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

Zh Vasilieva, I Gaponenkov, M Vasekha and T Ivanova

1 Murmansk State Technical University, Murmansk, Russia
2 Peter the Great St. Petersburg Polytechnic University, Russia

Abstract. Extracellular polymeric substances (EPS) are efficient and environmentally friendly bioflocculants 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 NH₄OH/EDTA method; the one for extracting polysaccharides – the HCHO/NaOH method, and the one for extraction of humic acids – the CH₃NO/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.

| 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 CH₃NO/NaOH and CH₃NO/EDTA methods [31], the NH₄OH/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 1. EPS liquid fractions
Figure 2. EPS Lyophilized Fractions: (a) the HCHO/NaOH and NH$_4$OH/EDTA methods, unbound ESP fraction; (b) the CH$_3$NO/NaOH and CH$_3$NO/EDTA methods, unbound ESP fraction; (c) the HCHO/NaOH method, loosely bound EPS fraction; (d) the CH$_3$NO/NaOH and CH$_3$NO/EDTA methods, loosely bound EPS fraction; (e) the NH$_4$OH/EDTA method, loosely bound EPS fraction; (f) the HCHO/NaOH method, tightly bound EPS fraction; (g) the CH$_3$NO/NaOH method, tightly bound ESP fraction; (i) the NH$_4$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 NH$_4$OH/EDTA method; for extracting the polysaccharides – the HCHO/NaOH method; for the humic acids – the CH$_3$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 |
|-----------|--------|--------------------------|-----------------------------|-------------------------------------|-------------------------------------|

4
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 bioflocculants 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$_3$NO/NaOH and CH$_3$NO/EDTA methods. The NH$_4$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$_3$NO/NaOH method.

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

| Method                  | Concentration, g/l | Treatment efficiency |
|-------------------------|--------------------|----------------------|
| Soluble EPS HCHO/NaOH   | 5.95 ± 0.65        | 38.26 ± 5.77         | 13.21 ± 4.59 | 16.03 ± 1.84 |
| Soluble EPS CH$_3$NO/NaOH | 7.21 ± 0.23        | 45.49 ± 3.5          | 19.22 ± 2.82 | 20.68 ± 1.32 |
| Soluble EPS CH$_3$NO/EDTA | 7.21 ± 0.23        | 45.49 ± 3.5          | 19.22 ± 2.82 | 20.68 ± 1.32 |
| Soluble EPS NH$_4$OH/EDTA | 5.95 ± 0.65        | 38.26 ± 5.77         | 13.21 ± 4.59 | 16.03 ± 1.84 |
| Loosely bound EPS HCHO/NaOH | 11.59 ± 0.86       | 72.53 ± 4.95         | 21.33 ± 1.41 | 35.57 ± 3.96 |
| Loosely bound EPS CH$_3$NO/NaOH | 11.36 ± 0.45      | 63.45 ± 1.23         | 23.81 ± 1.76 | 35.41 ± 4.55 |
| Loosely bound EPS CH$_3$NO/EDTA | 11.36 ± 0.45      | 63.45 ± 1.23         | 23.81 ± 1.76 | 35.41 ± 4.55 |
| Loosely bound EPS NH$_4$OH/EDTA | 18.73 ± 0.21      | 125.58 ± 3.09        | 36.52 ± 2.47 | 43.17 ± 6.58 |
| Tightly bound EPS HCHO/NaOH | 62.14 ± 0.34       | 472.79 ± 4.33        | 157.71 ± 4.94 | 72.63 ± 2.31 |
| Tightly bound EPS CH$_3$NO/NaOH | 57.52 ± 0.69       | 459.37 ± 6.61        | 116.37 ± 3.88 | 73.67 ± 0.52 |
| Tightly bound EPS CH$_3$NO/EDTA | 56.34 ± 0.52       | 508.71 ± 0.62        | 49.95 ± 3.18 | 78.84 ± 0.00 |
| Tightly bound EPS NH$_4$OH/EDTA | 57.75 ± 0.73       | 494.46 ± 5.78        | 88.46 ± 5.65 | 68.76 ± 1.31 |
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 bioflocculants 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.

