method was the most effective for the loosely bound EPS fraction. The tightly bound EPS fraction showed the best result with the use of the CH<sub>3</sub>NO/NaOH method.

Table 3 Efficiency of using EPS obtained by different methods for wastewater treatment

		EPS						
		concentration, g/l	12,6	15,2	19	25	38	76
Soluble EPS	HCHO/NaOH and NH <sub>4</sub> OH/EDTA	Treatment efficiency	0,6%	0,8%	1,0%	1,5%	1,7%	2,2%
	CH <sub>3</sub> NO/NaOH and CH <sub>3</sub> NO/EDTA	EPS concentration, g/l	15,3	18,4	23	30,6	46	92
Loosely bound EPS		Treatment efficiency	1,0%	1,5%	2,2%	3,6%	1,4%	1,9%
	HCHO/NaOH	EPS concentration, g/l	24,6	29,6	37	49,3	74	148

Tightly bound EPS	CH <sub>3</sub> NO/NaOH and CH <sub>3</sub> NO/ EDTA	Treatment efficiency	2,6%	3,3%	3,5%	3,9%	4,9%	5,2%
		EPS concentration, g/l	24,2	29	36,2	48,3	72,5	145
	NH <sub>4</sub> OH/EDTA	Treatment efficiency	0,2%	0,4%	1,1%	1,6%	2,1%	2,5%
		EPS concentration, g/l	39,8	47,8	59,7	79,6	119,5	239
	HCHO/NaOH	Treatment efficiency	12,8%	15,6%	24,3%	27,5%	17,5%	24,4%
		EPS concentration, g/l	132,2	158,6	198,3	264,3	396,5	793
	CH <sub>3</sub> NO/NaOH	Treatment efficiency	3,6%	11,5%	11,7%	21,0%	5,1%	0,3%
		EPS concentration, g/l	122,3	146,8	183,5	244,6	367	734
	CH <sub>3</sub> NO/ EDTA	Treatment efficiency	31,0%	42,8%	46,4%	62,6%	50,8%	56,7%
		EPS concentration, g/l	119,8	143,8	179,8	239,6	359,5	719
	NH <sub>4</sub> OH/EDTA	Treatment efficiency	18,0%	21,0%	21,8%	25,8%	27,6%	0,5%
		EPS concentration, g/l	122,8	147,4	184,25	245,6	368,5	737
		Treatment efficiency	5,3%	6,6%	16,7%	19,1%	24,9%	45,4%

### 3. Conclusion

Thus, it can be asserted that the EPS extraction method is important not only for extraction effectiveness, but also for the possibility of using extracted biopolymers for wastewater treatment. The most efficient EPS extracting method did not prove to be invariably the best in terms of obtaining effective biofloculants for wastewater treatment.

The solution to this problem may lie in selection and combination of methods that will reduce or exclude damage to flocculation-effective EPS molecules. The solution may be also based on modified methods that will include stage-by-stage extraction of different fractions of the effective EPS with their subsequent joint use for increasing the flocculation efficiency of wastewater pollution with the help of EPS.

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