References

[1] More T, Yadav J and Yan S 2014 Extracellular polymeric substances of bacteria and their potential environmental applications Journal of Environmental Management p 25
[2] Wingender J et al 1999 Microbial Extracellular Polymeric Substances: Characterization, Structure and Function. Springer-Verlag Berlin Heidelberg p 258
[3] Staudt C et al 2004 Volumetric measurements of bacterial cells and extracellular...
polymeric substance glycoconjugates in biolms Biotechnology and Bioeng 88 p 585-592
[4] Platt R M et al 1985 Isolation and partial chemical analysis of firmly bound exopolysaccharide from adherent cells of a freshwater sediment bacterium Can. J. Microbiol 31 p 675-680
[5] Frølund B et al 1996 Extraction of extracellular polymers from activated sludge using a cation exchange resin Wat. Res p 1749-1758
[6] Wassels S et al 2004 The lactic acid bacteria, the food chain, and their regulation Trends in Food Science and Technology 15 p 498-505
[7] Shatskaya N et al 1994 Technological aspects of the production of dairy products with low allergic properties and hypoallergenic foods Problems of Nutrition 1(2) p. 15
[8] Richert L et al 2005 Characterization of Exopolysaccharides Produced by Cyanobacteria Isolated from Polynesian Microbial Mats. Current Microbiology 51 p 379-384
[9] Parkinson I S et al 1979 Fracturing dialysis ostendostrophy and dialysis encephalopathy. An epidemiological survey The Lancet 1 p 406-409
[10] McLachlan D et al. 1991 Would decreased aluminum ingestion reduce the incidence of Alzheimer’s disease? Can. Med. Assoc. J. 145(7) p 796-804
[11] Rudén C 2004 Acrylamide and cancer risk-expert risk assessments and the public debate Food and Chemical Toxicology 42 p 335–349
[12] Gamboa da Costa G et al 2003 DNA adduct formation from acrylamide via conversion to glycidamide in adult and neonatal mice Chem. Res.Toxicol. 16(10) p 1328-1337
[13] Tapan K 2012 Microbial Extracellular Polymeric Substances: Production, Isolation and Applications Journal of Pharmacy p 276-281
[14] Sheng G Yu H and Yu Z 2005 Extraction of the extracellular polymeric substances from photosynthetic bacterium Rhodopseudomonas acidophila Appl.Microbiol. Biotechnol 67 p.125–130
[15] Ma F Zheng L N and Chi Y 2008 Applications of Biological Flocculants (BFs) for Coagulation Treatment in Water Purification: Turbidity Elimination Chem. Biochem. Eng. Q. 22(3) p 321–326
[16] Amy S Gong et al 2009 Extraction and Analysis of Extracellular Polymeric Substances: Comparison of Methods and Extracellular Polymeric Substance Levels in Salmonella pullorum SA 1685, Environmental Engineering Science 26 10
[17] Tchobanoglous G et al 2003 Wastewater engineering – treatment and reuse (4th edition): Metcalf and Eddy. Rev (New York: McGraw-Hill) p 823
[18] Prianikova A A et al 2018 Evaluation of the Waste Processing System in the Concept of Management of the Sustainable Development of the Arctic Zone of the Russia Proceedings of the 2018 IEEE International Conference &quot;Management of Municipal Waste as an Important Factor of Sustainable Urban Development&quot; WASTE 2018 8554102 p7-9
[19] Govorova N et al 2018 Russian Arctic: problems of international cooperation, Contemporary Europe -Sovremennaya Evropa 1 pp 156-159
[20] Salehizadeh H and Shokaosadati S A 2001 Extracellular biopolymeric flocculants: recent trends and biotechnological importance. Bioeng. Adv. 19 p 371–385
[21] 1977 The method of technological control of the sewage treatment plant operation (Moscow: Stroyizdat) p 299
[22] 1997 Methods for measuring the contents of suspended solids and total impurities in samples of natural and treated wastewater by the gravimetric method: HDPE F 14.1.2.110-97 p16
[23] 1997 Methods for measuring the mass concentration of dry residue in samples of natural and treated wastewater by the gravimetric method: HDPE F 14.1.2.114-97 p 20
[24] Lowry O 1951 Protein measurement with the Folin phenol reagent J. Biol. Chem p 265–275
[25] DuBois M et al 1956 Colorimetric method for determination of sugars and related substances.
**Anal. Chem.** **28** p 350–356

[26] Kononova M M and Belchikova N P 1961 Accelerated methods for determining the composition of humus mineral soils. *Soil Science* **10** 75–87

[27] Eaton A 2005 Standard methods for the examination of water and wastewater, 21st edition *Washington DC: American Public Health Association* p 541

[28] Kurane R et al 1986 Screening for and Characteristics of Microbial Flocculants. *Agric. BiolChem.* p 7

[29] 2005 Quantitative chemical analysis of waters. Methods for measuring the chemical oxygen consumption in drinking, natural and wastewater samples by the photometric method: 14.1: 2:4.210-05 p 19

[30] 1997 Quantitative chemical analysis of waters. Methods for measuring the mass concentration of dissolved oxygen in samples of natural and waste waters by the iodometric method: HDPE F 14.1: 2:3.101-97 p 20

[31] Liang Z et al 2010 Extraction and structural characteristics of extracellular polymeric substances (EPS), pellets in autotrophic nitrifying biofilm and activated sludge *Chemosphere* **81**(5) p 626 - 632

[32] Sato T and Ose Y 1984 Floc-forming substances extracted from activated sludge with ammonium hydroxide and edta solutions *Dept. of Environmental Hygiene* p 517-529

[33] Pellicer-Nàcher C et al 2013 Critical assessment of extracellular polymeric substances extraction methods from mixed culture biomass *Water Res.* **47** p 5564–5574